TOLL2008: RECENT ADVANCES IN PATTERN RECOGNITION

CASCAIS, PORTUGAL

SEPTEMBER 24-27, 2008

SCIENTIFIC ORGANIZING COMMITTEE

Neal Silverman, University of Massachusetts Medical School,
Worcester, MA USA

Alan Ezekowitz, Merck and Co., Rahway, NJ, USA

Douglas Golenbock, University of Massachusetts Medical School

Eicke Latz, University of Massachusetts Medical School

Egil Lien, University of Massachusetts Medical School

Jules Hoffmann, Institut de Biologie Moleculaire et Cellulaire, Strasbroug, France

Miguel Soares, Instituto Gulbenkian de Ciência, Oeiras, Portugal

Executive Secretariat

Forum d'Ideias
toll2008@forumdideias.com

Susann Paul
University of Massachusetts Medical School
susann.paul@umassmed.edu

The Organizing Committee would like to thank Bernardo Simões Franklin for his help in editing this abstract book.

SPONSORS

MERCK & CO.

GlaxoSmithKline

MEDIMMUNE

DYNAVAX

EISAI RESEARCH INSTITUTE

IDERA PHARMACEUTICALS

PFIZER PHARMACEUTICALS

UEHARA MEMORIAL FOUNDATION

BURROUGHS WELLCOME FUND

COLEY PHARMACEUTICAL GROUP

GENENTECH

INNATE PHARMA

NOVARTIS PHARMACEUTICALS CORPORATION

OPSANA THERAPEUTICS

VentiRx Pharmaceuticals

WYETH RESEARCH

ALEXIS BIOCHEMICALS

IMGENEX CORPORATION

IMMUNETICS, INC.

InvivoGen

eBioscience

Cell Press

SABiosciences

Table Contents

Abstract Number	Neighborhood	First Author	Abstract title	Page
116.	Inflammatory processes in health and disease	Lawrenz, M.	TLR-mediated inflammatory responses of the intestine	144
117.		Amaral, F.A.	Commensal microbiota is fundamental for the development of inflammatory pain	145
118.		Astudillo, A.M.	Regulation of TLR-mediated eicosanoid production in macrophages by phospholipases A2	146
119.		Cassel, S.L.	The Nalp3 inflammasome is essential for the development of silicosis	147
120.		Chaturvedi, A	The B Cell Receptor governs the subcellular location of TLR9 leading to hyperresponses	148
121.		de Vos, A.F.	Role of CD14 in the recognition of smooth and rough LPS in the lung	149
122.		Grela, F	Toll-like receptor 7 stimulation activates directly Natural Killer T cells: implications in allergic asthma	150
123.		Mancek-Keber, M	MICROVESICLES ACTIVATE TOLL-LIKE RECEPTORS AND CONTRIBUTE TO INFLAMMATORY PROCESSES	151
124.		Månsson, A.	Nasal CpG Administration Induces Local Airway Inflammation	152
125.		Marchlik, E.	Anti-inflammatory roles for TBK1 in innate and adaptive immunity	153
126.		Nugent, A.S.	TLR-9 senses human fetal DNA	154
127.		Palmer CD	Prolonged Protein Kinase C phosphorylation following macrophage stimulation in Crohn's Disease - a possible link to apoptosis?	155
128.		Pulskens, W.P.C.	TLR-4 IS INVOLVED IN TUBULO-INTERSITIAL INJURY FOLLOWING UNILATERAL URETHER OBSTRUCTION	156
129.		Raby, A.-C.	Negative regulation of TLR2-mediated pro-inflammatory responses by soluble TLR2	157
130.		Rahman, F.	Impaired bacterial clearance in Crohn's Disease results from attenuated macrophage pro-inflammatory cytokine release following TLR stimulation	158

Abstract Number	Neighborhood	First Author	Abstract title	Page
143.		Gribar, S.C.	Toll-like receptor 9 (TLR9) activation attenuates TLR4 signaling in enterocytes via IRAK-M and attenuates the severity of intestinal inflammation.	171
144.	Sepsis and immune responses to bacteria	Hoogerwerf, J.J.	Sepsis-Induced Suppression Of Lung Host Defense Is Mediated By St2	173
145.		Dejonckheere, E.	The Role Of Matrix Metalloproteinase-8 In Endotoxemia And Immunity	174
146.		Maciej Lech1	Tir8/Sigirr Prevents Murine Lupus By Suppressing The Immunostimulatory Effects Of Lupus Autoantigens.	175
147.		Roger, T.	Protection From Lethal Gram-Negative Septic Shock By Anti-Toll-Like Receptor 4 Antibodies	176
148.		Schmaler, M	In Sepsis Host Protection Via Myd88-Mediated Inflammation Is Opposed By S. Aureus Lipoproteins, Which Enable Bacterial Growth Via Iron Uptake	177
149.		Talbot, S	The Toll-Like Receptor 4 Signaling Pathway And Salmonella Infections: A Complex Relationship	178
150.		Alvarez-Arellano, L	Tlrs-Dependent Release Of IL-8 From Human Neutrophils H. Pylori-Infected	179
151.		Wright, J.	The Response Of Intracellular Salmonella Enterica To TLR4 Modulation Of The Macrophage Environment	180
152.		Buß, C.	Role Of IRF3 And IRF7 In Chlamydophila Pneumoniae-Mediated IFN-Beta Response And Control Of Bacterial Replication In Endothelial Cells	181
153.		Camorlinga-Ponce, M.	Expression Of Toll-Like Receptors In Gastric Mucosa Of Children Infected With Helicobacter Pylori.	182
154.		Carneiro, L.A.	Nod1 Protects Non-Myeloid Cells From Shigella Flexneri-Induced Cell Death By Preventing Mitochondrial Damage	183
155.		Wieland, C.W.	TLR4 Is Of Primary Importance In Host Defense Against Gram-Negative Infection With Klebsiella Pneumoniae But TLR2 "Help" Is Needed When Bacterial Numbers Are High	184

Abstract Number	Neighborhood	First Author	Abstract title	Page
247.	Linking innate and acquired immunity	Hutchinson, S.R.	Functional Role Of Murine TLR8 In Autoantibody Production	280
248.		Cottalorda, A.	Direct Involvement Of TLR2 In The Maintenance Of Memory CD8 Cells	281
249.		Magalhaes, J.G.	Nod1 And Nod2-Dependent Th2 Polarization Of Antigen-Specific Immunity Is Dependent Of Radio-Resistant Cells.	282
250.		Mercier, B.C.	Tlr2 Engagement On Cd8 T Cells Enables Generation Of Functional Memory Cells In Response To Suboptimal Activation	283
251.		Pargmann, D.	T-Cell Co-Stimulation By TLR7/8 Ligands Is Dependent On The Cellular Environment	284
252.		Stein, K.	The Allergy Protective Lactococcus Lactis Strain G121 Requires Intracellular Signaling For Dendritic Cell Activation	285
253.		Uematsu, S.	Regulation Of Humoral And Cellular Gut Immunity By Lamina Propria Dendritic Cells Expressing Toll-Like Receptor 5	286
254.		Jakob Loschko	Role of TLR7 and IRAK1 for pristane-induced autoantibody production and development of lupus nephritis	287

Innate immune responses to parasites

ABSTRACT 1

Th2 ANTIGEN RESPONSE IN MICE CAN BE ENHANCED BY GLYCOINOSITOLPHOSPHOLIPID FROM Trypanosoma cruzi, IN A TOLL-LIKE RECEPTOR 4-DEPENDENT PROCESS.

Ramon Lemos[1], Adriana Bonomo[1], Maria-Ignez G. Elsas[2], Pedro P. X. Elsas[1], José O. Previato[3], Lúcia Mendonça-Previato[3], Alberto Nóbrega[1] and Maria Bellio[1].
[1]Instituto de Microbiologia Prof. Paulo de Góes, Universidade Federal do Rio de Janeiro, Brazil;

[2]Instituto Fernandes Figueiras, FIOCRUZ, Rio de Janeiro, Brazil.;

[3]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil.

The surface of trypanosomatid parasites contains large amounts of glycoinositolphospholipid (GIPL). We have recently demonstrated that GIPL from Trypanosoma cruzi, the etiologic agent of Chagas' disease, is a TLR4 agonist with proinflammatory effects and that spleen cells from GIPL-injected mice display an enhanced production of IL-4 following in vitro stimulation by anti-CD3. In the present study we investigated whether GIPL administration could also modulate the adaptive response toward a defined antigen. Our data demonstrate that 8 days after receiving a single GIPL injection i.p., 24 h prior to a secondary OVA/alum immunization, GIPL-treated mice have higher OVA-specific IgE serum levels and an augmented frequency of CD4+IL-4+ lymphocytes. These mice also have augmented eosinopoiesis, revealed by bone marrow cultures in the presence of rIL-5. Moreover, the increase in eosinophil precursor numbers in the bone marrow of GIPL-injected mice is dependent on IL-4 and on TLR4 expression. Together these results indicate that GIPL may skew the acquired response toward a Th2 profile in a TLR4-dependent manner.

Supported by: FAPERJ, CNPq, CAPES and the Millennium Institute for Vaccine Development and Technology (CNPq - 420067/2005-1).

ABSTRACT 2

INTERACTION BETWEEN Paracoccidioides brasiliensis AND HUMAN NEUTROPHILS MODULATES TLR2 AND TLR4 EXPRESSION

Luciane A. Dias-Melicio1, Michele J. Acorci1, Érika Nakaira-Takahagi1, Marjorie A. Golim2, Ana P. Bordon-Graciani1, Maria Terezinha S. Peraçoli1, and Ângela M. V. C. Soares1
1 Dept. Microbiology and Immunology, Biosciences Institute, São Paulo State University, Botucatu-SP, Brazil,

2 Botucatu Blood Center, School of Medicine, São Paulo State University, Botucatu-SP, Brazil.

Paracoccidiodomycosis (PCM) is a systemic mycosis caused by P. brasiliensis that is endemic in Latin America and presents a wide spectrum of clinical manifestations. Because of the great number of neutrophils (PMNs) found in P. brasiliensis granuloma, studies have been done to evaluate the role of these cells during the development of the infection. Thus, in the present study, we investigated whether interaction between P. brasiliensis and PMNs could modulate TLR2 and TLR4 expression in these cells. First, PMNs were incubated or not with GM-CSF, IL-15, TNF-alpha, IFN-gamma and LPS during 18h, with subsequent challenge with P. brasiliensis during 4h, and after, evaluated for TLR2 and TLR4 expression by flow cytometry. Our results showed that the constitutive TLR4 expression on PMNs was much higher than TLR2. All stimuli, mainly GM-CSF and LPS, were able to increase TLR2 and TLR4 expression. Striking results were obtained when PMNs and activated PMNs were challenged with the fungus, showing a significant increase mainly in TLR4 expression. Once neutrophils are the first cells recruited to infection sites, the capacity of P. brasiliensis to induce mainly TLR4 expression on these cells could be an important process in the induction of inflammatory cytokines in the early immune response against this fungus.
* Dias-Melicio and Acorci contributed equally.

ABSTRACT 3

MyD88 is a mediator of protective immunity induced by Plasmodium radiation attenuated sporozoites

Ana França1, Maria M. Mota1,2, and António Coutinho1

1Instituto Gulbenkian de Ciência, Oeiras, Portugal, 2Instituto de Medicina Molecular, Lisbon, Portugal

Sterile protective immunity against malaria can be elicited through immunization with radiation-attenuated sporozoites. This experimental model has been unraveling essential defense mechanisms involved in the establishment and maintenance of protective immunity. However, these have not been fully elucidated.Toll-like receptors (TLRs) play a critical role in the activation of innate immunity by the recognition of parasite specific molecules. In signaling pathways via TLRs, MyD88 is a common adaptor which is essential for the production and release of inflammatory cytokines. In order to determine whether these host immune players are involved in the protection against Plasmodium liver infection, TLR2-/-, TLR4-/-, TLR9-/- and MyD88-/- mice were immunized with radiation-attenuated sporozoites. Upon immunization with one or two doses of irradiated sporozoites, MyD88-/- mice are able to develop some degree of protection, although to a smaller extent when comparing to wild type mice. Nevertheless, this was not enough to confer full protection after challenge with viable sporozoites. Accordingly, MyD88-/- mice failed to develop protective immunity against Plasmodium berghei sporozoites upon three doses of irradiated sporozoites, whereas in immunized wild-type, TLR2-/-, TLR4-/- and TLR9-/- mice, parasite development was arrested. These findings point out the essential but not exclusive role of MyD88 (TLR2, TLR4 and TLR9 independent) in the protection elicited by irradiated sporozoites, emphasizing the relevance of innate immune mechanisms in the development of protective immunity to Plasmodium.

ABSTRACT 4

Pro-Inflammatory Priming in Febrile Malaria Patients: Interferon-γ-Inducible Genes mediate Hyper-Responsiveness of Toll-Like Receptors

Bernardo S. Franklin,2* Peggy Parroche,1* Fanny Lauw,1# Catherine Ropert, 2 Dhelio Pereira,3 Paulo Nogueira,3Σ Luiz Hildebrando Pereira da Silva,3 Harry Björkbacka,4 Douglas T. Golenbock,1,2π and Ricardo T. Gazzinelli1,2,5π

1- Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical School, 364 Plantation Street, LRB 208, Worcester, MA 01605, USA;

2- Immunopathology Laboratory, René Rachou Institute, FIOCRUZ, Av. Augusto de Lima 1715, Belo Horizonte, MG 30190, Brazil;

3- Centro de Pesquisas em Medicina Tropical de Rondônia, Br 364, Km 4.5, Porto Velho, RO 78900, Brazil;

4- Experimental Cardiovascular Research, Lund University, Malmö SE 205 02, Sweden;

5- Department Biochemistry and Immunology, Biological Sciences Institute, Federal University of Minas Gerais, Av. Antonio Carlos 6627, Belo Horizonte, MG 31270, Brazil

Malaria-induced sepsis is associated with an intense pro-inflammatory cytokinemia for which the underlying mechanisms are poorly understood. It has been demonstrated that experimental infection of humans with Plasmodium falciparum primes TLR-mediated pro-inflammatory responses. Nevertheless, the relevance of this phenomenon during natural infection and, more importantly, the mechanisms by which malaria mediates TLR hyper-responsiveness are unclear. Here we show that TLR responses are boosted in febrile patients during natural infection with P. falciparum. Microarray analyses demonstrated that an extraordinary percentage of the up-regulated genes, including genes of TLR signaling pathway, had sites for IFN-inducible transcription factors. To further define the mechanism involved in malaria-mediated "priming," we infected mice with P. chaubadi. The human data was remarkably predictive of what we observed in the rodent malaria model. Malaria-induced priming of TLR responses correlated with increased expression of TLR mRNA in a TLR9, MyD88- and IFNγ-dependent manner. Acutely infected wild-type mice were highly susceptible to LPS-induced lethality while TLR9-/-, and to a greater extent, IFNγ-/- mice were protected. Our data provide unprecedented evidence that TLR9 and MyD88 are essential to initiate. IFNγ responses and favor host hyper-responsiveness to TLR agonists resulting in overproduction of pro-inflammatory cytokines and the sepsis-like symptoms of acute malaria.

ABSTRACT 5

Unc93b1 mutant mice are highly susceptible to infection with Toxoplasma gondii despite the unimpaired production of pro-inflammatory cytokines

Mariane B. Melo, Douglas Golenbock, Egil Lien, and Ricardo T. Gazzinelli

Dept. of Medicine, University of Massachusetts Medical School, Worcester, MA, USA; Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; Laboratório de Imunopatologia, CPqRR, FIOCRUZ, Belo Horizonte, Minas Gerais, Brazil.

Unc93b1 is essential for signaling through intracellular Toll-like receptors (TLRs) in both humans and mice. This ER-resident protein specifically interacts with TLR3, 7 and 9 and regulates their trafficking to endolysosomes, where the receptors trigger signaling cascades via MyD88/TRIF-dependent pathways. Here we show that mice displaying a recessive point-mutation in Unc93b1 (H412R), known as 3d mice, are highly susceptible to infection with the intracellular protozoan Toxoplasma gondii. In contrast with control littermates that display 100% survival, infection of 3d mice with the avirulent ME-49 parasite strain resulted in 100% mortality within 10 days, while MyD88-/- and MyD88/TRIF-/- animals succumbed between days 15-20 post-infection. Microscopic examination of peritoneal exudates from infected 3d mice demonstrated that mortality was associated with uncontrolled parasite replication. FACS analysis of spleen cells from acutely infected 3d mice indicated decreased expression of activation markers and no expansion of both monocyte and T lymphocyte populations. Further, MHC I and II expression, as well as IL-12p40 and nitric oxide production, all known to be induced by IFN gamma, were reduced in infected 3d mice. Surprisingly, contrasting with MyD88-/- and MyD88/TRIF-/- mice, we observed an unimpaired TNFalfa, IFN gamma, IL-6, MCP-1 and RANTES production in infected 3d mice. Further, mortality rates, parasitism and cytokine production by TLR3-/-, TLR7-/-, TLR9-/-, and TRIF-/- (as well as IL-1R-/-) mice compared with those from wild type C57BL/6 mice. Thus, our findings provide evidences that a TLR-independent event regulated by Unc93b1 plays a critical role in the immune response and host resistance to infection with T. gondii.

ABSTRACT 6

THE GLICOPROTEIN (GP43) OF Paracoccidioides brasiliensis MODULATES TLR2 AND TLR4 EXPRESSION AND CYTOKINE PRODUCTION BY HUMAN MONOCYTES

Erika Nakaira-Takahagi1, Marjorie A. Golim1, Rosana Puccia2, Michele J. Acosci1, Camila F. Bannwart1, Renata Cristofalo1, Luciane A. Dias-Melicio 1, Ângela M.V.C. Soares1, Maria T.S. Peraçoli1.

1São Paulo State University, Botucatu, S.P., Brazil; 2Federal University of São Paulo, São Paulo, SP, Brazil

Toll-like receptors recognize distinct components of fungi to initiate the innate immune response. We examined whether P. brasiliensis (Pb) or its immunodominant antigen gp43 can modulate in vitro TLR2 and TLR4 expression and induce the production of tumor necrosis factor-alpha (TNF-alpha) and interleukin-10 (IL-10) by human monocytes. Monocytes from healthy individuals were incubated with gp43, lypopolysaccharide (LPS) or Pb yeast cells in a ratio of 50 monocytes per fungal cell at 37oC for 4h and 18h. The expression of TLR2 and TLR4 on monocyte surface and TNF-alpha and IL-10 production were determined by flow citometry and ELISA respectively. The results showed that monocyte stimulation with LPS or Pb induces an up-regulation of the TLR2 and TLR4 expression on monocyte in relation to the non-stimulated cells at both 4h and 18h of culture. High TLR4 and low TLR2 expression were elicited by gp43 at 4h of culture. However they changed to high TLR2 and low TLR4 expressions at 18h. The up-regulation of TLR4 or TLR2 expression induced by gp43 was associated respectively with higher levels of TNF-alpha at 4h or IL-10 at 18h of culture. The up-regulation of TLR2 associated with higher levels of IL-10 produced by monocytes after stimulation with gp43 might allow the fungus to establish infection in the host.

Supported by FAPESP, proc. no. 06/53366-9

ABSTRACT 7

Differential response of bone marrow -derived murine macrophages deficient in TLR2, MyD88 or CD14 to Mycobacterium avium and Mycobacterium tuberculosis.

EDUARDO P. AMARAL, ELENA LASUNSKAIA, THEREZA L. KIPNIS
Universidade Estadual do Norte Fluminense

M. tuberculosis and M. avium are important human pathogens. M. tuberculosis is responsible for infection of the one -third of world population, whereas M. avium is able to infect immunodeficient persons predominantly. TLRs are involved to the innate recognition and macrophage (MF) activation by mycobacteria. To address the importance of TLR - mediated pathogen recognition in anti -mycobacterial MF responses, we examined the bacterial capacity to intracellular growth and MF response to M. tuberculosis and M. avium strains. Bone marrow -derived MF were infected at a MOI of 1:1, 10:1 and 50 bacteria per cell, and the number of bacterial colony -forming units (CFU), macrophage viability and proinflammatory mediators, NO and TNF -a, production were quantified within 6 days after infection. M. avium strains, 27TL and 104TL, grew significantly faster in TLR2 -/- and MyD88 -/- MF than in CD14 -/- and macrophages obtained from wild type (WT) mice. In contrast to M. avium, the growth of M. tuberculosis was significantly faster only in MyD88 -/- MF, whereas TLR2 or CD14 deficiency had no effect on the intracellular replication of these bacteria. Cell viability was significantly impaired in TLR2 -/- and MyD88 -/-, but not in CD14 -/- or WT MF cultures infected with both mycobacterial spec ies.
M. tuberculosis was much more cytotoxic in these MF, provoking the death of 80% infected cells, in contrast to 30% of cell death induced by M. avium. Viable bacteria, neither M. tuberculosis, nor M. avium, induced no NO or TNF -a production in studied MF of any genetic background, in contrast to lysed bacteria. The obtained results demonstrated that TLR2/MyD88 pathway is important for the control of intracellular growth of M. avium, whereas M. tuberculosis growth inhibition was MyD88 -, but not TLR2-depe ndent.

ABSTRACT 8

Toll-like receptor-2 in the modulation of regulatory T cells during Leishmania infantum infection

Olivia Rodrigues1, Marta Soares-Clemente1, Sandra Gomes-Pereira2, Shizuo Akira3, Gabriela Santos-Gomes1

1 UL, LA-CMDT, IHMT, Universidade Nova de Lisboa, Portugal, 2 LMII, IBMC, Universidade do Porto, Portugal, 3 RIMD, Osaka University, Osaka, Japan

Toll-like receptor (TLR) signalling is known to directly or indirectly regulate immunosuppressive function of regulatory T cells (Treg) and subsequently influence outcome of immune response. However, which TLR are involved in L. infantum infection and which regulate Treg is still poorly defined. Here, we investigated the role of TLR-2 in the modulation of Treg and the generation of protective immunity to L. infantum. Gene-disrupted TLR-2-/- mice were more susceptible than wild-type C57Bl/6 mice to L. infantum. Higher rates of parasite multiplication, observed in spleen and liver of TLR-2-/- mice showed evidence of non-healing chronic infection.

CD4+CD25+Foxp3+ T cell numbers that were also GITR+ and CD103+ were reduced in TLR-2-/- early during infection. The generation of CD4+CD25- effector T cells in spleen was significantly increased and accompanied by an early Th2 response with expression of higher levels of IL-4 in addition to the presence of immunosuppressive IL-10 and TGF-b as compared to wild-type. Later during infection, although total Treg number did not show important variations, CD4+CD25+Foxp3+CD103+ T cells with migratory phenotype was seen to be increased in TLR-2-/- as compared to C57Bl/6 mice with simultaneous detection of high levels of IFN-g and IL-10-expressing spleen cells. Our data suggests that the absence of TLR-2 triggering may favor IFN-g expression. However, this Th1 response associated to the retention of suppressive Treg at sites of Leishmania infection may result in a balancing effect between these cell subsets that reduces the efficiency of protective immunity, contributing to parasite expansion and increased susceptibility of TLR-2-/- mice to L. infantum.

Funding: FCT-POCI2010-EU Fund (project POCI/CVT/55113/2004, PhD grant SFRH/BD/12250/2003)

ABSTRACT 9

Differential activation of Toll-like Receptors (TLRs) in macrophages and dendritic cells exposed to Trypanosoma cruzi: implications in host innate immune responses and host resistance to infection.

Catherine Ropert1, Douglas T Golenbock2, Ricardo T. Gazzinelli1,2,3

1-Laboratory of Immunopathology, René Rachou Institute, FIOCRUZ, Belo Horizonte, MG,BRAZIL. 2-Division of Infectious Disease and Immunology, University of Massachusetts Medical School, Worcester, MA, USA.

3-Department of Immunology and Biochemistry, Federal University of Minas Gerais, Belo Horizonte, MG, BRAZIL

Different components from *Trypanosma cruzi* have been identified as TLR2, TLR4 and TLR9 agonists. We now demonstrate that similarly to TLR2, TLR6 is critical in innate immune responses to T. cruzi parasites and trypomastigote derived mucin-like glycoproteins linked to glycosylphosphatidylinositol anchors (tGPI-mucin). When we compared the involvement of TLRs in pro-inflammatory responses at the cellular level, it appears that TLRs located at the cell surface membrane (i.e. TLR2) play a predominant role in cytokine release by macrophages in a MAL/TIRAP-dependent manner, while TLR9, located at the endo-lysosomal compartment, is the main receptor responsible for IL-12 release by dendritic cells (DCs) when exposed to live T. cruzi parasites. In this context, we are currently investigating the modulation of the expression of TLR2 and TLR9 in both cells during the infection with T.cruzi. Interestingly, in vivo, the production of IL-12 and IFN-g was under negative and positive control of TLR2 and TLR9, respectively. Importantly, the augmented IFN-g response in infected TLR2-/- mice was not observed in double TLR2/TLR4-/- or MAL-/- infected mice suggesting that TLR4 may also play a positive role in inducing IFN-g during infection with T. cruzi. Thus, while TLR2/TLR6, TLR4 and TLR9 are all involved in trypomastigote recognition and influence each other in the initial induction of pro-inflammatory response, TLR9 appears to have the predominant role in inducing protective immunity, controlling parasite replication, and preventing host lethality in mice acutely infected with T. cruzi.

ABSTRACT 10

Plasmodium falciparum AT-rich DNA induces type I IFN *via* NALP3, but does not require the inflammasome components ASC and caspase-1

Nadege Goutagny[1] , Peggy Parroche[1], Zhaozhao Jiang[1], Daniella C. Bartholomeu[2], Luis Hildebrando Pereira da Silva[3], Rosanne DeOliveira[1], Daniel R. Caffrey[4], Harry Björkbacka[5], Ricardo T. Gazzinelli [1,2], Katherine A. Fitzgerald [1] and Douglas T. Golenbock [1,2].

[1]Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01605.

[2]Universidade Federal de Minas Gerais, Departamento de Parasitologia, Av. Antonio Carlos 6627, Pampulha, Belo Horizonte, MG 31270-901, Brazil;

[3]Centro de Pesquisas em Medicina Tropical de Rondonia, Br 364, Km 4.5, Porto Velho, RO 78900, Brazil

[4]Pfizer Research Technology Center, 620 Memorial Drive, Cambridge, MA 02139.

[5]Experimental Cardiovascular Research, CRC, Lund University, Malmö University Hospital, SE-205 02 Malmö, Sweden.

Malaria is the world's most common infectious disorder affecting hundreds of millions of people annually in developing countries. The molecular mechanisms by which *Plasmodium falciparum* activates immune cells are not well understood. Since the malaria genome is 80% AT-rich, this encouraged us to consider the possibility that additional innate sensing systems for malarial DNA exist. In this study, we demonstrate that AT-rich DNA derived from the malaria genome as well as human genome triggers IFNβ gene transcription *via* cytosolic sensing mechanisms both in human and mouse cell system. Using splenocytes from various deficient mice and shRNA approaches, we demonstrated that induction of IFNβ by AT-rich DNA is not regulated by known cytosolic receptors such as RIG-I, MDA-5 or DAI, neither by the interferon regulatory factor -3 or -7. In contrast, this requires a new signalling pathway involving TBK1, IRF1 and more surprisingly the NLR protein, NALP3. Intriguingly, IFNβ production does not require ASC, caspase-1 or IL-1□ signaling, although AT-richDNA was capable of inducing IL-1β production via caspase-1. Collectively, these data reveal a previously unknown role for NALP3 in transcriptional regulation of IFNβ responses.

Vaccines and adjuvants

ABSTRACT 11

A LOCALLY ADMINISTERED TOLL-LIKE RECEPTOR-7 AGONIST DRIVES SYSTEMIC ANTI-TUMOUR IMMUNE RESPONSES THAT ARE ENHANCED BY ANTI-CD40 IMMUNOTHERAPY

Steve Broomfield1, Amy Prosser1, Robbert van der Most1, Sathish Mahendran1, Bruce Robinson1 and Andrew Currie1

1. Tumour Immunology Group, School of Medicine and Pharmacology, University of Western Australia, Australia

Direct application of select toll-like receptor (TLR) agonists such as the TLR7 agonist imiquimod, to tumors, mimics local viral infection and can thereby activate significant CD8 T cell responses. Typically, responses to topically applied TLR7 agonists have been investigated at the local tumor site or in single tumor models. However, a key problem clinically is the need to promote a systemic response to the tumor, in order to slow or eradicate progression of metastases as well as the primary. We therefore investigated the potential of locally delivered imiquimod, to stimulate an effective systemic immune response in an established mouse tumor model of malignant mesothelioma (AB1-HA) with primary or both primary and distal tumors (dual tumor model). Prolonged delivery of imiquimod into the primary tumor significantly retarded tumor growth in all treated mice compared to a vehicle control. This local anti-tumor immune response required both CD8 T cells and NK cells, but not CD4 T cells, and was reliant on type I IFN induction. In-vivo CTL studies and Ly6A/E staining of lymphocytes suggested that local imiquimod treatment had indeed induced a systemic, antigen-specific CD8 response. However, notably this response was not sufficient/capable of retarding the growth of distal tumors in our dual tumor model. Since local imiquimod treatment did not induce significant CD4 T cell responses, we investigated the efficacy of combining imiquimod with agonistic CD40 antibody (by-passing the need for activated CD4 T cells). Combination of locally delivered imiquimod with systemic anti-CD40 immunotherapy not only significantly enhanced the local anti-tumor response, with 90% complete resolution, it was also effective at significantly retarding growth of distal tumor (45% resolution). These results demonstrate that anti-tumor responses induced by locally delivered TLR7 agonists can be harnessed systemically for treating distal tumors.

ABSTRACT 12

Identification of Dendritic Cell ligands for vaccine development

Dominique De Wit1, Séverine Thomas1, Marcelle Van Mechelen2, Philippe Hermand2, David Johnson3 and Michel Goldman1

1Institute for Medical Immunology, Gosselies, Belgium

2GlaxoSmithKline Biologicals, Rixensart, Belgium

3GlaxoSmithKline Biologicals, Hamilton, MT, USA

Vaccine adjuvants derived from LPS, such as monophosphoryl lipid A (MPL) have proven to be safe and effective in inducing immune responses to heterologous proteins in animal and human vaccines. We investigated the effects of a distinct family of lipid A mimetics, the aminoalkyl glucosaminide phosphates (AGP), in which the length of the secondary acyl chain has been varied. Of the synthetic AGPs tested, CRX-527 (10-10-10) was found to induce MyD88- and TRIF-dependent cytokines by human monocyte-derived dendritic cells (DCs). In contrast, naturally derived MPL is a poor inducer of TRIF-dependent genes in man.

On human cells, cytokine profiles induced by LPS, MPL and CRX-527 were shown to be clearly distinct, providing evidence that the mode of action of these molecules through TLR4 is related to their structure. Analysis of the relationship between structure and function of TLR4 ligands will allow the design of further improved vaccine adjuvants.

ABSTRACT 13

Aluminum Hydroxide Adjuvants Activate the Immune System Through the Nalp3 Inflammasome

Stephanie C. Eisenbarth (1,2), Fayyaz S. Sutterwala (3), Richard A. Flavell (2)
(1) Department of Laboratory Medicine, (2) Department of Immunobiology, Yale University School of Medicine, New Haven, CT, 06504, (3) Inflammation Program, Department of Medicine, University of Iowa, Iowa City, IA 52241

Aluminum adjuvants, typically referred to as "alum", are the only adjuvants licensed for use in human vaccines in the U.S., yet how they initiate an immune response remains unknown to-date. While alum is known to induce the production of proinflammatory cytokines in vitro, it has been repeatedly demonstrated that alum does not require intact Toll-like receptor signaling to activate the immune system. We have found that aluminum adjuvants activate an intracellular innate immune response system called the Nalp3 inflammasome, which senses microbial infection and disruption of cell integrity. Macrophages isolated from WT (C57BL/6) mice stimulated with alum in vitro produce the pro-inflammatory cytokines IL-1beta and IL-18 in a dose-dependent manner. In contrast, macrophages from mice deficient in components of the inflammasome (Nalp3, ASC or Caspase-1) do not produce IL-1beta or IL-18 in response to alum. We confirmed that macrophage death with resultant release of uric acid was not responsible for the inflammasome-dependent cytokine secretion seen with alum by treating macrophage cultures with uricase. Addition of uricase blocked the ability of uric acid to induce IL-1beta but had no effect on alum-induced IL-1beta production. In vivo, IL-1 has been shown to be a potent stimulus for T cell proliferation and antibody production; therefore, we evaluated the adaptive immune response to alum immunization in a model of asthma. We found that both Th2-mediated inflammation and antigen-specific antibody production were reduced in Nalp3-deficent mice. These findings help elucidate how alum regulates immune responses to vaccine components and have implications for the design of new, more effective adjuvants.

ABSTRACT 14

Distinct molecular signatures of human Monocytes and Dendritic Cells predict adjuvant activity and pyrogenicity of TLR agonists.

Richard Kamgang Kenmoe, Inês Ramos, Lurdes Duarte, Mascia Ghielmetti, Marina Freudenberg, Clemens Dahinden and Elisabetta Padovan.

Lisbon Medical School and Instituto Gulbenkian de Ciência, Oeiras, Portugal; University of Bern, Bern, Switzerland; Max Planck Institute for Immunobiology, Freiburg, Germany; Bern Institute of Immunology, Bern, Switzerland.

We present a systematic study that defines molecular profiles of adjuvanticity and pyrogenicity induced by agonists of human Toll-like receptor molecules in vitro.
Using P3CSK4, Lipid A and Poly I:C as model adjuvants we show that all three molecules enhance the expansion of IFNγ+/CD4+ T cells from their naïve precursors following priming with allogeneic DC in vitro. In contrast, co-culture of naive CD4+ T cells with allogeneic monocytes and TLR2/TLR4 agonists only resulted in enhanced T cell proliferation. Distinct APC molecular signatures in response to each TLR agonist underline the dual effect observed on T cell responses. Using protein and gene expression assays, we show that TNF-α and CXCL10 represent DC-restricted molecular signatures of TLR2/TLR4 and TLR3 activation respectively, in sharp contrast to IL-6 produced by monocytes upon stimulation with P3CSK4 and Lipid A. Furthermore, although all TLR agonists are able to upregulate proIL-1β specific gene in both cell types, only monocyte activation with Lipid A results in detectable IL-1β release. These molecular profiles, provide a simple screen to select new immune enhancers of human Th1 responses suitable for clinical application.

ABSTRACT 15

Inflammasome activation by Alum and Alum's adjuvant effect are mediated by NLRP3

Hanfen Li*, Stephen B. Willingham#, Jenny P.-Y. Ting#, and Fabio Re*

*Department Molecular Sciences, University of Tennessee Health Science Center, Memphis, TN 38163, USA, and #Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, NC 27599, USA

Alum is the only adjuvant approved for routine use in humans, although the basis for its adjuvanticity remains poorly understood. We have recently shown that alum activates caspase-1 and induces secretion of mature IL-1? and IL-18. Here we show that in human and mice macrophages, alum-induced IL-1?, IL-18, and IL-33 secretion is mediated by the NLR protein NLRP3 (Nalp3/Cryopyrin) and its adaptor ASC, but not by NLRC4 (Ipaf). Other particulate adjuvants, such as QuilA and chitosan, induced inflammasome activation in a NLRP3-dependent fashion, suggesting that activation of the NLRP3-inflammasome may be a common mechanism of action of particulate adjuvants. Importantly, we demonstrate that antigen-specific antibody production elicited by vaccines that contain alum is significantly impaired in NLRP3-deficient mice. Activation of the NLRP3-inflammasome by alum was not mediated by release of uric acid and was blocked by NADPH-oxidase inhibitors, suggesting that generation of reactive oxygen species regulates inflammasome activation. Our results demonstrate for the first time a role for the NLRP3-inflammasome during development of the immune response elicited by alum-enhanced vaccination and suggest that therapeutic intervention aimed at NLRP3 may improve adjuvant efficacy.

ABSTRACT 16

Development of TLR8 agonists as potential neonatal vaccine adjuvants

Victoria J Philbin, PhD1, Eugenie E Suter, MA1, Leighanne Gallington, BSc1, Ariel Shuckett, M.P.H1, Amity Paye1, Richard L Miller, PhD2, Keith Mansfield, DVM3, Sarah Davies, DVM4, Guadalupe Cortes-Garcia, PhD1, Isaac Kohane5, Kamila Naxerova5 and Ofer Levy, PhD, MD1.

1Medicine and 5Endocrinology, Childrens Hospital Boston, Boston, MA, USA. 23M Pharmaceuticals, St Paul, MN, USA. 3New England Primate Research Centre, Southborough, MA, USA.4California National Primate Research Centre, U.C. Davies, CA, USA.

Human newborns are functionally immunodeficient and mount poor memory responses creating an unmet medical need for effective neonatal vaccine adjuvants. Human neonatal APCs show impaired TLR-mediated activation in response to agonists of TLRs 1-7, reflecting the inhibitory effect of high cAMP levels in neonatal cells. In contrast, TLR8 agonists are capable of activating TNF production from APCs, suggesting efficacy as neonatal vaccine adjuvants. Development of TLR8 agonists as neonatal vaccine adjuvants requires a fuller understanding of their immune-activating effects and mechanisms.

We have demonstrated that TLR8 agonists in neonatal APCs induce: a) broader and more robust activation of the TLR transcriptome and of cytokine protein production, b) co-stimulatory molecule upregulation, c) selective Btk phosphorylation and inhibition using a Btk inhibitor (terreic acid), d) inhibition of TNF production in the presence of terreic acid, e) refractoriness to the inhibitory effects of db-cAMP, f) IFN-α expression (TLR7/8 agonist) and g) greater TNF production than other TLR agonists in neonatal and infant Rhesus macaque whole blood. Furthermore selective mRNA knock-down of TLR8 reveals its importance for 3M002 induced TNF production in human neonatal APCs. The unique ability of TLR8 agonists to trigger robust innate immune responses in neonatal APCs may reflect engagement of Btk signaling pathways refractory to cAMP-mediated inhibition and suggests that these agents may be effective vaccine adjuvants for neonates and infants.

ABSTRACT 17

Efficacious Recombinant Influenza Vaccines Produced by High Yield Bacterial Expression: A Solution to Global Pandemic and Seasonal Needs

Langzhou Song1*, Valerian Nakaar2, Uma Kavita1, Albert Price2, Jim Huleatt2?, Jie Tang2, Andrea Jacobs2, Ge Liu1, Yan Huang2 Priyanka Desai2, Gail Maksymiuk2, Virginia Takahashi2, Scott Umlauf1, Lucia Reiserova1, Rodney Bell1, Hong Li1, Yi Zhang1, William F. McDonald2, T. J. Powell2, Lynda Tussey1

1 VaxInnate Corporation, 3 Cedar Brook Drive, Cranbury, NJ 08512, USA
2 VaxInnate Corporation, 300 George Street, New Haven, CT 06511, USA
? Current address: Sanofi Pasteur, Discovery Drive, Swiftwater, PA 18370, USA.

It is known that physical linkage of TLR ligands and vaccine antigens significantly enhances the immunopotency of the linked antigens. We have used this approach to generate novel influenza vaccines that fuse the globular head domain of the protective hemagglutinin (HA) antigen with the potent TLR5 ligand, flagellin. These fusion proteins are efficiently expressed in standard E. coli fermentation systems and the HA moiety can be faithfully refolded to take on the native conformation of the globular head. In mouse models of influenza infection, the vaccines elicit robust antibody responses that mitigate disease and protect mice from lethal challenge. These immunologically potent vaccines can be efficiently manufactured to support pandemic response, pre-pandemic and seasonal vaccines.

ABSTRACT 18

Intra-nasal instillation of monomeric endotoxin:human MD-2 complex protects mice from pneumonic plague

Athmane Teghanemt1, Theresa Gioannini1, Suzana Hadina2, DeSheng Zhang1, Peter Thorne2, Ashok Chopra3, and Jerrold Weiss1

1The Inflammation Program and 2Environmental Health Science Research Center, University of Iowa and VAMC, Iowa City, and 3Departments of Microbiology and Immunology, University of Texas Medical Branch

Virulent Yersinia pestis is the cause of pneumonic plague. Evasion of Toll-like receptor (TLR) 4 signaling is a major determinant of Y. pestis virulence. We therefore tested if activation of airway TLR4 activity by intranasal (i.n.) instillation of a potent TLR4 agonist could protect against lethal airway Y. pestis infection. Monomeric endotoxin (E):MD-2 complexes are unique in their ability to activate, at pM concentrations, cells expressing mCD14/MD-2/TLR4 (e.g., alveolar macrophages) or TLR4 alone (e.g., airway epithelial cells). E:MD-2 induced dose-dependent, TLR4-dependent acute airway inflammatory responses including recruitment of neutrophils and mobilization of chemokines and cytokines into the airway lumen. Maximum responses were induced within 4-24 h by 15 ng of E:MD-2. A complex of E:MD-2F126A that activates macrophages but not airway epithelial cells induced ca. 30% less airway inflammation, suggesting that effects of instilled wt E:MD-2 were due to TLR4-dependent activation of macrophages and epithelial cells. I.n. administration of 15 ng (E) of wild-type E:MD-2 48 h and 24 h before i.n. administration of virulent Y. pestis CO92 (10x LD50 dose) resulted in survival of 90% (9/10) of the infected mice, whereas only 1/30 mice survived infection when E:MD-2 was administered at either the time of infection, 24 h after or not at all. These findings suggest that E:MD-2 could provide a novel approach to immuno-prophylaxis in outbreaks of pneumonic plague as could occur following a bio-terrorist attack.

ABSTRACT 19

Immunization with Francisella tularensis LPS with the TLR2 ligand, neisserial PorB, induces a high level of protection against respiratory challenge and triggers formation of organized BALT in lungs of mice

Damiana Chiavolini1, Susan Weir1, Javier Rangel-Moreno2, Joseph Alroy3, Troy Randall2 and Lee Wetzler1

1Department of Medicine, Division of Infectious Diseases, Boston University School of Medicine, Boston, MA, 2Trudeau Institute, Saranac Lake, NY, 3Department of Pathology, Tufts University Medical School and Tufts Medical Center, Boston, MA, USA.

Francisella tularensis causes severe pneumonic disease that can lead to fatality if left untreated. Due to the potential use of F. tularensis as a biological weapon, research is being conducted to understand correlates of protection and to develop an effective vaccine against the pneumonic form of the disease. Efforts are also being made to select and study adjuvant molecules able to generate a better and long-lasting protective effect. PorB from Neisseria meningitidis is a well established TLR2 ligand and has been shown to be a promising vaccine adjuvant candidate due to its co-stimulatory capacity both in vitro and in vivo.

BALB/c mice were immunized three times with lipopolysaccharide isolated from F. tularensis live vaccine strain (LVS) (Ft-LPS) mixed to neisserial PorB and challenged intranasally with 106 CFU of LVS. Survival was observed and lungs were dissected from mice at different time points post-infection, and processed for histopathology and immunohistochemistry to determine tissue changes.

Seventy percent of the animals immunized with Ft-LPS+PorB were fully protected from respiratory challenge. Lungs from mice recovering from infection presented prominent lymphoid aggregates, which revealed to be areas of induced bronchial associated lymphoid tissue (iBALT) as shown by the presence of proliferating B cells, germinal centers and T cell infiltration in perivascular areas. Organization of iBALT, lymphocyte proliferation and lymphoid follicle size were found to be increased over time. Interestingly, vaccinated survivors sacrificed at later time points post-challenge also presented classic BALT, which is mostly present around blood vessels in the upper bronchi.

Innate immune responses to viruses

ABSTRACT 20

Cytokine co-expression by poxviruses in Toll-Like Receptor GKO mice reveals immuno-biology of Toll-Like Receptors and cytokines in vivo.

Duncan Sutherland, Klaus Matthaei, Simon Phipps, Kate Stacey, Charani Ranasinghe, Stephanie Day, Paul Foster, Ian Ramshaw

Division of Immunology and Genetics, The John Curtin School of Medical Research, Australian National University, Canberra, ACT, 2601, Australia.

We demonstrate that Toll-Like Receptors (TLR) are essential in controlling pox-virus infection. Mice deficient in MyD88 (adaptor protein for all TLR's except TLR3) are highly susceptible to an attenuated strain of ectromelia virus (mouse pox) while wild-type mice are resistant. Viral load measured in liver at day-6 post infection was 3 logs higher in MyD88-deficient mice than in wild-type mice. This increased susceptible phenotype was also observed in TLR9 deficient mice.

Mice deficient in MyD88 or TLR9 lack the capacity to generate effective antigen-specific CD8+ T cells expressing gamma interferon on primary infection with vaccinia virus (member of the poxvirus family). Co-expression of Type I interferon-beta by recombinant vaccinia virus, however, restored CD8+T cell effector function in MyD88 and TLR9 deficient mice. This result provides in vivo evidence that the potent anti-viral cytokine IFN-beta can overcome TLR based immune deficiency. By contrast, co-expression of the cytokine interleukin-2, a potently attenuated recombinant virus in all previously tested animal strains, is highly pathogenic and not attenuated in MyD88 deficient mice thus demonstrating the dependence of interleukin-2 on TLR signaling for antiviral immune activation. Specifically, we show that the innate antiviral immune function of interleukin-2 is TLR-2 dependent.

Finally, we demonstrate that TLR deficient and wild-type mice can be protected against lethal ectromelia virus infection via co-expression of IFN-B with recombinant vaccinia virus. In conclusion, we have established a powerful in vivo platform, by combining recombinant viral delivery vectors in TLR GKO mice, for the study of TLR/ cytokine immuno-biology.

ABSTRACT 21

THE TYROSINE KINASE C-SRC MODULATES ANTIVIRAL REPONSES INDUCED BY THE CYTOPLASMIC RNA HELICASE RIG-I

Ingvild B. Johnsen1, Thuy Nguyen1, Bjarte Bergstroem1, Katherine A. Fitzgerald2, and Marit W. Anthonsen1

1Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Norway, and 2The University of Massachusetts Medical School, Worcester, MA, USA.

We have previously shown that the nonreceptor tyrosine kinase c-Src is implicated in dsRNA-elicited, TLR3-dependent antiviral immune responses. In this study we show that c-Src additionally participates in RIG-I-mediated antiviral signaling. Sendai virus (SV), which activates endogenous RIG-I, induced c-Src activation. Functional impairment of c-Src through chemical inhibition or genetic depletion strategies using c-Src siRNA or c-Src deficient cells attenuated IRF-3 activation, IP10 and IFN-β production after SV infection. Also, overexpression of RIG-I or its mitochondrial adapter protein MAVS elicited IP10 and IFN-β synthesis that was attenuated by coexpression of a kinase-inactive c-Src mutant. This indicates that c-Src acts downstream of RIG-I and MAVS in activation of IRF-3. We also found that c-Src interacted with proximal and distal components of the RIG-I pathway: RIG-I, MAVS, TRAF3, and TBK1. Moreover, from results obtained using TRAF3 deletion mutants, we suggest that the interaction between c-Src and TRAF3 occurs within the RING domain of TRAF3. Hence, our previous and present results suggest that c-Src is implicated in both TLR-dependent and cytoplasmic RIG-I-mediated pathways, acting at the level of TRAF3, thus modulating immune signaling through two distinct antiviral receptors.

ABSTRACT 22

LOWER EXPRESSION OF TLR7 AND TLR8 IN PBMC FROM RSV-INFECTED INFANTS

Bendelja K1, Gagro A1, Baće A2, Čepin-Bogović J3, Mlinarić-Galinović G4, Rabatić S1

1Institute of Immunology, 2Dr Fran Mihaljević University Hospital for Infectious Diseases, 3Childrens Hospital, 4Croatian National Institute of Public Health, Zagreb, Croatia.

Toll-like receptors (TLRs) are part of the innate immune system able to recognize pathogen-associated molecular patterns and activate immune system upon pathogen challenge. TLR7 and TLR8 recognize single-stranded viral RNA and initiate antiviral control mechanisms. A RNA virus particularly detrimental in infancy is respiratory syncytial virus (RSV). RSV can cause severe lower respiratory tract disease and recurrent infections related to inadequate development of anti-viral immunity. The reason for frequent RSV re-infections in infancy could be inadequate TLR7 and TLR8 engagement by viral RNA resulting in lower synthesis of anti-inflammatory mediators and antigen presentation, possibly due to a lower TLR expression in peripheral blood mononuclear cells (PBMC) of a still developing immune system. We determined TLR7 and TLR8 expression in PBMC subsets from infants with primary RSV infection and their healthy controls using multi-parameter flow cytometry. We found the expression of TLR7 and TLR8 in monocytes lower in infected infants than in healthy controls. Also, the comparison of TLR7 and TLR8 expression between different age groups revealed that they are expressed at lower level in monocytes from infants (both diseased and healthy) when compared to healthy adults and newborns. Unexpectedly, TLR8 in T cells and NK cells was detected in lower percentage in infected infants when compared with healthy controls. Difference in percentage of both cell subsets positive for TLR8 between healthy and infected infants emphasize possible role in mounting anti-RSV immunoreactions. In conclusion, difference in TLR7 and TLR8 expression stresses out their importance in early antiviral immune mechanisms responsible for the control of RSV replication and generation of an adaptive immune response.

ABSTRACT 23

Alterations in expression of TLR signaling pathway genes in HepG2 cells during dengue virus infection

Thaís M. Conceição1, Leandro S. Costa1, Tatiana El-Bacha1, Camila S. A. Villas-Bôas1, Lorena Chávez2, Jorge Ramírez2, Monica Montero-Lomeli1 and Andrea T. Da Poian1*
1.Instituto de Bioquímica Médica - UFRJ – Brazil

2.Instituto de Fisiologia Celular - UNAM – Mexico

Dengue virus (DV) causes one of the most important mosquito borne diseases in the world. Infection with DV produces a spectrum of disease severity ranging from an asymptomatic to a fatal hemorrhagic disease. Since several evidences show that liver dysfunction is a characteristic of severe dengue infection we evaluated the transcriptome of a hepatic lineage (HepG2) after 6, 24 and 48 hours of infection, using a human library containing 10.000 oligonucleotide probes for the best annotated human genes. The results showed that DV infection leads to expression alterations in numerous genes that constitute important signaling pathways in HepG2 cells, including a range of genes that are part of toll-like receptors (TLR) pathway. Giving the importance of innate immune response to infection, we observed strictly the expression pattern of these genes through real-time PCR experiments. Results showed an up-regulation in TLR3 and TLR8 mRNA expression in infected cells. IRF3, a transcription factor downstream in TLR3 pathway, and MYD88, an adapter protein downstream in TLR8 signalization, had their expression down-regulated after infection. Some of the final products of TLR pathway were also investigated. IFN-beta presented no mRNA detectable levels upon infection. IL1-beta and TNF-alfa presented mRNA levels not different from control cells. Just IL-6 mRNA presented a progressive increase during infection. Finally, no difference was observed in mRNA expression of TLR7, TLR4 and CD14. Although further analysis is needed, we propose that DV infection is altering signalization of innate immune response in HepG2 cells, which might interfere with the progression of DV replication in the liver. Supported by: ICGEB, Faperj and CNPq.

ABSTRACT 24

ZBP1/DAI Contributes to Cytomegalovirus-Mediated Induction of Interferon Beta

Victor R. DeFilippis, David Alvarado, Stephan Rothenburg, and Klaus J. Früh
Oregon Health and Science University

National Institutes of Health

Human cytomegalovirus (HCMV) is a member of the Herpesvirus family that possesses a large (>220kb) dsDNA genome and infects hosts in a persistent, lifelong manner. A hallmark of HCMV replication is the rapid induction of interferon beta synthesis following host cell entry via activation of interferon regulatory factor 3 (IRF3) and nuclear factor kB (NFkB). While NFkB activation has been attributed to cellular detection of viral components by TLR2, the receptor(s) and pathway triggered by HCMV that lead to IRF3 activation are unknown. Here we show that stable overexpression in human fibroblasts of the recently described cytoplasmic sensor of dsDNA (ZBP1/DAI) enhances HCMV-mediated, IRF3-dependent interferon beta synthesis. siRNA-mediated knockdown of the ZBP1/DAI transcript was also found to diminish, but not abolish, this effect from both ZBP1/DAI-transfected and untransfected fibroblasts. Furthermore, siRNA-directed knockdown of IPS1 was found to have no effect on either normal or ZBP1/DAI-enhanced HCMV-mediated interferon beta induction. This process also occurs independently of TRIF-dependent pathways, which are not operable in these cells. Thus, we are currently exploring the possibility that HCMV-mediated activation of IRF3 occurs via an uncharacterized cellular pathway that includes ZBP1/DAI but not the adaptor molecules TRIF or IPS1. Studies are also underway that aim to identify the HCMV-associated molecular pattern(s) necessary for this activation including understanding the role of viral genomic DNA.

ABSTRACT 25

Role of Toll-like receptor (TLR), RIG-I-like receptor (RLR) and Nod-like receptor (NLR) pathways in innate immune responses elicited by poxvirus

Julie Delaloye, Quynh-Giao Steiner-Tardivel, Mariano Esteban*, Thierry Roger and Thierry Calandra

Infectious Diseases Service, CHUV & University of Lausanne, Lausanne, Switzerland and *Centro Nacional de Biotecnología, Ciudad Universitaria Cantoblanco, Madrid, Spain

Background: Modified Virus Ankara (MVA) is a replication-deficient poxvirus currently used as a vaccine vector against a broad spectrum of diseases, including HIV and malaria. Whilst this vector has been shown to be immunogenic and safe in humans, the innate immune pathways it triggers remain largely unknown. The aim of this project was to characterize TLR-dependent and TLR-independent (RLR and NLR) pathways activated by MVA in human macrophages.

Method: Human THP-1 monocytic cells and THP-1 cells stably transduced with lentivirus expressing shRNA directed against TLR2, IPS-1, NALP3, caspase-1, ASC or laminin were infected with MVA. Cytokines and chemokines mRNA and protein expression were quantified by real-time PCR, ELISA and Luminex technology. The activation of transcription factors was analyzed by EMSA, Western blotting and transient transfection using multimeric kB- and interferon b (IFNb) promoter-dependent luciferase reporter vectors.

Results: MVA infection stimulated the production of abundant amounts of chemokines (MIP-1a, MIP-1b, MCP-1, IL-8, IP-10, RANTES) and low levels of cytokines (TNF, IL-1b, IL-6, IL-12) by THP-1 cells. TLR2 was found to be required for the expression of cytokines and IFNb-independent chemokines (IL-8, MIP-2), whereas IPS-1 was involved in the expression IFNb and IFNb-dependent chemokines (IP-10, RANTES). Moreover, MVA activated the production of IL-1b in a NALP3-dependent manner. In agreement with these findings, MVA increased NF-κB DNA-binding activity, IRF3 phosphorylation and NF-kB- and IRF3-dependent transcriptional activity.

Conclusion: MVA poxvirus activates innate immune responses of human monocytic cells via TLR2, RLR (RIG-I or MDA5) and the NALP3 inflammasome.

ABSTRACT 26

Epstein-Barr virus oncoprotein Latent Membrane Protein 1 down-regulates TLR9: a possible mechanism by EBV to escape innate immunity

Ikbal Fathallah1, Henri Gruffat2, Hanna Johansson1, Peggy Parroche1, Claudia Zannetti1, Ishrak Hussein1, Evelyne Manet 2, Massimo Tommasino1, Bakary Sylla1, Uzma A Hasan1

1 International Agency for Reasearch on Cancer, 150 Cours Albert Thomas. 69008 Lyon, France

2Laboratoire de biologie moléculaire des g-herpesvirus INSERM U758, ENS-Lyon IFR 128 Bioscience Lyon Gerland 46 allée d'Italie F-69364 lyon, France

The common feature of human carcinogenic viruses is their ability to induce cellular transformation and evade the immune system. These two events are required for cancer development. Epstein-Barr Virus (EBV) is strongly associated with various human cancers such as Burkitt´s lymphoma, nasopharyngeal carcinoma and gastric cancer and. EBVs' carcinogenicity is mediated through its oncoprotein Latent Membrane Protein 1 (LMP-1). LMP-1 highly activates the Nuclear Factor-κB (NF-κB) signalling pathway, through its C-terminal Activation Region 1 (CTAR1) and 2 (CTAR2). LMP1-induced NF-κB activation is essential for EBV-mediated B cell immortalisation. In a normal setting NF-κB is important for the control of immune response, inflammation, cell survival and cell proliferation.

We have recently shown that the oncoproteins E6 and E7 from human papillomas virus type 16 potentially escapes the innate immune response by down-regulating Toll Like Receptor 9 (TLR9) through NF-κB. To determine if this event is a crucial element in viral oncogenesis, we asked whether LMP-1 from EBV is also capable to deregulate TLR9 expression and function.

Expression of LMP-1 alone in B cell line (RPMI 8226) induces down-regulation of the TLR9 transcript as well as protein levels. In addition, TLR9 suppression was decreased when LMP-1 mutants deficient in activating NF-κB pathways were expressed, indicating that LMP-1 alters TLR9 expression through the NF-κB pathway. These data were corroborated in that infection of RPMI 8226 cells as well as human primary B cells with the entire virus also inhibits TLR9 transcription and hence functional loss of TLR9-regulated pathways. Furthermore losing the expression of LMP-1 in EBV positive cells restored TLR9 levels. This study reveals a novel mechanism used by EBV to potentially escape the immune recognition through TLR9 down-regulation, and highlights further the importance of immune evasion in the carcinogenic events mediated by tumour inducing viruses.

ABSTRACT 27

The E7 oncoprotein from HPV16 inhibits TLR9 transcription: Switching the site of the NF-kB complex on a promoter

Uzma A Hasan, Claudia Zannetti, Ishraq Hussain, Ikbal Fathallah, Peggy Parroche, Bakary S. Sylla, Massimo Tommasino

International Agency for Reasearch on Cancer, 150 Cours Albert Thomas. 69008 Lyon, France

Evidence from epidemiological studies indicates that viruses cause 15% of human cancers. Chronic infection, as a result of ineffective immune control, is a condito sine qua non for cancer development. Thus, carcinogenic dsDNA viruses have developed strategies to avoid innate immune recognition. Toll Like Receptors (TLRs) are sentinels of the innate immune system sensing pathogen-derived products. We have previously reported that E6/E7 oncoproteins from high-risk mucosal human papillomavirus (HPV) type 16, one of the causing agents of cervical cancer, is able to suppress the transcription activity of TLR9. In the present study we show that the oncoprotein HPV16E7 turns down TLR9 expression through NF-kB. During a normal TLR9 response following stimuli by CpG, NF-kB is activated, binds to the TLR9 promoter at--418bp upstream from the ATG and temporarily suppresses the expression of the receptor. However, in human keratinocytes expressing HPV16E7, but not E6, the NF-kB pathway is constitutively activated. Interestingly, the activated NF-kB composed by p65 and p50 was found on TLR9 promoter at position -1270bp, instead of -418bp. In normal keratinocytes where TLR9 is expressed, NF-kB p65 binding to -418bp was observed. Taken together our findings highlight a new mechanism by which oncogenic viruses down regulate the expression of an immune sensor, such as TLR9, by modulating the affinity of a transcription factor to a particular promoter region. This event is may be a crucial step involved in the carcinogenic events mediated by the virus.

ABSTRACT 28

Expression of Toll-like receptors is modulated by opportunistic infections in HIV-1-infected patients

Juan Hernández1, Georges Laurent2, Ajit Kumar2, Danielle Hernandez-Verdun3, Silvio Urcuqui-Inchima1
1Grupo Inmunovirología - Universidad de Antioquia, Colombia.

2Biochemistry, Molecular Biology and Genetics - Georges Washington University, USA. 3Nuclei and Cell Cycle - Institut Jacques Monod, France

The ability of triggering an immune response against co-pathogens associated to HIV-1 infection has been demonstrated to depend on innate recognition molecules, such as TLR, which mediate activation of innate immunity and influence the development of adaptive immunity. In addition to cytokine-mediated HIV-1 replication, the interaction of PAMPs with TLR may also result in direct activation of HIV-1 transcription. However, there is no evidence if HIV-1 would modulate TLR expression.

Our investigation was aimed to evaluate by flow cytometry the expression of TLR2 and TLR4 on monocytes, mDCs and pDCs from 50 HIV-1-infected individuals classified according to viral load, HAART treatment and the presence of opportunistic infections (OI); 16 healthy subjects were evaluated as controls.

Our findings demonstrated an increased expression of TLR2 and TLR4 on mDCs, and of TLR4 on pDCs, in HIV-1+ patients with OI (mostly without HAART) when compared to both HIV-1+ patients without OI and healthy subjects. This up-regulation of TLR expression in HIV-1+ patients with OI was more pronounced on mDCs and pDCs derived from those individuals co-infected with M. tuberculosis or fungi. A positive correlation between TLR2 expression (as mean fluorescence intensity) and viral load was observed in monocytes from HIV-1+ patients with OI, but not between TLR expression and CD4+ T cell count in peripheral blood.

ABSTRACT 29

Polymorphisms in Rig-I like helicases and their influence on the course of Hepatitis C Virus infection

Franziska Hoffmann1, Andreas Schmidt1, Johannes Hellmuth1, Jürgen Glas2, Matthias Folwacny2, Stefan Endres1, Simon Rothenfusser1

1 Division of Clinical Pharmacology, Department of Medicine University of Munich
2 Department for preventive Dentistry and Periodontology, University of Munich

Hepatitis C, is a major cause of liver cirrhosis, liver failure and hepatocellular carcinoma in humans. Hepatitis C Virus (HCV) infects about 3 % of the human population. Interestingly the course of infection varies widely among patients. Approximately 15-20% of the infected individuals clear the infection spontaneously, whereas in 80% the infection presides in a chronic state. Virus-related factors and clinical risk factors of the host such as sex, alcohol consumption and age at infection do not allow to predict the development of a chronic status. It is therefore suspected that the susceptibility to chronic HCV infection is determined by genetic polymorphisms in the host's immune system. The RIG-I like helicase (RLH) RIG-I was recently shown to be responsible for the triggering of innate immune responses after infection with HCV. This led us to examine single nucleotide polymorphisms (SNPs) in Rig-I and other components of the Rig-I-like helicase pathway and correlate them with the outcome after HCV-infection. We focused our analyses on non-synonymous SNPs in coding regions and followed two parallel approaches. We compared the distribution of SNPs in patient groups consisting of healthy controls, patients with chronic Hepatitis C infection and patients that had spontaneously cleared their HCV infection. In addition we made expression constructs of RLHs and analyzed in complementary in vitro assays the functional consequences of the analyzed SNP-polymorphisms.

ABSTRACT 30

A PROVIRAL ROLE FOR CpG IN CYTOMEGALOVIRUS INFECTION

Ann-Charlotte Iversen[1], Bjørg Steinkjer[1], Nadra Nilsen[1], Janne Bohnhorst[1], Siv Helen Moen[1], Randi Vik[1], Phil Stephens[2], David W. Thomas[2], Chris A. Benedict[3], and Terje Espevik[1]
[1]Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway, [2]Wound Biology Group, School of Dentistry, Cardiff University, Cardiff, UK, [3]Division of Molecular Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, USA

TLR9-dependent signaling in plasmacytoid dendritic cells (DCs) is a key contributor to innate immune defense to mouse CMV (MCMV) infection. Here we aimed to study the expression and potential contribution of TLR9 signaling in human CMV (HCMV) infection of primary fibroblasts. HCMV infection strongly induced TLR9 expression in two of three fibroblast types tested. Furthermore, the TLR9 ligand CpG-B induced a strong proviral effect when added shortly after HCMV infection, enhancing virus production and cell viability. However, not all CpG classes displayed proviral activity, and this correlated with their IFN-β-inducing ability. The proviral effect of CpG-B correlated completely with concurrent viral up-regulation of TLR9 in fibroblasts. Importantly, the timing of CpG addition was a critical parameter; in striking contrast to the proviral effect, CpG addition at the time of infection blocked viral uptake and nearly abolished HCMV production. The contrasting and time-dependent effects of CpG on HCMV infectivity reveal a complex interplay between CpG, TLR9 and HCMV infection. Additionally, the data suggest a potentially harmful role for CpG in the promotion of HCMV infection.

ABSTRACT 31

A role for IRF-5 in TLR 9-mediated induction of the antiviral gene viperin by agonists of TLR9.

Cathrine F. Knetter1,2, Ekambar Kandimalla3, Harry Bjørkbacka4, Mason Freeman5, Martina Severa2,6, Sudhir Agrawal3, Katherine A. Fitzgerald2, Terje Espevik1 and Egil Lien1,2
1Institute of Cancer Research and Molecular Biology, Norwegian University of Science and Technology, Trondheim, Norway. 2University of Massachusetts Medical School, Division of Infectious Disease, Worcester, MA, USA. 3Idera Pharmaceuticals, Cambridge, MA, USA. 4Experimental Cardiovascular Research, CRC, Lund University, Malmö University Hospital, Malmö, Sweden. 5Mass General Hospital, Harvard Medical School, Cambridge, MA, USA. 6Department of Infectious, Parasitic, and Immunomediated Diseases, Istituto Superiore di Sanita` Rome, Italy.

Toll like receptor 9 (TLR9) is responsible for immune recognition of CpG motifs, which are present in bacterial and viral DNA. Here we conducted a gene expression profiling study of GM-CSF matured bone marrow cells from C57Bl/6, MyD88 and TLR9 deficient mice. Cells were stimulated with a 22-mer agonist of TLR9 containing two 5'-ends and synthetic Cp7-deaza-G immune stimulatory motif for 4 hours. All responses were entirely dependent on both TLR9 and MyD88. In the wild-type mDC, a high induction of the antiviral gene viperin was observed upon stimulation with TLR9 agonist. Viperin is an interferon inducible gene, and upregulation of gene expression has previously been shown by different viruses like Cytomegalovirus and Sendai Virus, in addition to the TLR4 and TLR3 ligands; LPS and Poly I:C.

Reporter studies with TLR9-expressing HEK293 cells revealed a robust activation of the viperin promoter upon stimulation with TLR9 agonist. Viperin promoter reporter activity was induced upon MyD88 overexpression via IRF5. Moreover, GM-CSF matured bone marrow cells from IRF1, 3, 5 and 7 deficient mice revealed an important role for IRF5 in TLR9 induced viperin gene activation.

The rapid induction of viperin by TLR ligands such as CpG DNA suggests that viperin may be an important component of the innate response to pathogens which are sensed by TLR9.

ABSTRACT 32

Respiratory viruses and Toll-like receptor 7: Antibodies contribute to TLR7-driven anti-viral innate immunity

Evelyn A. Kurt-Jones, Robert W. Finberg, Peter E. Newburger, Jennifer P. Wang
Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01605 USA

Neutrophils are recruited to the airways during infection with influenza A virus (FLU) and respiratory syncytial virus (RSV) and are likely activated as a result of cytokines and inflammatory molecules released by virally infected cells. Human neutrophils express TLRs 1-10, with the exception of TLR3. We have determined that respiratory viruses activate neutrophils via their TLR receptors. Neutrophils were challenged with FLU or RSV and the neutrophil cytokine response was measured by ELISA and by single cell analysis using FACS. These studies revealed that neutrophils were activated for cytokine production by viruses and by ligands for TLR2, TLR4, TLR7/8 and TLR9. The neutrophil cytokine response to FLU was dependent on TLR7/8, i.e., the response could be inhibited by Bafilomycin A1 and was minimal in neutrophils from TLR7 knockout mice. In addition, activation of neutrophils by FLU required virus entry but not virus replication.

TLR7-dependent activation of plasmacytoid dendritic cells (pDC) requires virus entry, but this could be bypassed by Fc-receptor mediated delivery of virus to pDCs suggesting a novel pathway for delivering virus to an intracellular compartment containing TLRs. pDC activation by antibody-virus complexes was TLR7- and FcR-gamma chain-dependent and resulted in enhanced secretion of IFN-alpha. Engagement of TLR7/8 creates a delicate balance: while a strong IFN response is likely protective to the host, excessive inflammation can be detrimental. Such inflammatory responses likely contribute to the high morbidity and mortality observed with newly emergent influenza strains. By determining which influenza virulence factors trigger excessive innate immune responses by the host, further steps can be made towards the development of innovative therapeutic agents targeting influenza.

ABSTRACT 343

Proapoptotic role of STAT3 in hepatocytes during acute inflammatory hepatitis

Chien-kuo Lee, Priscilla Lee, I-Ting Chen, Wei Lee and Lo Kuan

Graduate Institute of Immunology, National Taiwan University College of Medicine 1, Jen-Ai Road, Section 1, Rm 513, Taipei 100, Taiwan

Con A-induced acute inflammatory hepatitis is a well documented mouse model for fulminant hepatitis. However, the detailed mechanisms have yet to be determined. STAT3, a critical signal mediator of cytokines, has been proposed to be hepatoprotective in this system. However, we reported here that STAT3 is actually proapoptotic for hepatocytes during acute inflammation induced by Con A and other liver injury agents such as LPS plus D-gal. Using conditional knockout mice lacking STAT3 in the liver, we show that the mutant mice are more resistant to Con A-induced hepatitis with reduced AST/ALT levels in the serum and less severe liver damage. The resistant phenotype in STAT3 mutant mice is not due to impaired immune responses as proinflammatory cytokines such as TNFα, IL-6, and IFNγ and numbers of liver-infiltrated leukocytes are comparable between wild-type and mutant mice, suggesting that the effector factors can not account for by the reduced hepatitis in the mutant mice. Instead, we find that STAT3-deficient hepatocytes are more resistant to inflammation-induced apoptosis. First, using intra-hepatic leukocytes (IHL) of wild type or mutant mice to mix with primary hepatocytes of these two mice, we demonstrate that STAT3-deficient hepatocytes are more resistant to the killing of IHL from either mouse in vitro. Second, TNFα-induced apoptosis in wild-type primary hepatocytes is enhanced by pretreatment of IL-6 in a dose-dependent manner. However, STAT3-deficient hepatocytes are more resistant to cytokine-mediated apoptosis. To understand the molecular mechanisms underlying the apoptosis-resistant phenotype, microarray analysis is performed. We have identified two hepatoprotective genes whose expression is highly induced in hepatocytes lacking STAT3. The detailed mechanisms of these two genes for protecting hepatocytes from apoptosis will be reported.

ABSTRACT 34

Vaccinia virus protein A49 is a novel inhibitor of the NF-kB pathway

Daniel S. Mansur, 1Leonie Unterholzner, Antonio Postigo, 1Andrew Bowie, Geoffrey L. Smith. Department of Virology, Faculty of Medicine, Imperial College London, St Mary's Campus, Norfolk Place, London w2 1PG, UK and 1Department of Biochemistry, Trinity College Dublin, Eire.

Recognition of vaccinia virus (VACV) infection by the innate immune system involves pathogen recognition receptors such as TLR 2 , 3, 9, and the dsDNA sensor protein DAI. VACV contains a large dsDNA genome of around 200 kb and encodes many immunomodulatory proteins. These include the intracellular virulence factors A46, A52, N1 and B14, which help the virus evade the innate immune response by inhibiting signaling to nuclear factor kappa B (NF-kB). Here we report that VACV strain Western Reserve protein A49 is a novel suppressor of innate immune activation. A49 is an 18.8-kDa intracellular protein without sequence similarity to proteins outside the poxvirus family. Immunofluorescence analysis revealed that HA-tagged A49 is predominantly localized in the nucleus. Expression of A49 in HEK 293 T cells blocks poly I: C-induced activation of the interferon (IFN)B promoter while also inhibiting NF-kB activation following stimulation with tumour necrosis factor (TNF)a. As this suggested A49 could be interfering with the NF-kB activation pathway, we co-expressed A49 with TRAF 2, 6, TAB3, IKKa, IKKB and p65. In all cases, A49 suppressed induction of the NF-kB promoter, indicating that A49 inhibits a downstream component of the pathway. Furthermore, A49 prevented p65 translocation to the nucleus following TNFa treatment. In summary, A49 is novel VACV suppressor of the NF-kB pathway.

ABSTRACT 35

TLR-mediated NF-kappaB-dependent cytokine production is differently affected by HIV therapeutics

Jesper Melchjorsen1, Søren R. Paludan2, Trine H. Mogensen1, Martin Tolstrup1, Lars Østergaard1
1 Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark
2 Institute of Medical Microbiology and Immunology, Aarhus University, Aarhus. Denmark

Pathogen-recognizing Toll-like receptors (TLR)2 and TLR4 are known to recognize a number of pathogens, including E.coli, S. pneumoniae and N. meningitidis. We have studied whether a number of anti-HIV therapeutics affect immediate proinflammatory cytokine responses in cell cultures. Our results suggest an opposing effect of two nucleotide and nucleoside reverse transcriptase inhibitors. While Tenofovir strongly inhibited TLR-induced cytokine expression, Retrovir (AZT) potentiated this response. We present data on the molecular mechanisms behind drug-mediated remodeling of innate immune activation and how the drugs affect early host-pathogen interactions.

ABSTRACT 36

Assessment of selected Toll-like receptors in children with chronic viral hepatitis C

Iwona Mozer-Lisewska, Gregorz Dworacki, Mariusz Kaczmarek, wojciech Służewski, Jan Żeromski

Dept Infectious Dis & Child Neurol and Clin.Immunol. University of Medic. Sci, Poznan, Poland

Pattern recognition receptors (PRRs) are sensors of molecular structures, so called pathogen associated molecular patterns (PAMPs) present in a large variety of microbial pathogens such as bacteria, viruses and fungi. PRRs are expressed on most cells of the immune system but also on various epithelia. Recognition of PAMPs by PRR leads to activation of mechanisms of innate immunity and indirectly have an impact on adaptive response. It takes place by the activation of biochemical machinery in cell interior leading to up-regulation of transcription factors entering cell nucleus, activation of several genes with subsequent production of proinflammatory agents such as cytokines etc.

The aim of this study was to search PRRs member, Toll-like receptors (TLRs) in liver and blood of children with chronic viral hepatitis C (HCV). The study group comprised of 15 boys and 8 girls in the age from 8 to 16 years. Mean duration of HCV infection confirmed by viral and molecular criteria was 3.5 years. Uninfected children of comparable age and sex (n=5) served as control ones.

Liver biopsy frozen tissue sections were subjected to ABC immunohistochemistry using polyclonal antibodies vs. TLR2, TLR3 and TLR4. Blood white cells (WBC) obtained from HCV+ patients and control ones were searched for expression of TLRs as above, by means of fluorochrome labeled monoclonal antibodies and multicolor flow cytometry.
It was found that all tested TLRs could be demonstrated in infected liver cells, showing uneven focal pattern. Flow cytometry studies permitted to discern differences in TLRs expression on various subsets of WBC between HCV infected and uninfected children. These data suggest that PRRs participate in the pathogenesis of chronic HCV infection in juvenile patients.

ABSTRACT 37

Proteome characterization of Influenza A virus infected human primary macrophages

Tiina Öhman[1], Johanna Rintahaka[2], Tuula A. Nyman1 and Sampsa Matikainen[2]
[1]Institute of Biotechnology, University of Helsinki, P.O. Box 65, FI-00014 University of Helsinki, Finland and [2]Finnish Institute of Occupational Health, Helsinki, Finland

During viral infection, multiple cell signaling cascades are activated resulting in the production of interferons and other cytokines. In addition, programmed cell death, apoptosis, of virus-infected cells is initiated. Influenza A virus is one of the most important causes of respiratory infection. Here we have characterized the cytosolic and mitochondrial proteomes of Influenza A virus-infected macrophages by using two-dimensional electrophoresis for protein separation and mass spectrometry for protein identification. In cytosolic proteomes, the expression of several heat shock proteins and fragments of cytoskeletal proteins was clearly up-regulated. In addition, the cytoplasmic level of Rho GDP dissociation inhibitor 2 (Ly-GDI) as well as cathepsins B and D were down-regulated upon Influenza A infection. In mitochondrial proteomes, the expression of actin and its fragments were highly up-regulated during Influenza A virus infection. This was followed by translocation of molecules of RIG-I/MAVS signaling pathway, including RIG-I, TRIM25, and IKK-epsilon to the mitochondrial outer membrane. Mitochondrial co-localization of RIG-I/TRIM25 signalling complex was verified by co-immunoprecipitation experiments further emphasizing the role of mitochondria in antiviral response. Furthermore, actin fragmentation and translocation of these fragments to mitochondrial outer membrane was associated with caspase-3 activation suggesting a role for actin in the regulation of apoptosis.

ABSTRACT 38

dsRNA dependent protein kinase R (PKR) is essential to control pathogenicity of Rift Valley Fever virus and is inhibited by the virus NSs protein

Andreas Pichlmair (1, 2), Matthias Habjan (2), Giulio Superti-Furga (1), Hermann Unger (3) and Friedemann Weber (2)

(1) CeMM-Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria; (2) Department of Virology, University of Freiburg, Germany; (3) Institute for Tropical medicine, University of Veterinary Medicine, Vienna, Austria

Rift Valley Fever Virus (RVFV) is a zoonotic arbovirus that causes epidemics of hemorrhagic fever in Africa, Saudi Arabia and Yemen. The pathogenicity of the virus is caused by the viral interferon antagonist 'Nonstructural protein S' (NSs) that prevents induction of antiviral cytokines like type-I interferon. Viruses lacking NSs are attenuated and considered safe vaccine candidates. Here we demonstrate that beside its known effect, NSs interferes with the function of the pattern recognition receptor 'double-stranded RNA induced Protein Kinase R' (PKR). PKR is not necessary for induction of type-I interferon in response to RVFV infection but blocks virus replication in vitro and in vivo. Further, mice lacking PKR are highly susceptible to attenuated RVFV lacking the NSs protein. This suggests that the NSs protein of RVFV has pleiotropic functions that support virus growth and pathogenicity. Moreover, vaccination of immune-compromised individuals with NSs-deleted attenuated RVFV strains may potentially be fatal.

ABSTRACT 39

The Role of IRF-5 in the antiviral and inflammatory response.

Paula M. Pitha[1], Mumtaz Y. Balkhi[1] ,Jasmin C. Lien[1] and Katherine A. Fitzgerald[2]
[1]Johns Hopkins University School of Medicine, Baltimore, MD and [2]University of Massachusetts Medical School, Worcester, MA, USA

Recognition of viral pathogens by cellular receptors leads to activation of signaling pathways culminating in the activation of latent transcription factors of the NFkB and IRF-families. Members of the IRF family play a critical role in the activation of the innate antiviral response. While viral infections activate latent IRF-3 and IRF-7 and the induction of Type I IFN, IRF-5 has been implicated both in the innate antiviral and inflammatory response. IRF-5 is activated downstream of TLR7 and TLR9 via the MyD88 pathway, where it interacts with both MyD88 and the E3 ubiquitin ligase, TRAF6. We showed that IRF5 is subjected to TRAF6 mediated K63-linked ubiquitination, which is important for IRF5 nuclear translocation and transcription of target genes. In contrast, IRF-5BMv, a variant, containing an internal deletion of 288 nucleotides is not subject to such ubiquitination. Lysine residues at position 410 and 411 in a putative TRAF6 consensus-binding domain of IRF-5 are the targets of K63 linked ubiquitination. Finally, we show that IRAK1 associates with IRF5, and that this interaction precedes and is required for IRF5 ubiquitination and activation. MuIRF-5 is expressed as a single major transcript in spleen cells of several inbred strains of mice including C57/Bl6 mice. The analysis of the genetically modified C57/Bl6-IRF-5-/- mice have shown that induction of both Type IFN and inflammatory cytokines is impaired in NDV and VSV infected IRF-5-/- mice. Furthermore while CpG-DNA, poly (I-C) or LPS induced inflammatory cytokines are defective in CD11c+ splenic DCs from IRF5-/-mice, IFN levels were downregulated only in response to CpG DNA stimulation. The analysis of the lymphoid cell compartment in IRF-5-/- mice also revealed increased numbers of B cells and splenomegaly. Further characterization indicates that the B cells from the IRF-5-/- mice have an altered phenotype.

ABSTRACT 40

The innate immune receptors TLR3 and RIG-I differentially regulate inflammatory and antiviral responses to influenza A virus

POTHLICHET J., LE GOFFIC R., CHIGNARD M., SI-TAHAR M.

Unit "Innate Defense and Inflammation", Institut Pasteur, INSERM U874, Paris, France.

Influenza A virus (IAV) is the etiological agent of a highly contagious acute respiratory disease that causes epidemics and considerable mortality annually. The viral replicative intermediate double-stranded RNA (dsRNA) is considered critical for the outcome of IAV infection but is also considered a potent activator of the innate anti-viral immune response. Bronchial epithelial cells are the primary target and the principal host for IAV. We previously used an in vitro approach to establish a role for the pattern-recognition Toll-like receptor (TLR)3 in the immune response of lung epithelial cells to IAV-derived dsRNA (J. Biol. Chem., 2005) and we demonstrated that in vivo, IAV-TLR3 interaction critically contributes to viral pathology (PLoS Pathog., 2006). However, additional cellular dsRNA-recognition proteins have lately been implicated as key sensors of viral infection. These include two caspase recruiting domain (CARD) containing RNA helicases, i.e. the retinoic acid-inducible gene I (RIG-I) protein and the melanoma differentiation-associated gene (MDA-5) protein. In such context, we aimed at delineating the respective role of TLR3 vs. RIG-I/MDA-5 in the respiratory mucosa infected by IAV using human bronchial epithelial (BEAS-2B) cells that were stably transfected either with a control plasmid or with a vector encoding a dominant-negative, non-functional, form of TLR3. Our findings demonstrated that the sensing of IAV by TLR3, RIG-I and MDA-5 clearly differs. Indeed, TLR3 activation appears critical for the induction of a pro-inflammatory response whereas RIG-I (but not MDA-5) activation is rather essential for the induction of a type I interferon-dependent antiviral response (J. Immunol., 2007). Finally, we recently established that the innate immune response triggered by IAV is negatively regulated by the Suppressor of Cytokine Signaling (SOCS)1 and SOCS3 proteins through a RIG-I/IFNAR1-dependent pathway (J. Immunol., 2008).

ABSTRACT 41

Innate Immune Recognition and Activation of Type I Interferons in Tick borne Flavivirus Infections

Katrin Ramsauer and Christian W. Mandl

Clinical Institute of Virology, Medical University of Vienna

The pathogenicity of the flavivirus tick-borne encephalitis virus (TBEV) has been the subject of intense investigations, however little is known about the interaction of this virus with the host innate immune system, and in particular interaction with Type I Interferons (IFN). Here we investigate signal transduction events leading to the activation of Type I IFN expression.

Infection of primary mouse macrophages and mouse embryonic fibroblasts with TBEV activates expression of Type I IFNs. Recognition of the virus and subsequent expression of IFNs is strongly dependent on virus replication. In mouse embryonic fibroblasts the cytoplasmic RNA virus pattern recogmition receptors retinoic acid inducible gene I (RIG-I) and melanoma differentiation associated gene 5 (MDA5) are both utilized to recognize TBEV RNA. The downstream transcription factor interferon regulatory factor 3 (IRF3) plays a main role in early activation of Type I IFNs, IRF7 is dispensable at early stages of infection. In addition, IRF3 inhibits virus replication but not spread of viral particles to neighbouring cells.

ABSTRACT 42

Activation of innate immune defence mechanisms in Polyomavirus associated Nephropathy

Andrea Ribeiro1, Markus Wörnle1, Hans J. Anders1, Hans Nitschko2, Matthias Sauter1, Clemens D. Cohen3, Matthias Kretzler4, Hermann J. Gröne5, Detlef Schlöndorff1, and Holger Schmid1

1Medizinische Poliklinik, University of Munich, Germany, 2Max von Pettenkofer Institut, Munich, Germany, 3UniversitätsSpital, Zürich, Switzerland, 4Department of Medicine, University of Michigan, Ann Arbor, USA, 5Department of Cellular and Molecular Pathology, DKFZ, Heidelberg, Germany

Background: Polyomavirus associated nephropathy (PVAN) is a significant complication after kidney transplantation, often leading to premature graft loss. Here we analysed a potential activation of the viral dsRNA recognition receptors Toll-like Receptor 3 (TLR3) and RIG-I in allograft biopsies with PVAN and in cultured human collecting duct cells (HCDC) after infection with polyomavirus BK (BKV).
Methods: Protein and mRNA expression of TLR3, RIG-I, cytokines (IL-6, IL-8) and chemokines (RANTES, MCP-1 and IP-10) as well as IFN-β were analysed in renal allograft biopsies and cultured HCDC. To show the specific effect of the viral dsRNA receptors siRNA was used.

Results: In allograft biopsies with PVAN, BKV and TLR3 could be co-localized by double staining in tubular epithelial cells of distal cortical tubules and the collecting duct. In microdissected tubulointerstitial compartments TLR3 and RIG-I mRNA expression was increased. HCDC expressed TLR3 and RIG-I and activation of these viral receptors by poly(I:C) significantly enhanced the mRNA expression of IL-6, IL-8, RANTES, MCP-1, IP-10 and IFN-β. This was significantly counteracted by siRNA specific for TLR3 and RIG-I. Finally, the infection of HCDC with BKV further enhanced the expression of the selected cytokines and chemokines.

Conclusion: These results suggest that in PVAN, the activation of innate immune defence mechanisms via TLR3 and RIG-I are involved in the antiviral and anti-inflammatory response leading to the expression of pro-inflammatory cytokines and chemokines.

ABSTRACT 43

Molecular mechanisms of virus recognition by RIG-I-like helicases

Wolgang Hamm*1, Tobias Schwerd*1, Johannes Hellmuth*1, Andreas Schmidt1, Hendrik Poeck1, Sheng Cui2, Karl-Peter Hopfner2, Stefan Endres1, Simon Rothenfusser1
1 Division of Clinical Pharmacology, Department of Internal Medicine, University of Munich, Germany

2 Department of Chemistry and Biochemistry, Gene Center, University of Munich

* authors contributed equally to this work

Viral infections are a constant threat to the integrity of human beings. Survival of the host organism depends on the rapid induction of innate immune responses including the production of interferon type I. The newly described family of cytoplasmic RIG-I-like helicases sense viral infection via the recognition of viral RNA, and trigger a potent antiviral defense. This family comprises Retinoic acid-inducible gene I (RIG-I) and melanoma differentiation associated gene 5 (MDA-5) two non-redundant signalling receptors and the regulatory protein Lgp-2. RIG-I was recently shown detect RNA of negative stranded RNA viruses via a virus-specific 5'-triphosphate modification absent in normal cytoplasmic RNAs. This provided a hint to the structural basis for the distinction of self- and non-self RNA. However, recent work has challenged the view that 5'-triphosphate modification is the only feature that can be specifically recognized by RIG-I. This prompted us to reinvestigate the requirements for RIG-I activation in detail. We found that 5'-triphosphate is required but not sufficient for an RNA to activate RIG-I. We employed small RNA cloning and found that double-stranded structures are another essential feature of short RNA ligands for RIG-I. Thus, we propose that only together double-strandedness and 5'-modification form a pattern sufficient for RIG-I signalling.

ABSTRACT 44

Molecular pathways in virus-induced apoptosis

Michael Wenzel1, Andreas Schmidt1, Hendrik Poeck1, Robert Besch2, Tobias Schwerd1, Johannes Hellmuth1, Stefan Endres1 and Simon Rothenfusser1
1Division of Clinical Pharmacology, Department of Internal Medicine, Ludwig-Maximilian-University, 80336 Munich, Germany

2Division of Dermatology and Allergology, Ludwig Maximilian University, 80337 Munich, Germany

Apoptosis has evolved into a complex decision-making-system that is found throughout the animal kingdom allowing cells to die physiologically in response to different endogenic and exogenous stimuli. The detection of invading microorganisms depends on a limited number of receptors that recognize so called pathogen-associated-molecular-patterns (PAMPs). Certain virus-specific RNA-motifs are recognized as PAMPs by the recently described RIG-I-like helicase (RLH) family consisting of retinoic acid-inducible gene I (RIG-I), melanoma differentiation associated gene 5 (MDA-5) and the regulatory protein Lgp-2. It is established that binding of viral nucleic acids by RLHs in the cytoplasm leads to the initiation of intracellular cascades causing proinflammatory processes like the production of type-I-interferon. Current studies support the idea that recognition of viral nucleic acids and an increased rate of apoptosis could be linked. However, the molecular basis for the enhanced disposition of virus-infected cells to undergo programmed cell death is not understood. In this study we try to define the mechanisms leading to apoptosis after infection with RNA-viruses or stimulation with synthetic mimics of viral RNAs. Using gen-deletion mutant Jurkat-T-Cell-Lymphoma cell lines and an siRNA approach in melanoma cells we demonstrate activation of the intrinsic pathway in virus-induced apoptosis and identify crucial steps involved.

ABSTRACT 45

Novel recombinant Hepatitis C virus-like particle ISVAC stimulates serum interferon alpha and spleen B cells without common interferon-induced side effects.

Igor G. Sivov1, Irina Kirillova1, Boris. V. Pinegin2, Evgeny I. Samokhvalov3, Dmitry M. Kulish1,4

1DKIS LLC (Moscow, Russia), 2National Research Center Institute of Immunology, FMBA (Moscow, Russia), 3Ivanovsky Institute of Virology, Russian Academy of Medical Sciences (Moscow, Russia),4NEUROK PHARMA LLC (San Diego, USA)

Preparation ISVAC, based on novel recombinant virus-like particles built of structural proteins of HCV, was found to strongly stimulate serum interferon in whole human blood and healthy human volunteers, without causing the usual interferon-alpha side effects. Furthermore, ISVAC strongly stimulated B cells in murine spleen. Both effects were similar to or exceeded those of respective known analogues: interferon inducers such as poly I:C and CpG ODN, as well as B cell mitogens such as polyacrylate. Since both interferon production and B-cell activation are mediated by TLRs, we suggest that ISVAC may stimulate TLRs expressed in interferon-producing and B cells. Current finding that TLR9-driven interferon induction in plasmacytoid dendritic cells requires CpG ODN being delivered by a nanoparticle (Kerkmann et al., 2005) allows us to propose that ISVAC stimulates TLRs by delivering to them some nucleic acids incorporated during its production. However, considering the total absence of the common side effects in response to ISVAC-induced interferon, it is possible that ISVAC may also stimulate and/or inhibit additional pathways, abrogating the negative impact of interferon alpha overproduction by balancing interferon pool induction; thus making ISVAC a drug of choice for treating diseases, commonly treated by recombinant interferon alpha.

ABSTRACT 46

EXPLORING THE INHIBITORY MECHANISMS OF THE VACCINIA VIRUS TIR-DOMAIN CONTAINING PROTEIN, A46

Julianne Stack*, Clare Bryant**, Ricardo Núñez Miguel***, and Andrew Bowie*
*School of Biochemistry and Immunology, Trinity College, Ireland, **Department of Veterinary Medicine, University of Cambridge, UK, ***Department of Biochemistry, University of Cambridge, UK

Accumulating evidence shows that many TLRs are involved in the detection of viruses. Both viral proteins and nucleic acids have been shown to activate anti-viral signalling pathways via TLRs, leading to cytokine and interferon induction. Highlighting their importance in anti-viral immunity, a number of viral proteins which interfere with TLR signalling have been identified. The vaccinia virus protein, A46, was the first viral protein identified to inhibit TLR signalling. A46 can interact with the TIR adaptor molecules Mal, MyD88, TRAM and TRIF, but not SARM, via its TIR domain. This allows A46 to block multiple signalling pathways emanating from TLR complexes. Here we have explored the mechanism whereby A46 inhibits TLR4 signalling. A46 can inhibit LPS-induced NF-kappaB and IRF activation, and for TLR4 these pathways are controlled by Mal and TRAM respectively. When transfected into cells, A46 inhibited the TLR4-Mal and TLR4-TRAM interactions in a dose-dependent manner. Further, structural modelling and molecular docking analysis of the A46-Mal interaction has revealed residues on A46 particularly important for TIR interactions. The role of these residues in TLR inhibition by A46 is currently being examined. This study provides the molecular basis for pathogen subversion of TLR signalling.

(This work was supported by funds from Enterprise Ireland.)

ABSTRACT 47

Low susceptibility of IRF7-deficient mice to MCMV infection despite lack of IFN-a production

Katharina Tittel*, Christian Steinberg*, Olaf Gross, Frank Schmitz, Wolfgang Reindl, Jürgen Ruland and Anne Krug

(* authors contributed equally)

Medical Department, Technical University Munich, Germany

Pattern recognition receptors (PRRs) detect invading viruses and trigger the innate antiviral immune response including type I interferon (IFN) production, which is regulated by interferon regulatory factors (IRFs). IRF7 was described as the master regulator of systemic IFN responses. In addition to type I IFN, NK cell responses are involved in the control of viral replication during the acute phase of infection. To investigate the role of IRF7 in the context of a viral infection, which induces a strong NK cell response, the murine cytomegalovirus (MCMV) infection model, in which both type I IFN and NK cell responses are critical for innate antiviral resistance, was used. Wild-type (WT), IRF7-KO and IRF3/IRF7-double KO mice were infected with MCMV and type I IFN bioactivity was measured in the serum at different time points. The type I IFN response was greatly reduced in IRF7-KO mice compared to WT mice. However, we detected residual type I IFN production in both IRF7-KO and IRF3/IRF7-double KO, excluding a role of IRF3 for type I IFN production in response to MCMV. Neutralization of IFN-b in serum samples led to abrogation of the residual type I IFN activity observed in the serum of IRF7 and IRF3/IRF7 KO mice. Thus, during systemic MCMV infection the systemic IFN-a response to MCMV is mediated by IRF7, whereas IFN-b is induced independently of both IRF3 and IRF7 suggesting the involvement of other IRFs. Despite the severe defect in IFN-a production, IRF7 KO mice were able to control MCMV replication quite efficiently and were only slightly more susceptible to MCMV than WT mice. Furthermore, activation of NK cell cytotoxicity, was unaffected and IFN-gamma production was significantly enhanced in IRF7KO mice. Thus, IRF7-dependent antiviral immune responses are not essential for the resistance to MCMV infection in vivo.

ABSTRACT 48

HPV16 up regulates TLR8 expression and function in keratinocytes: The search is on for the responsible factors.

Claudia Zannetti1, Francois Bonnay1, Ikbal Fathallah1, Peggy Parroche1, Fumihiko Takeshita2, Massimo Tommasino1 and Uzma A Hasan1

1 International Agency for Research on Cancer, 150 Cours Albert Thomas. 69009 Lyon, France

2 Yokohama City University School of Medicine 3-9 Fukuura, Kanazawaku. Yokohama 236-004 Japan

Cervical cancer development is linked to the persistent infection of high-risk mucosal human papillomaviruses (HPVs) types. We have shown that HPV16, the most oncogenic type, interferes with innate immunity by affecting the expression of Toll Like Receptors (TLRs). Infection of primary keratinocytes with oncoproteins from HPV16 inhibits TLR9 transcription, but strikingly TLR8 expression and function was up regulated. In the case of TLR9, its down- regulation may be due to viral mechanisms of immune evasion. In contrast, no plausible hypothesis can be easily envisaged for TLR8 up-regulation. This is primarily due to the fact that little is known about the regulation of this receptor. What is known is that TLR8 appears to play a more predominant role in ssRNA viral recognition in human. In fact, ssRNA sequences recognised by human TLR8 are also the natural ligand for mouse, but not human TLR7. In order to understand the biological significance of TLR8 regulation in HPV16 infection, we have to understand how TLR8 expression is regulated in the normal immune setting. For this purpose we have identified a fragment of 3.5 kb located upstream from the TLR8 translational start site and cloned this fragment in the pGL3-Basic reporter vector. A monocyte cell line (THP-1) transfected with the TLR8 promoter displayed a 10 fold increase in luciferase activity in comparison with the empty vector. To identify the proximal region on the promoter, progressively deleted mutants were constructed. Reporter analysis showed that a fragment spanning 800bp from the ATG is sufficient for the maximal promoter transactivation. In addition, the treatment of THP-1 cells with and IL-6 or the TLR8 ligand R848 led to an cytokines such as IFN gamma increase of TLR8 mRNA. The effects of these compounds on the TLR8 promoter showed that IFN gamma activates the promoter probably through STAT-1 activation, while by contrast R848 and IL-6 seemed to induce the promoter indirectly, through non-identified factors. In order to isolate the potential transcription factors involved in TLR8 regulation, we used expression plasmids for several trans - elements and found that C/EBP delta is a strong activator of the TLR8 promoter. Other elements such as RelA (p65) also play a role in trans-activating the promoter. The identification of the pathways involved in TLR8 regulation will firstly set the stage for understanding its regulation in the normal immune setting. Secondly, and more importantly, we can clarify the physiological significance of its increased expression in the context of the carcinogenic events mediated by HPV16 infection.

ABSTRACT 49

Innate Immune response to HSV-1: role of TLR2 and TLR9

G. K. Lima1, D. S. Mansur1, R. G. Astigarraga2, B.H.F. Lima2, D. T. Golenbock2/3, R. T. Gazzinelli2/3, M.C.C. Brandi2, G.P.P. Zolini2, E. G. Kroon1, M. A. Campos2

1Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, 2Instituto René Rachou, FIOCRUZ, Belo Horizonte, Brazil, 3Division of Infectious Diseases and Immunology, University of Massachusetts, Massachusetts, MA, United States

Herpes simplex virus 1 (HSV-1), is a neurotropic human DNA virus. This virus is the major cause of sporadic lethal encephalitis and blindness in humans. The balance of the immune response against HSV-1 is essential to the evolution of disease. Previously, we and others separately have shown the importance of Toll-like receptors (TLRs) in the immune response to HSV-1 infection. In this study, we have further investigated the role of TLR2 and TLR9 in the innate immune response against HSV-1. The production of TNF alpha and IL-12 p40, was assessed by ELISA in the supernatants of bone marrow dendritic cells (BMDCs) and macrophages derived from knockout (KO) mice, after stimulation with HSV-1. Production of IL-12 p40 in BMDCs derived from TLR2 or TLR9 KO mice decreased when compared to cells derived from wild type mice. Interestingly, BMDCs derived from TLR9 knockout mice showed an even more diminished response when TLR2 was blocked by a specific antibody. Production of TNF alpha in BMDCs was dependent on TLR2 but not on TLR9. In macrophages, production of IL-12 p40 and TNF alpha was dependent on both, TLR2 and TLR9, as KO cells for either of these receptors have shown a decrease in production of the cytokines. Moreover, macrophages TLR2-TLR9 double KO had a major decrease in IL-12 p40 production when compared to each of the single KOs. Additionally, we observed that MyD88, TLR9, IL-12 p40, TNF alpha rp55, IFN gamma, or iNOS KO mice showed higher susceptibility to intranasal infection with HSV-1, when compared to wild-type mice. Our results suggest an important role of TLR2 and TLR9 as mediators of the innate immune response after HSV-1 infection.

Flies

ABSTRACT 50

Preliminary X-Ray Analysis and Function of the Serpin, Necrotic from Drosophila Melanogaster

So Young Lee, Danel Kortazar, Ane Fullaondo, María Lucas, Pablo Fernández-Millán, Luis Alfonso Martínez-Cruz and David Gubb

CIC bioGUNE, Parque Tecnológico de Bizkaia Edificio 801 A - 48160 DERIO, SPAIN
E. mail ; sylee@cicbiogune.es

The Nec serpin controls the proteolytic cascade that activates the Toll-mediated innate immune response in the fly. In null mutants of nec, the immune response to fungal infections is constitutively activated. Nec is synthesized in the fat body, much as α1-antitrypsin is secreted from the liver to form a circulating plasma protein. Nec carries a long (90 amino acid) N-terminal extension of the core fold which is cleaved following immune-challenge. The N-terminally deleted Nec core serpin (ΔN-Nec), has a modified target specificity in vitro (Pelte et al., 2006) and partially rescues the nec mutant phenotype in transgenic flies. We have crystals of the ΔN-Nec protein and expect the X-ray structures of ΔN-Nec will clarify the mechanism of activation of the innate immune response. When fitted to the antithrombin fold, Nec carries a series of 5 charged amino-acids which align to the heparan-sulphate-binding groove of antithrombin. These charged residues are critical for Nec activity in vitro (Robertson et al., 2006) and both the K68 residue of D. melanogaster and a long N-terminal sequence is conserved across the 9/12 Drosophila species for which we have sequence data. We postulate that the N-terminal sequence may interact with charged residues on the surface of serpin core to modify stability.

ABSTRACT 51

The Drosophila membrane-associated protein PGRP-LF prevents IMD/JNK pathways triggering by blocking PGRP-LC activation~

Frédéric Maillet, Julien Royet

Institut de Biologie du Développement de Marseille-Luminy (IBDML)

UMR 6216 CNRS, Université de la Méditerannée Aix-Marseille II

Parc Scientifique de Luminy-Case 907

F-13288 Marseille Cedex 9; France

It has long been apparent that mammalian immune response needs to be kept under tight control. Responses that are delayed or of insufficient vigor can lead to a failure to control infection. However, excessive or inappropriate inflammation can be harmful or event fatal. Using the fruitfly as a model, we show that such an immuno-modulation is also essential in invertebrates. Peptidoglycan Recognition Proteins are evolutionary conserved molecules derived from bacterial peptidoglycan cleaving enzymes. It has been previously shown that some Drosophila PGRPs have lost this enzymatic activity (PGRP-SA, PGRP-LC) and function as bacterial sensors upstream of the Toll and IMD immune pathways. Other Drosophila PGRPs which have conserved amidase enzymatic activity (PGRP-SC1/2, -LB) regulate the intensity of the immune response by cleaving PGN and therefore reducing the amount of available immunostimulatory muropeptides.
We present here evidence that PGRP-LF, a non-enzymatic member of the same family of proteins maintains the Drosophila IMD pathway silent by sequestering circulating PGN. Flies in which PGRP-LF is inactivated present a constitutive activation of both the IMD and the JNK pathway which lead to developmental defects. We propose that PGRP-LF plays, in flies, a role similar to that of Decoy Receptor for TNF-a in mammalian immune response.

ABSTRACT 52

Studies of natural host-pathogen interactions between Microsporidia and Drosophila melanogaster

Sebastian Niehus1, Frédéric Delbac2, Caspar Franzen3 and Dominique Ferrandon1
1Institut de Biologie Moléculaire et Cellulaire, UPR 9022 du CNRS, 67084 Strasbourg, France, 2Laboratoire Microorganismes: Génome et Environnement, UMR CNRS 6023, Université Blaise Pascal, 63177 Aubière, France, 3Department for Internal Medicine I, University of Regensburg, 93042 Regensburg, Germany

Microsporidia are a highly diverse group of obligate intracellular eukaryotic parasites including more than 1,200 species able to infect host species from almost all phyla of the animal kingdom. Once classified as primitive protozoan eukaryotes, it is now acknowledged that they are extremely specialized and highly reduced relatives of fungi. Due to its powerful genetics and lack of adaptive immune response, Drosophila melanogaster has emerged as a strong model to study host immunity to microbial infections, and has offered valuable clues to the vertebrate innate immune system. The current paradigm describes two pathways for NF-?B dependent gene expression (Toll and IMD), resulting in an effective basal immunity against the majority of Drosophila pathogens. However, a parasitic model of infection by a natural pathogen of Drosophila does not exist at present, whereas knowledge to intracellular pathogens remains particularly elusive. Recently, a new microsporidian species Tubulinosema ratisbonensis was described and isolated from Drosophila laboratory colonies. To this end we have established models of infections, either by oral route at larval and adult stages, or by direct injection into the body cavity. Hence, the development of an infection system with an obligate intracellular parasite in which we can apply the full power of Drosophila genetics, provides a useful model to study host-parasite interactions and could lead to important conceptual new advances. Furthermore, it may give rise to an improved understanding of dipteran human disease vectors.

ABSTRACT 53

Effects of microbial and allergic components on the immune system of the fruitfly Drosophila melanogaster

C. Warmbold1, A.J. Ulmer1, K. Röschmann1, A. Petersen1, T.Roeder2

1) Division of Cellular Immunology, Research Center Borstel, 23845 Borstel.
2) Division of Zoophysiology, Christian-Albrechts University Kiel, 24098 Kiel, Germany.

The fruitfly Drosophila melanogaster has been used as a powerful model to study basic aspects of the innate immune system. The two independent signal transduction pathways Toll and IMD are constituents of the fruitfly`s immune system. The Toll-pathway is involved in signal transduction in response to infection with Gram-positive bacteria, whereas the IMD-pathway is stimulated during infection with Gram-negative bacteria. We have now investigated whether isolated cowshed dusts as well as isolated bacteria and lipopeptides, which have never been tested before in this system, are able to activate the innate immune system. For our analysis we use the macrophage-like cell line SL2 isolated from embryos of the D.m. strain Oregon R. The results of our studies are based on real-time PCR and on a luciferase reporter assays. In this assay a Firefly-luciferase is under the control of the drosomycin-promoter as a read-out-system for the Toll-pathway and the diptericin-promoter for the IMD-pathway. The data are normalized by the constitutive Renilla-luciferase. In our experiments we found that isolated cowshed dusts and bacteria excluding L.lactis G121 can activate the fruitfly`s immune system. In the human system L.lactis G121 leads to a delayed immune response. In addition to these bacterial components we furthermore discovered that allergic proteins of Timothy grass pollen (Phl p1, Phleum pratense), namely Phl p 1 and housedust mite (Der p 1, Dermatophagoides pteronyssinus) are also able to activate the Toll- and IMD-pathway in Drosophila. By using RNAi-experiments and microarray-analysis we will confirm our current data.

(supported by DGF-SFB/TR 22, project A7)

ABSTRACT 54

Deciphering the role of Tep genes during the innate immune response in Drosophila melanogaster

Richard Bou Aoun, Nicolas Matt, Samuel Liegeois, Jules Hoffmann & Dominique Ferrandon
Institut de Biologie Cellulaire et Moléculaire - UPR 9022 du CNRS-15 rue René Descartes - 67084 Strasbourg-France

An analysis of the genome of Drosophila melanogaster led to the identification of genes sharing similarities with the C3/C4 (complement factors in mammalian immunity), which were named Tep for ThiolEster containing Proteins. These proteins also share some similarities with the α2-macroglobulins family of protease inhibitors. The thiolester active site typical of the C3/C4 and the α2-macroglobulins is present in Tep proteins. There are 6 Tep proteins in the genome of drosophila (Tep1-6), including Tep5 which is a putative pseudogene, and Tep6 (also named mcr: macroglobulin complement-related) in which the thiolester site is mutated.

Tep proteins have also been studied in the mosquito Anopheles gambiae. aTep1 interferes with the life cycle of Plasmodium bergeï by limiting the development of the parasite during its invasion of the intestine of anopheles. It constitutes an essential factor in the anti parasitic response: it is able to bind to the surface of ookinetes and facilitates their elimination.

First, we investigated the expression patterns of the drosophila Tep genes by performing in situ hybridization on whole mount (larvae and adults) and on sections (adults). We show that Tep genes are transcribed in a broad range of tissues that may be involved in the defense of the flies against microorganisms. Second, we confirm, by Q-RT PCR, that Teps transcription increases in adults after a microbial challenge. These findings indicate that Teps may play a role in the defense against microbes. In Drosophila melanogaster, very little is known about the molecular function of this family of proteins, with the exception of the Tep-like protein, Mcr. Mcr has been shown to be involved in the phagocytosis of Candida albicans by Schneider cells. We are studying the molecular function of Tep proteins in Drosophila using genetic analysis.

ABSTRACT 55

Regulation of the response to mycobacteria in Drosophila

Marc S Dionne, Martina Yildirim, Rebecca Clark

Departments of Craniofacial Development and Microbiology, King's College London, London, United Kingdom

The systemic transcriptional response to pathogenic mycobacterial infection in Drosophila is highly complex. At late stages of disease, it includes markers and effectors of pathogenesis, immune effectors, metabolic regulators, and many uncharacterized activities. Understanding the complex regulation underlying this response will help us understand the pathogen-detection and regulatory mechanisms that operate in this infection.

We have taken a paired bioinformatic and genetic approach to this problem, starting with a large set of microarray data generated by us and others. This has allowed us to define a set of transcription factors that apparently regulate the disease process. These include some expected activities, such as NF-kB, AP-1, and gata factors and the forkhead-box protein Foxo; they also include several surprises, including other forkhead-box proteins. In addition to revealing novel aspects of the regulation of later pathogenesis, this analysis has been informative with regard to the regulation of the immediate systemic immune response in the fly. Our technique, and the prospective roles of the identified factors, will be discussed.

ABSTRACT 56

Hormonal control of Drosophila Immune Responses

Florentina Rus[1], Thomas Flatt[2], Ermelinda Porpiglia[1], Chris Sherlock[1†], Rochele Yamamoto[2], Alina Garbuzov[2], Marc Tatar[2] and Neal Silverman[1]

[1]Department of Internal Medicine, University of Massachusetts Medical School, Worcester, MA, USA, [2]Department of Ecology and Evolutionary Biology, Brown University Providence, RI,

In insects, the steroid hormone 20-hydroxy-ecdysone (20E) and the terpenoid juvenile hormone (JH) interact to regulate metamorphosis, reproductive maturation, and aging. Also, both hormones modulate immune responses in *Drosophila,* but how these hormones interact to regulate the immune response remains unclear. Our data show that JH and 20E have antagonistic effects on the induction of antimicrobial peptide (AMP) genes in *Drosophila*. Pretreatment of S2* cells with 20E promoted a robust induction of AMP genes following immune stimulation. On the other hand, a JH analog (methoprene) inhibited the ability of 20E to promote immune responsiveness. RNA silencing of either partner of the ecdysone receptor heterodimer (*EcR* or *Usp*) prevented the 20E-induced immune potentiation. Our results clearly demonstrate that 20E and JH play major roles in the regulation of gene expression in response to immune challenge. To identify potential downstream targets of 20E/EcR that are involved in modulating the immune response, a gene profiling approach was used. Microarrays (Affymetrix DrosChip 2.0) were probed in triplicate from *Drosophila* S2* cells treated with 20E alone, methoprene alone, both 20E and JHa, or no hormones. The microarray results reveal a set of ~100 genes modulated by 20E and JH, in an opposing manner. This list represents likely targets through which reproductive hormones regulate fly innate immunity. Further, we plan to determine whether any of these candidate genes have important functions in the pathway through which 20E regulates the immune responsiveness.

ABSTRACT 57

BACTERIAL STRATEGIES TO AVOID RECOGNITION OF THEIR PEPTIDOGLYCAN BY THE DROSOPHILA PGRP-SA PROTEIN

Magda Atilano*1, James Yates*1, Petros Ligoxygakis2 and Sérgio R. Filipe1
1 Instituto de Tecnologia Química e Biológica / Universidade Nova de Lisboa, Av. da República (EAN), Oeiras, Portugal; 2 Genetics Unit, Department of Biochemistry, University of Oxford, Oxford, UK.
*These authors contributed equally to this work.

Bacterial peptidoglycan is recognized by different mechanisms in multicellular organisms. Recognition triggers an inflammatory response that aims to eliminate the invading bacteria.

Gram-negative bacteria have a thin layer of peptidoglycan, which is masked from the external environment by an outer membrane, consisting mainly of phospholipids and LPS. In contrast, the cell wall from Gram-positive bacteria is directly exposed to the environment. It consists of a thick layer of peptidoglycan with which other polysaccharides and proteins are associated via either covalent or non-covalent interactions.

We are interested in furthering the understanding of how Staphylococcus aureus, a gram-positive pathogenic bacterium, synthesizes and degrades its peptidoglycan while simultaneously avoiding recognition by the infected host.

We have constructed different isogenic null mutant S. aureus strains that are unable to produce a normal, mature peptidoglycan. Cell wall and peptidoglycan preparations were purified from these strains and their ability to be recognized by the Drosophila peptidoglycan detector protein, PGRP-SA, was determined. We have shown that PGRP-SA binds with a higher efficiency to S. aureus peptidoglycan with a low degree of crosslinking. Using fluorescent microscopy we have also shown that PGRP-SA binds to live and dividing bacteria at specific sites. Furthermore our results indicate that in mature peptidoglycan, certain sites are masked and are not accessible to PGRP-SA. These results suggest that a coordinated synthesis of cell wall may have a key role in the strategies bacteria use to evade host detection.

ABSTRACT 58

Rudra Interrupts Receptor Signaling Complexes to Negatively Regulate the IMD Pathway

Kamna Aggarwal[1], Florentina Rus[1], Christie Vriesema-Magnuson[1], Deniz Ertürk-Hasdemir[1], Nicholas Paquette[1], and Neal Silverman[1*]

1. Divison of Infectious Diseases, Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, MA 01605, USA.

Insects rely primarily on innate immune responses to fight pathogens. In *Drosophila,* antimicrobial peptides are key contributors to host defense. Antimicrobial peptide gene expression is regulated by the IMD and Toll pathways. Bacterial peptidoglycans trigger these pathways, through recognition by peptidoglycan recognition proteins (PGRPs). DAP-type peptidoglycan triggers the IMD pathway via PGRP-LC and PGRP-LE, while lysine-type peptidoglycan is an agonist for the Toll pathway through PGRP-SA and PGRP-SD. Recent work has shown that the intensity and duration of the immune responses initiating with these receptors is tightly regulated at multiple levels, by a series of negative regulators. Through two-hybrid screening with PGRP-LC, we identified Rudra, a new regulator of the IMD pathway, and demonstrate that it is a critical feedback inhibitor of peptidoglycan receptor signaling. Following stimulation of the IMD pathway, *rudra* expression was rapidly induced. In cells, RNAi targeting of *rudra* caused a marked up-regulation of antimicrobial peptide gene expression. *rudra* mutant flies also hyper-activated antimicrobial peptide genes and were more resistant to infection with the insect pathogen *Erwinia carotovora carotovora*. Molecularly, Rudra was found to bind and interfere with both PGRP-LC and PGRP-LE, disrupting their signaling complex. These results show that Rudra is a critical component in a negative feedback loop, whereby immune-induced gene expression rapidly produces a potent inhibitor that binds and inhibits pattern recognition receptors.

Signaling

ABSTRACT 59

Inhibition of ASC-dependent caspase-1 activation by a *Yersinia* type III secreted protein

Igor E. Brodsky[1], Saheli Sadanand[1], Michelle Ryndak[3], Fayyaz Sutterwala[1,4], Richard Flavell[1,2], James Bliska[3], and Ruslan Medzhitov[1,2]

[1]Department of Immunobiology and [2]Howard Hughes Medical Institute

Yale University School of Medicine, New Haven, CT 06520

[3]Department of Molecular Genetics and Microbiology, Center for Infectious Diseases, School of Medicine, SUNY Stony Brook, Stony Brook, NY 11794-5222

[4]Current address: Division of Allergy and Immunology, University of Iowa, Iowa City, IA 52242

Activation of caspase-1 requires the assembly of multi-protein complexes known as inflammasomes. In addition to caspase-1, inflammasome complexes contain an NLR family protein that is thought to act as a sensor, and often include the adaptor protein ASC. Stimuli such as pore-forming toxins and infection with bacterial pathogens that possess type III secretion systems activate caspase-1 through particular inflammasomes characterized by unique NLR proteins. Successful pathogens have evolved numerous mechanisms of interfering with many aspects of host defense. However, whether type III secreted proteins of bacterial pathogens might modulate or inhibit inflammasome activation has not been demonstrated. Here, we show that macrophages infected with the bacterial pathogen *Yersinia pseudotuberculosis* can activate caspase-1 via at least two pathways, one of which is ASC-independent, while the other is ASC-dependent. We further describe a *Yersinia* type III secreted protein that has the novel function of specifically inhibiting the ASC-dependent pathway of caspase-1 activation. *Yersinia* lacking this protein are attenuated during in vivo infection, suggesting that inhibiting caspase-1 activation is a key aspect of *Yersinia* virulence. We propose that modulation of caspase-1 activation is likely to be a common theme in the interaction of microbial pathogens with eukaryotic cells.

ABSTRACT 60

A role for IRAK-2 in Toll-like receptor signalling to NF-κB via activation of TRAF6 ubiquitination.

Sinéad Keating, Geraldine Maloney, Ellen Moran and Andrew Bowie

School of Biochemistry and Immunology, Trinity College Dublin, Dublin 2, Ireland.

Although discovered over 10 years ago, the particular role of IL-1 receptor-associated kinase-2 (IRAK-2) in TLR signalling has remained elusive. Here we demonstrate a broad requirement for IRAK-2 in NF-κB activation by multiple TLRs. A52, a poxvirus-encoded TLR antagonist, inhibited the induction of NF-κB via TLR2, 3, 4, 5, 7 and 9 by targeting IRAK-2. siRNA knock-down of IRAK-2 expression in human cell lines impaired NF-κB activation by TLR3, TLR4 and TLR8 and led to reduced IL-8 production in primary human cells stimulated with LPS. Expression of IRAK-2 was sufficient to trigger polyubiquitination of TRAF6, an event critical for NF-κB activation, but IRAK-1 expression was not. Moreover, exogenous IRAK-2 could induce this TRAF6 ubiquitination in cells deficient in IRAK-1, demonstrating for the first time a distinct dichotomy in function for these proteins. IRAK-2 contains two putative TRAF6 binding motifs and mutation of the first binding site (E528A) completely abrogated the ability of IRAK-2 to activate all downstream signalling pathways tested. Conversely, mutation of the second motif (E559A) had no effect. While both mutants could still bind TRAF6, E528A could no longer initiate ubiquitination of TRAF6. Thus, this single residue is essential for IRAK-2-mediated TRAF6 ubiquitination and the induction of TRAF6 ubiquitination is a crucial downstream function of IRAK-2. An essential requirement for IRAK-2 in the TLR/NF-κB signalling axis has now also been demonstrated in mice (Kawagoe et al., 2008) confirming a critical role for IRAK-2 in TLR signalling to NF-κB.

ABSTRACT 61

FUNCTIONAL ROLE OF TOLL-LIKE RECEPTOR 4 DOMAIN INTERFACE FOR SIGNALLING

Mireille Treeby Premus1, Jozica Vasl1, Jozica Friedrich1, Roman Jerala1,2

1Department of biotechnology, National institute of chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia; 2Faculty of chemistry and chemical technology, University of Ljubljana,1000 Ljubljana, Slovenia

We hypothesized that specific segments of TLR4 inhibit association of the receptor in the inactive state and thus also receptor activation. Binding of LPS to ectodomain-bound MD-2 causes a conformational change which overcomes this inhibition and brings about interaction between two membrane receptors. In order to study which segment of TLR4 is responsible for this inhibition, we engineered flexible inserts (between 2-10 amino acids long) between various structural domains of TLR4 (TIR, TM and within LRR). All mutants were expressed at comparable levels as wild-type TLR4. Insertion of a linker into the two positions of membrane-proximal ectodomain impaired signaling most significantly within the C-terminal cysteine-rich segment, most likely due to disruption of the correct fold. Insertion of a linker between the TM and cytosolic TIR domain did not have any affect on activation, indicating that for activation the orientation of TIR domains does not need to be tightly constrained. In contrast, the insertion of a linker between the TM and ectodomain decreased activation proportionally with the length of the linker. This suggests tight coupling between the ecto- and transmembrane domain. These results will contribute to the refinement of our understanding of the molecular mechanism of Toll-like receptor signaling.

ABSTRACT 62

CROSS TALK BETWEEN THE TLR AND JAK/STAT PATHWAYS

Kristie A. Jenkins1, Luke A.J. O'Neill2 Paul J. Hertzog1 and Ashley Mansell1

1 Centre for Innate Immunity and Infectious Diseases, Monash Institute of Medical Research, Monash University, Melbourne, Victoria, Australia

2 School of Biochemistry and Immunology, Trinity College, Dublin, Ireland

While both the Jak/STAT and TLR pathways have been intensively studied, the role of cross talk between these two pathways is not well defined.

Suppressor of Cytokine Signaling (SOCS) proteins are known to play a crucial role in the regulation of the immune system via the Jak/STAT pathway. However, we and others have demonstrated a direct role for SOCS1 in TLR signaling, with SOCS1 described as targeting proteins via SH2 recognition of phosphoylated tyrosine residues. Although we have shown SOCS1 targets Mal for degradation, mutation of any of Mals 6 tyrosines fails to abrogate SOCS1 interaction or SOCS1 mediated degradation. This suggests SOCS1 does not directly bind Mal.

We have since found that SOCS1 may bind tyrosine phosphorylated TLR2 and TLR4. Mutation of TLR2 Y761F (TLR4 Y794F) results in potentiated TLR2 mediated activation of NF-κB and ablated SOCS1 or TLR2 induced polyubiquitination of Mal. This data suggests a novel mechanism of SOCS1 negative regulation in binding TLR2/4 to proximally associate with Mal and facilitate Mal degradation.

The role of STATs in the secondary response pathway following both TLR2 and TLR4 stimulation is well characterised. However, it has now been shown that TLR simulation results in the rapid Ser727 phosphoylation of STATs.

Our lab has identified putative TRAF6 binding sites in both STAT 1 and 3 that may provide the mechanism for the rapid phosphoylation observed. We have further demonstrated a direct interaction between TRAF6 and STAT 1 and 3 respectively.

ABSTRACT 63

Contributions of IPS-1- and TRIF-dependent pathways to poly IC-mediated immune responses

Taro Kawai1,3, Himanshu Kumar1,3, Shohei Koyama1,3, Ken J Ishii2,3, Shizuo Akira1,3
1Department of Host Defense, 2Department of Molecular Protozoology, Research Institute for Microbial Diseases, 3WPI Immunology Frontier Research Center, Osaka University, Osaka, Japan

Viral infection is initially sensed by innate immune system. Host cells express sensors that detect viral components such as nucleic acids, which induce type I interferon (IFN) and inflammatory cytokines as well as up-regulation of co-stimulatory molecules that result in elimination of viruses. Poly IC has been extensively used to mimic viral double stranded RNA, a byproduct of viral replication, and is recognized by two distinct pathways. One pathway is mediated by TLR3, which is localized in the endosomes. The other pathway is mediated by a cytoplasmic RNA helicase, Mda5. Upon recognition, TLR3 elicits signaling through the recruitment of an adapter TRIF whereas Mda5 recruits an adapter IPS-1. However, the functional differences between IPS-1- and TRIF-dependent pathways on polyIC-mediated immune responses are unclear. In this study, we have investigated relative contributions of IPS-1 and TRIF with respect to the generation of adaptive immune responses, using mice deficient for IPS-1 or TRIF.

ABSTRACT 64

The MAPK-activated kinase RSK controls TLR mediated responses in dendritic cells~

Rossana Zaru and Colin Watts

College of Life Sciences, Division of Cell Biology and Immunology, University of Dundee, UK

TLR engagement by microbial products induces several acute responses in dendritic cells (DC) as well as longer term transcriptional changes. One acute response is characterized by a transient increase in antigen uptake by macropinocytosis (West MA et al Science, 2004). This actin based process of fluid phase uptake is regulated during TLR stimulation through Erk1/2 or p38 pathways. Recently, we have identified the kinase RSK (p90 ribosomal S6 kinase) as one of the key regulators downstream of both ERK1/2 and p38 required for the TLR induced macropinocytosis (Zaru et al. Nature Immunology, 2007). We are currently investigating how RSK regulates this process. Two potential candidates have been identified: the actin cross-linker Filamin A (FLNa) and the Na+/H+ exchanger protein NHE1. Both are involved in the remodelling of the actin cytoskeleton which is essential for macropinosome formation and RSK phosphorylates FLNa upon TLR4 engagement. Moreover, phosphorylated FLNa is present at the site of macropinosome formation.

In parallel, we have also used a wider approach to identify potential RSK substrates. We took advantage of the fact that 14-3-3 proteins recognize consensus sequences on proteins phosphorylated by RSK. This technique combined with SILAC labelling of DC and the use of a specific RSK inhibitor has allowed us to identify new potential RSK substrates.
Besides regulating TLR induced macropinocytosis, we are also assessing whether RSK is also required for other responses in DC. Using either specific inhibitors or RSK deficient mice we found that RSK is required for the production of the pro-inflammatory cytokines IL-6 and IL-12. These preliminary results suggest that RSK could be an important regulator of multiple DC functions.

ABSTRACT 65

Characterisation of the death domain signalling complex of the IRAK4 and MyD88 proteins that are important for the innate immune response.

Gugu Motshwene1, Murali Ayaluru2, Cheng Kao2, Luca Pellegrini1, Nick Gay1.

1Department of Biochemistry, University of Cambridge, Cambridge, UK.

2Department of Biochemistry & Biophysics, Texas A&M University, Texas, USA.

Innate immune signalling is activated when transmembrane toll-like receptors (TLRs) bind to their ligands. Ligand binding triggers the recruitment of several intracellular proteins and activates a signalling cascade which eventually leads to the transcription of genes required to elicit an immune response. The death domains of two such intracellular proteins, namely Interleukin-1-receptor kinase 4 (IRAK4) and MyD88, were expressed in E.coli with the aim of studying their interaction. A stable oligomeric complex was reconstituted and its molecular weight was determined by gel filtration chromatography and analytical ultracentrifugation (AUC). The size of the IRAK4-MyD88 complex was larger than expected at 160kDa. The complex was further characterised by dynamic light scattering (DLS) and was found to be monodisperse. The determination of the low-resolution structure of the complex by single-particle cryo electron microscopy is currently underway. The result of this analysis will provide important insights into the structure and function of the IRAK4-MyD88 complex.

ABSTRACT 66

Functional proteomics of endosomal TLR signaling complexes

Irene Aspalter, Christoph Baumann, Tilmann Bürckstümmer, Andreas Pichlmair, Keiryn Bennett, Jacques Collinge and Giulio Superti-Furga

Center for Molecular Medicine, CeMM

Toll-like receptors (TLRs) are trans-membrane proteins that mediate the recognition of pathogen associated molecular patterns (PAMPs). Subsequently, TLRs induce an intracellular signaling cascade to activate transcription factors which initiate the expression of cytokines, interferons and host defense proteins. Ten human, structurally highly conserved TLRs have been assigned to structurally very dissimilar ligands. The complexity of a PAMP-TLR interface is best understood for the recognition of LPS (Lipopolysaccharide) which requires a receptor complex of at least four proteins (LBP-Cd14, TLR4-MD2). Hence, our central hypothesis is that TLRs generally function in protein complexes. To identify receptor associated co-receptors and co-ligands we started to systematically search for endosomal Toll-like receptor complexes required for the innate immune response to viral infection. We employ an interdisciplinary strategy combining biochemistry, cell biology, bioinformatics and proteomics. To this end, the full length murine, endosomal TLRs 3,7,8,9 have been C-terminally tagged with the GS- tandem affinity purification (TAP) cassette. Signaling competent RAW264.7 cells were virally transduced and stable, TLR expressing cell lines have been created. To assess the functionality and proper processing of the TLR-TAP fusion proteins we analyzed their glycosylation state and, moreover, are currently testing their ability to induce interferon in functional assays in HEK293 cells. Finally, we perform tandem affinity purifications of non-stimulated and selected, stimulated TLRs. Following the analysis of the eluates by MS, candidate interactors will be further evaluated using cell biological, biochemical and immunological methods. This project will help to broaden our understanding of Toll-like receptor mediated recognition of viral pathogens by the innate immune system and autoimmune disease.

ABSTRACT 67

An investigation into the interactions between Mal,

TLR1, TLR2 and TLR6

Kenny EF & O'Neill LA, School of Biochemistry and Immunology, Trinity College, Dublin 2, Ireland.

Toll-like receptors (TLRs) are key sensors of microbial pathogens that initiate the innate and subsequently the adaptive immune responses. TLRs utilise four adapter proteins to mediate their signalling pathways. These adapters are MyD88, Mal, Trif and Tram. MyD88 binds all TLRs with the exception of TLR3 and Mal has a role in TLR2 and TLR4 signalling. TLR2 senses bacterial products through its ability to bind TLR1 and 6. The TLR1/2 complex binds triacylated lipopeptides and the TLR2/6 complex binds diacylated lipopeptides.

Our investigations into the role of Mal have shown that it may not be key to the signalling initiated through TLR2. Yeast-two hybrid assays and confocal microscopy confirmed that Mal interacts with TLR2 but importantly not TLR1 and 6, which only bind MyD88. MyD88 was also shown to interact with TLR2 as expected. Mal-deficient cells show only a slight reduction in their ability to produce an immune response when stimulated with the TLR1/2 ligand Pam_3CSK4 and the TLR2/6 ligand Malp-2 at a high dose. Interestingly, at low doses of the TLR2 ligands the Mal-deficient cells lose their ability to respond. In comparison MyD88-deficient cells are unable to produce an immune response to the TLR2 ligands at both high and low doses. Interestingly MyD88- and Mal-deficient cells were hyper-responsive to the TLR3 ligand PolyI:C

These data therefore suggest that Mal binds TLR2 and this is required for TLR2 to bind MyD88. However, signalling through these complexes can still occur in the absence of Mal at high ligand dose. This may be due to TLR1 or 6 binding MyD88 and bypassing the need for Mal. This study shows that Mal may only be crucial for TLR4 signalling and is in fact used in TLR2 signalling to sensitise cells to low dose ligands or low pathogen load.

This work is supported by Science Foundation Ireland and the Irish Council for Science, Engineering and Technology.

ABSTRACT 68

Heme amplifies the innate immune response to microbial molecules dependently of ROS generation

Patricia L. Fernandez1,2, Fabianno F. Dutra1, Guilherme B. Fortes1, Letícia Alves1, Rodrigo T. Figueiredo1, Leonardo H. Travassos1, and Marcelo T. Bozza1.

1Laboratório de Inflamação e Imunidade, Departamento de Imunologia, Instituto de Microbiologia; UFRJ, Brazil; 2Instituto de Investigaciones Científicas Avanzadas y Servicios de Alta Tecnología INDICASAT, Ciudad de Panamá, Panamá.

Infectious diseases that cause hemolysis are among the most threatening human diseases. In these conditions high amounts of hemeproteins are released, but how it affects the innate inmune response to molecules of microorganisms is unknown. We have previously shown that heme induces the production of TNF by macrophages dependently of TLR4. However, ROS generation, neutrophil recruitment and HO-1 expression induced by heme, are independent on a functional TLR4. Here we show that heme enhances the secretion of cytokines by macrophages stimulated with all the agonists of TLRs and NLRs tested, irrespectively of the subcellular location of the receptor. This amplifying effect of heme was independent of TLR4, MyD88, ASC or RICK proteins. The over-expression of heme oxygenase (HO)-1, treatment with biliverdin or bilirubin reverted the effect of heme. Moreover, the amplifying effect of heme was dependent of ROS generation produced by NADPH oxidase and mitochondria. Challenge with heme or heme released after hemolysis induced by phenylhydrazine, increased the lethal effects of LPS. Together, these results indicate a critical and previously unrecognized role of heme as a deleterious molecule, affecting the inflammatory response during sepsis.

ABSTRACT 69

The induction of IL-12 production by the lectin KM+ depends on TLR2 and MyD88 molecules.

Camila F. Pinzan1, Kely C. Coltri2, Ademilson Panunto-Castelo2, Leandro L. Oliveira3, Maria Cristina Roque-Barreira1.

1 Department of Cellular and Molecular Biology, Ribeirão Preto Medicine School

niversity of São Paulo, Brazil.

2 Department of General and Specialized Nursing, Ribeirão Preto Nursing School, University of São Paulo, Brazil.

3 Department of Clinical Analysis, Toxicology and Bromatology, Ribeirão Preto School of Pharmaceutical Sciences, University of São Paulo, Brazil

KM+ is a mannose-binding lectin extracted from jackfruit seeds (Artocarpus integrifolia) that induces interleukin (IL)-12 production by antigen-presenting cells and confers protective Th1 immune response against intracellular pathogens.

To determine whether this KM+ property could be affected by MyD88, we determined the in vitro IL-12p70 production by spleen macrophages from MyD88-/- or WT mice following KM+ stimulation. When derived from MyD88-/- mice, KM+ stimulated macrophages were not able to release IL-12 in culture supernatants, suggesting that the IL-12 production induced by KM+ can be dependent on TLRs/MyD88 signaling pathway. In order to investigate the possible role of TLR2 or TLR4 in the induction of IL-12 production by KM+, we compared the IL-12p70 concentrations released by KM+-stimulated macrophages from TLR2-/- versus TLR2+/+ mice, or from TLR4 deficient versus proficient mice. Macrophages from mice TLR2-/-, but not from TLR4 deficient mice and control mice, failed to produce IL-12 in response to KM+, as well as in response to zymosan. Moreover, IL-12 production by KM+ stimulated macrophages was inhibited with 50 mM D-mannose, the KM+ specific monosaccharide. These results indicate that TLR2, but not TLR4, plays a critical role in IL-12 production mediated by KM+ and the cell stimulation occurs in a carbohydrate recognition-dependent manner.

ABSTRACT 70

Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis

Miguel A. Sanjuan, Christopher P. Dillon, Stephen W. G. Tait, Simon Moshiach, Frank Dorsey, Samuel Connell, Masaaki Komatsu, Keiji Tanaka, John L. Cleveland, Fabien Llambi, Sebo Withoff & Douglas R. Green

St. Jude Children's Research Hospital

Phagocytosis and autophagy are two ancient, highly conserved processes involved respectively in removal of extracellular organisms and the destruction of organisms in the cytosol. Autophagy, for either metabolic regulation or defense, involves the formation of a double membrane called the autophagosome, which then fuses with lysosomes to degrade the contents, a process that shows similarities with phagosome maturation. TLR engagement activates a variety of defense mechanisms within phagocytes including facilitation of phagosome maturation, and also engages autophagy. Therefore we speculated that TLR signaling might link these processes to enhance the function of conventional phagosomes. Here we show that a particle that engages TLRs while it is phagocytosed triggers the autophagosome marker LC3 to be rapidly recruited to the phagosome in a manner that depends on the autophagy pathway proteins ATG5 and ATG7, and is preceded by recruitment of Beclin-1 and Phosphoinositide-3 Kinase activity. Translocation of Beclin-1 and LC3 to the phagosome was not associated with observable double membrane structures characteristic of conventional autophagosomes, but was associated with phagosome fusion with lysosomes, leading to rapid acidification and enhanced killing of the ingested organism.

ABSTRACT 71

DUBLIN is a novel negative regulator of RIG-Like Helicase Signaling.

Kristie Jenkins1, Jing Jing Khoo1, Jordi Minguillon1, Giacinto Gaetano2, Phillip

Nagley2, Paul Hertzog and Ashley Mansell1

Centre for Innate Immunity and Infectious Disease, Monash Institute of Medical Research, Monash University, Melbourne, Australia

1Centre for Innate Immunity and Infectious Disease,

Monash Institute of Medical Research, Monash University, Melbourne, Australia

2Department of Biochemistry, Monash University, Melbourne, Australia

The RIG-like helicase (RLH) family of cytosolic viral nucleic acid receptors undergo ligand-induced polyubiquitination and signal via the mitochondrial localized adapter protein MAVS (also known as VISA/Cardif/IPS-1). RLH interaction with MAVS initiates a potent antiviral response via activation of NF-kappaB and expression of Type I interferon. While the mitochondrial localized protein NLRX-1 has been proposed as a negative regulator of MAVS signaling by disrupting this complex, little is known of the mechanism of RLH regulation.

Here we describe a novel gene we have termed DUB-Like INhibitor (Dublin) which contains a putative CARD domain and localizes to the outer mitochondrial membrane. Overexpression of Dublin inhibits RLH-induced NF-kappaB- and IFNbeta-promoter activity, while depletion of Dublin by siRNA produces potentiated RLH-mediated inflammatory responses. Crucially, Dublin appears to mediate this negative regulation by promoting specific deubiquitination of RIG-I and MDA-5, yet has no effect on MAVS. Importantly, NLRX-1 does not induce this effect.

Interestingly, Dublin directly interacts with MAVS, but not RIG-I, Mda-5 or NLRX-1 suggesting the mitochondrial localization of Dublin and association with MAVS is designed to specifically target the activated RLH complex once localized to the mitochondria to facilitate negative regulation.

ABSTRACT 72

The DEAD-box helicase DDX3X is a critical component of the TANK-binding kinase 1-dependent innate immune response

Didier Soulat1*, Tilmann Bürckstümmer2*, Sandra Westermayer1, Adriana Goncalves2, Angela Bauch2, Adrijana Stefanovic2, Oliver Hantschel2, Keiryn L Bennett2, Thomas Decker1 and Giulio Superti-Furga2.

1 Max F. Perutz Laboratories, University of Vienna, Dr. Bohr-Gasse 9/4, 1030 Vienna, Austria
2 Research Center for Molecular Medicine, Austrian Academy of Sciences, Lazarettgasse 19, 1090 Vienna, Austria

* These authors contributed equally to this work.

Tank-binding kinase 1 (TBK1) is of central importance for the induction of type-I interferon (IFN) in response to pathogens. In order to identify new components of the signaling machinery that regulates IFN production, we purified TBK1 by tandem affinity purification and identified interacting proteins by mass spectrometry analysis. One of the interactors is the DEAD-box helicase DDX3X. RNAi-mediated reduction of DDX3X expression led to an impairment of IFN production elicited by various stimuli, suggesting that DDX3X is required for IFN induction in general. Conversely, coexpression of DDX3X with TBK1 triggered the synergistic activation of the IFN promoter. Chromatin immunoprecipitation indicated that DDX3X can be recruited to the interferon promoter upon infection with Listeria monocytogenes, suggesting a transcriptional mechanism of action. DDX3X was found to be a TBK1 substrate in vitro and in vivo. Phosphopeptide mapping in combination with site-directed mutagenesis led to the identification of several candidate phosphorylation sites. Phosphorylation-deficient mutants of DDX3X failed to synergize with TBK1 in their ability to stimulate the IFN promoter. Overall, our data imply that the DDX3X is a critical effector of TBK1 that is necessary for type I interferon induction.

ABSTRACT 73

Class A CpG ODN activates B cells in a TLR9-dependent manner upon BCR stimulation

Avalos AM1, Chaturvedi A2, Tian JA3, Coyle AJ3, Pierce S2, Lenert P4 and Marshak-Rothstein A1

1Department of Microbiology, Boston University School of Medicine, Boston, MA, 2Laboratory of Immunogenetics, NIAID, NIH, Rockville, MD, 3Inflammation and Autoimmunity, MedImmune Inc, Gaithersburg, MD 4Department of Internal Medicine, University of Iowa, Iowa City, IA

Systemic autoimmune diseases such as SLE are characterized by the production of autoantibodies directed to DNA and RNA. Using the BCR transgenic AM14 mouse model, our laboratory has previously shown that activation of autoreactive B cells by mammalian DNA-containing immune complexes is TLR9-dependent. Synthetic oligonucleotides containing CpG sequences (CpG ODNs) have been shown to induce proliferation and cytokine production by B cells, macrophages and DCs in a TLR9-dependent fashion. CpGA and CpGB display contrasting activation characteristics depending on the cell type: CpGA induces IFNα production by pDCs, while CpGB induces strong proliferation in B cells. Differential localization of CpG ODNs may account for their divergent function as CpGA localizes to endosomes in pDCs and CpGB is found in lysosomes (Honda et al, Nature 434:1035). Here we show that B cells take up very low levels of CpGA, and CpGA does not colocalize with CpGB. However, CpGA is much more efficiently internalized by B cells when delivered though the BCR in the form of an immune complex (CpGA IC). This increased uptake translates into a strong TLR9-dependent AM14 B cell proliferative response. Moreover, CpGA induces proliferation of AM14 B cells in a TLR9-dependent manner when added in conjunction with a protein IC, suggesting a BCR signal is sufficient to increase CpGA internalization and translocation to a TLR9-containing compartment. Addition of CpGA IC at picomolar concentrations induces robust B cell proliferation; comparable concentrations of CpGB have no effect. These data imply that BCR internalization of ODNs is highly efficient, and the efficacy of TLR9 inhibitor ICs is explored.

ABSTRACT 74

LPS and ATP induced interleukin-1beta release from macrophages is Zn2+ dependent

David Brough, Nadia M. Luheshi and Nancy J. Rothwell

Faculty of Life Sciences, the University of Manchester, Manchester, UK.

Interleukin-1beta (IL-1beta) is a pro-inflammatory cytokine involved in host-defence and in the pathogenesis of diverse diseases. It is expressed and secreted by cells of the innate immune system in response to the activation of both membrane and cytosolic pattern recognition receptors. Secretion of IL-1beta is generally regarded to require two sequential stimuli. The first priming step induces expression of an inactive precursor called pro-IL-1beta. This is commonly achieved by treatment of the cells with bacterial lipopolysaccharide (LPS) that activates TLR4. The second stimulus activates the protease caspase-1 and requires the activation of cytosolic pattern recognition receptors such as those of the NOD like receptor family (NLR's) in a multi-protein complex called the inflammasome. Activation of the P2X7-receptor by ATP is known to activate caspase-1 via the NLR NALP3. Using this LPS / ATP model we have investigated the importance of transition metal ions in the processing and secretion of IL-1beta.
We have identified that Zn2+ is crucial for the activation of caspase-1 in response to LPS and ATP. K+ ionophore (nigericin) induced IL-1 processing was also dependent on Zn2+. Zn2+ chelation did not inhibit in vitro inflammasome assembly and ATP did not induce increases in the free cellular Zn2+ level. Thus our data suggest that Zn2+ regulates the activity of a protein upstream of inflammasome assembly and that this is a crucial step in the activation of this innate response

ABSTRACT 75

Interaction of Adaptor Proteins with the Cytoplasmic TIR domain of Toll-Like Receptor 4

Farhana H. Ahmadi1, Heather J. Brooks2, Tom P. Monie1, Clare E. Bryant2, Nicholas J. Gay2

1Department of Biochemistry, Cambridge University, Cambridge, UK, 2Department of Vetenerary Medicine, Cambridge University, Cambridge, UK.

Toll-like receptors (TLRs) are key regulators of the immune system, recognizing pathogen associated molecular patterns (PAMPs). TLR4 is the best-characterized member of the TLR family, which initiates intracellular signaling with the recruitment of TIR-domain-containing adaptor proteins to the cytoplasmic domain. Four main TIR domain-containing adaptors are involved in propagating TLR signaling: MyD88, Mal, TRAM and TRIF. These adaptors link activated TLRs with 'downstream' kinases of the IL-1 receptor-associated kinase and mitogen-activated protein kinase families. Activation of these enzymes leads to the activation of two distinct signaling pathways involving transcriptional regulators such as NFκB, AP-1 and several interferon regulatory (IRFs), which induce hundreds of genes involved in immune defense. TLR4 is activated by lipopolysaccharide (LPS) derived from Gram-negative bacteria, and induces downstream signaling by lingand-induced dimerization. Recent studies show that this homodimerization can cause conformational changes in the receptor leading to self-association of the cytoplasmic TIR signaling domain, providing a new scaffold that is able to bind downstream signaling adaptor proteins. Structure modeling studies of TLR4 TIR domain suggest that both Mal and TRAM adaptors bind at a two symmetry-related sites at the homodimer surface. This study aims to determine a model for TLR4 homodimerization and interactions with adaptor proteins by functional assays carried out in HEK (Human Embryonic Kidney) and MEF (Mouse Embryonic Fibroblasts) cells. This involves transfection of these cells with signaling complexes harboring mutations in the TIR domains of both TLR4 and adaptor proteins, aiming to fully characterize the interface at which TIR domains of TLR4 and adaptor proteins interact.

ABSTRACT 76

DISSECTING THE MECHANISMS BY WHICH CASPASE-1 ACTIVATED BY NOD-LIKE RECEPTORS TRIGGERS THE CONTROL Legionella pneumophila INFECTION

Tatiana N. Silveira, Marcelo S.F. Pereira and Dario S. Zamboni

Department of Cell Biology and Microbial Pathogenesis, University of São Paulo, Medical School Ribeirão Preto, Brazil.

The intracellular bacterial pathogen Legionella pneumophila multiplies in macrophages and subverts host cell functions by using a type IV secretion system that delivers bacterial products in the host cell cytoplasm. Whereas injection of bacterial proteins by type IV secretion system is absolutely required for intracellular multiplication, the products secreted by such machineries can be detected in the cytoplasm by pattern recognition receptors such as the Nod-like receptors (NLRs). We have previously demonstrated that the NLRs Naip5 and Ipaf trigger caspase-1 activation in response to bacterial products delivered in the host cytoplasm. Indeed, activation of caspase-1 culminates with restriction of Legionella multiplication by mechanisms not identified yet. Here we used NLRs-deficient mice in combination with selected Legionella mutants to assess the mechanistic functions of the different caspase-1-containing inflammasome. Caspase-1 activation in response to Legionella requires a functional type IV secretion system, a bacterial flagellin in the host cell cytoplasm and the NLRs Ipaf and Naip5. In macrophages activation of caspase-1 culminates with: i) formation of a pore in the host cell membranes; ii) secretion of inflammatory cytokines; iii) an unusual form of host cell death, called pyroptosis and; iv) restriction of bacterial multiplication. Interestingly, these features are events not necessarily related, which require engagement of various inflammasome. As it will be demonstrated, activation of different inflammasome and engagement of distinct intracellular signaling pathways are required for caspase-1 effectors functions, as cell death, inflammation and restriction of Legionella infection in macrophages and in a murine model of Legionnaire's Disease.

ABSTRACT 77

Selection of molecular structure and delivery of RNA oligonucleotides to activate TLR7 versus TLR8 and to induce high amounts of IL-12p70 in primary human monocytes

Ablasser A. 1,°, Poeck H. 1,2,°, Berger M. 1, Schlee M. 2, Kim S. 1, Anz D. 1, Rothenfusser S. 1, Endres S. 1, Hartmann G. 2,° and Hornung V. 3,°
° Contributed equally

1 Division of Clinical Pharmacology, Department of Internal Medicine, Ludwig-Maximilians-University of Munich, Germany

2 Institute of Clinical Chemistry and Pharmacology, University Hospital, University of Bonn, Germany

3 Division of Infectious Diseases, Department of Medicine, University of Massachusetts, Worcester, MA, USA

Detection of non-self RNA by Toll-like receptors (TLRs) within endosomes and by RIG-I-like Helicases (RLH) in the cytosol is central to mammalian antiviral immunity. Here we used synthetic, pathway-specific agonists and targeted delivery to address RNA immunorecognition in primary immune cells. In human PBMCs plasmacytoid dendritic cells (PDCs) and monocytes were found to be responsible for IFN-α production upon immunorecognition of RNA. The mechanisms of RNA recognition within these cells however are distinct and our data reveal the following three pathways: (1) In PDCs recognition of single-strand and double-strand RNA oligonucleotides was exclusively TLR-7-dependent whereas a 5' triphosphate moiety (RIG-I ligand activity) did not contribute to IFN-α production. In monocytes the response to RNA oligonucleotides was mediated either by TLR or RIG-I: (2) Single-strand RNA oligonucleotides transfected into endosomes lead to robust IL-12 induction mediated by TLR-8. (3) In contrast 5' triphosphate RNA induced RIG-I-dependent IFN-α production when delivered into the cytosol. Dissecting these pathways revealed that selection of the appropriate structure and delivery of RNA oligonucleotides allows to elicit potent IL-12p70 production in human monocytes, a crucial Th1 cytokine - classically ascribed to myeloid dendritic cells - that can not be induced by CpG oligonucleotides in the human system.

ABSTRACT 78

The Role of Cellular Sialidase in Ligand-Induced Toll-Like Receptor Activation

S. Ray Amith1, Preethi Jayanth1, Rudi Beyaert2 and Myron R. Szewczuk1

1Queen's University, Kingston, Canada; 2VIB-Ghent University, Belgium

There is evidence indicating that TLRs are highly glycosylated. However, the precise role of TLR glycosylation in receptor activation and cell function is unknown. Previously, we have reported that cellular sialidase(s) (glycohydrolytic enzymes) plays a critical role in nerve growth factor induced TrkA tyrosine kinase receptor activation. Here, we propose that a similar sialidase-dependent mechanism is involved in ligand-induced TLR activation. A novel technique was developed to detect the sialidase activity of viable cells as assessed by fluorescence of the cleaved product of sialidase-specific substrate 2′-(4-Methylumbelliferyl)-α-DN-acetyl-neuraminic acid. Sialidase activity is only detected in TLR2- and TLR4/MD2-expressing cells when specific ligands bind their respective TLRs. Activated sialidase(s) hydrolyzes α-2,3-linked β-galactosyl-SA residues of TLR4 based on lectin colocalization and inhibition experiments using Maackia amurensis agglutinin (MAL-2, binds α-2,3-SA) and Sambucus nigra agglutinin (binds α-2,6-SA). Tamiflu blocks sialidase activity and inhibits ligand-induced NFκB activation as evidenced by immunocytochemistry, EMSA and supershift assays. In addition, MyD88 co-immunoprecipitates with TLR4 in lysates from LPS-treated HEK-TLR4/MD2 cells, but this is significantly inhibited in cells pretreated with either Tamiflu or MAL-2 lectin. Our findings indicate for the first time that TLR activation is dependent on sialidase induction upon TLR ligand binding, and that the hydrolysis of α-2,3-SA is a key step in facilitating MyD88/TLR4 interaction and downstream LPS-induced NFκB activation.

ABSTRACT 79

POLY(I:C) REGULATES THE EXPRESSION OF NOD-LIKE RECEPTORS AND INDUCE CASPASE-1 DEPENDENT IL-1BETA SECRETION IN HUMAN KERATINOCYTES

Szilvia Benkő#£, Aliz Varga£, Attila Balogh$, Gabriella Miklóssy§, Gabriella Emri$, Éva Remenyik$, József Tőzsér§, Éva Rajnavölgyi£

£ Institute of Immunology, § Department of Biochemistry and Molecular Biology, $Dermatology, Medical Health and Science Center, University of Debrecen, Debrecen, Hungary
present address: Laboratory of Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Toronto, Canada

Human keratinocytes are target of pathogens including viruses that contain pathogen associated molecular patterns, recognized by pattern recognition receptors. To study the involvement of the cytosolic Nod-like receptors in the innate immune response of keratinocytes, we treated human primary cells with poly(I:C) in vitro to mimic the effect of dsRNA generated during viral infection. We studied the expression of the receptor family members and the cytokines secreted by activated keratinocytes. We found that the expression of Nod and Nalp mRNAs and proteins are differentially regulated by poly(I:C) in this cell type. We detected robust induction of NLRC5 and strong induction of Nod2, while Nod1 was weakly induced and the transcription of NLRX1 mRNA did not change. Furthermore, we found that Nod and Nalp sensors that were inducible at mRNA level (Nod2, Nalp3) had weak basal protein expression but showed strong induction upon dsRNA treatment, while the protein of the non-inducible sensors (Nod1, Nalp1) were constitutively expressed in keratinocytes. Also, we found that poly(I:C) treatment induces the production of IL-1beta pro-inflammatory cytokine and this secretion is mediated by the activity of caspase-1.

ABSTRACT 80

STIMULATION OF TLR 3 AND TLR4 INDUCES CASPASE-1 INDEPENDENT IL-1b MATURATION

Rudi Beyaert, Jonathan Maelfait, Elisabeth Vercammen, Sophie Janssens, Peter Schotte, Mira Haegman

Unit of Molecular Signal Transduction in Inflammation, Department of Molecular Biomedical Research (VIB) and Department of Molecular Biology (Ghent University), Ghent, Belgium

The cytokine IL-1b is a key mediator of the inflammatory response and has been implicated in the pathophysiology of acute and chronic inflammation. IL-1b is synthesized in response to many stimuli as an inactive proIL-1b precursor protein that is further processed by caspase-1 into mature IL-1b, which is the secreted biologically active form of the cytokine. Although stimulation of membrane-bound Toll-like receptors upregulates proIL-1b expression, activation of caspase-1 is believed to be mainly initiated by cytosolic Nod-like receptors. Here we show that poly(I:C) and LPS stimulation of macrophages induces proIL-1b processing via a TRIF-dependent signaling pathway that is initiated by TLR3 and TLR4, respectively. RNAi mediated knockdown of the intracellular receptors NALP3 or MDA5 did not affect poly(I:C)-induced proIL-1b processing. Surprisingly, poly(I:C)- and LPS-induced proIL-1b processing still occurred in caspase-1 deficient cells. We will present evidence for another caspase playing a key role in the production of biologically active IL-1b in response to TLR3 and TLR4 stimulation.

ABSTRACT 81

COOPERATIVE INTERACTION OF THE CATIONIC ANTIMICROBIAL PEPTIDE, KLK AND THE TLR9-AGONIST, ODN1A WITH DENDRITIC CELLS

Michael C. Aichinger1, Michael Ginzler2, Karen Lingnau2, Alexander von Gabain2, Rudolf Schweyen1 and Tamás Henics1

1Department of Genetics, Max F. Perutz Laboratories, Vienna, Austria 2INTERCELL AG, Vienna, Austria

The cationic antimicrobial peptide, KLKLLLLLKLK (KLK) and the TLR9-agonist, oligo-dIC13 (ODN1a) function together as a potent vaccine adjuvant, termed IC31TM. We have previously demonstrated that KLK exerts non-pore forming membrane-interacting properties with prominent effects on membrane ultrastructure and fluidity. Furthermore, KLK intercalates with membranes and undergoes multiple conformational transitions, a function of membrane lipid composition. Here we show that KLK and ODN1a aggregates into a complex at the surface of dendritic cells (DCs). This process initiates peripheral enrichment and uptake of ODN1a into distinct compartments of the peripheral cytoplasm, while the peptide itself remains localized in the cell membrane vicinity. Following uptake and internalization, ODN1a does not show any obvious partitioning in either early or late endosomal compartments but co-localizes with the endoplasmic reticulum (ER) in both wild-type and TLR9 -/- DCs. We propose that KLK, due to its unique membrane active properties, stimulates association and a yet uncharacterized uptake of ODN1a into DCs and this process successfully targets ODN1a to the ER. These properties of KLK might fundamentally contribute to the potent immunostimulatory effects of the novel adjuvant, IC31TM.

ABSTRACT 82

A suggestive role for Naip5 on iNOS activation

Carina L. Buzzo; Rafael Leme-Souza; Alexandra Cassado; Liliana Massis; Momtchilo Russo; Gustavo P. Amarante-Mendes; Karina Ramalho Bortoluci.

Buzzo, C.L.; Leme-Souza, R.; Cassado, A.; Massis, L.; Ferreira, L.C.; Russo, M; Amarante-Mendes, G.P.; Bortoluci, K.R.

Department of Immunology; Institute of Biomedical Science; University of Sao Paulo, Brazil.

Innate immune system employs intracellular and extracellular pattern recognition receptors (PRRs) to detect microbial infections through the recognition of PAMPs, such as flagellin (Fli). It has been shown that Fli is recognized extracellularly by transmembranic TLR5 and intracellularly by Ipaf and Naip5. These two members of the cytosolic receptors of the NLR family are known to induce caspase-1 activation. To evaluate the relative contribution of TLR and NLR to Mø activation, peritoneal Mø from C57BL/6 mice were stimulated with free Fli or Fli inserted in DOTAP (FliDot), a cationic lipid vesicle that allows protein to be delivered to cytosol (0,6 ug of protein/5x105 cells). Stimulation was performed in the presence or absence of the inflammatory cytokines IL-12/IL-18 (2,5 ng/ml each), which are known to be induced by TLR and NLR, respectively. As expected, only FliDot lead to caspase-1-dependent IL-1β secretion (C:139±28; Fli:133±14; FliDot:413±50 pg/ml). Conversely, only Fli induced Nitric Oxide (NO) production (C:1,5±1; Fli:7,8±1; FliDot:1±0 μM). IL-12/IL-18 increased 4 times NO production in response to Fli and, surprisingly, high levels of NO were also found in response to FliDot (C12/18:5±1; Fli12/18:29±4; FliDot12/18:20±1 μM). Similar effect was observed with iNOS expression. Interestingly, in the presence of cytokines, Mø from A/J (Naip5 deficient) mice secrete high levels of NO in response to Fli whereas no additive effect was observed in response to FliDot (C12/18:4±1; Fli12/18:33±6; FliDot12/18: 8±5 μM). These results suggest the existence of an undescribed role for Naip5 on iNOS activation.

ABSTRACT 83

A NOVEL LEUCINE RICH REPEAT CONTAINING PROTEIN INVOLVED IN THE TLR4 SIGNALLING PATHWAY

Susan Carpenter, Aisling Dunne, Luke A.J O'Neill

School of Biochemistry and Immunology, Trinity College Dublin, Dublin 2, Ireland. Opsona Therapeutics, Lab 3.08, Institute of Molecular of Medicine, St. James Hospital, Dublin 8

Toll-like receptors are a family of proteins that act as the primary sensors of microbial products. These receptors recognise a range of ligands and activate a series of signalling pathways that lead to the induction of immune and inflammatory genes. TLR4 is the most characterised of all the TLRs, it recognises lipopolysaccharide (LPS), a component of the cell wall of gram negative bacteria. In order for TLR4 to recognise LPS it requires a number of accessory proteins, or co receptors. TLRs and the accessory proteins associated with them are characterised by the presence of extracellular leucine rich repeats. Here we identified a novel leucine rich repeat containing protein which is annotated as KIAA0644. The KIAA0644 gene maps to human chromosome 7p15. We have found that the protein is expressed and highly conserved across species. It contains 13 leucine rich repeats, a signal sequence and a putative transmembrane region. Expression is highest in brain, lung and ovary and this expression is enhanced by microbial products (e.g. LPS) suggesting that it may function as an immuno-modulator. We have found that overexpression of KIAA0644 enhances LPS induced signalling. In addition, knockdown of KIAA0644 using specific siRNAs abolishes LPS-induced signalling. KIAA0644 is capable of binding to TLR4 and LPS. This data demonstrates that KIAA0644 is a functional protein which may be acting as a co-receptor in TLR4 signalling.

ABSTRACT 84

A promoter complexity model for Trif-biased stimulation of TLR4 by Monophosphoryl Lipid A

Caglar Cekic1,2, Chelsea Eaves1,2, Carolyn R Casella2 and Thomas C Mitchell1,2
1Department of Microbiology and Immunology, University of Louisville, Louisville, KY, USA, 2Institute for Cellular Therapeutics, University of Louisville, Louisville, KY, USA.

We recently reported that monophosphoryl lipid A (MPLA) induces fewer TLR4/MyD88-dependent signaling outcomes than LPS, whereas activation of TLR4/Trif-dependent pathway was largely unimpaired. One caveat to a strictly Trif-biased view of MPLA's low toxicity, however, is that not all MyD88-dependent genes show reduced expression and not all Trif-dependent genes are fully active when comparing MPLA to LPS. Furthermore, both MPLA and LPS are heterogeneous mixtures of lipid A structures, which may have different effects on different kinds of cells.

We now report that homogeneous preparations of synthetic MPLA (sMLA) retained their Trif-bias when compared to synthetic diphosphoryl lipid A (sDLA) using BM-DC as targets, indicating that a change in a single phosporyl group is sufficient for the effect. One important difference from biologically derived MPLA and LPS involved Trif-dependent IFNβ, which showed impaired transcription 1-3h after stimulation with sMLA as compared to sDLA. Yet other Trif-dependent genes, such as IP-10 and IFIT1, were induced to similar levels. IP-10 and IFIT1 require fewer transcription factors for them to be active (IRF-3 and p65), whereas requirements for IFNbeta transcription are more complex (IRF-3, p50/p65, and AP-1). Immunoblotting experiments showed that sMLA induced weaker phospho-activation of p38, ERK1/2, p65 and IKKα/β. Thus, sMLA may drive largely unimpaired Trif-biased signaling because the promoters of its targeted genes are more likely to be 'simple', while those associated with MyD88 are more often complex. Initial experiments also indicate that sMLA and sDLA induce MyD88 recruitment with different kinetics which can be responsible for sMLA's Trif-biased signaling pattern via an unknown mechanism, which is under investigation

ABSTRACT 85

A Role for Tyrosine Kinase Syk in TLR Signaling

Anu Chaudhary

Los Alamos National Laboratory

A role for spleen tyrosine kinase (Syk) has recently emerged in TLR2 and TLR4 signaling. We have shown that Syk constitutively associates with TLR4 in human monocytic cells. Piceatannol, a pharmacological inhibitor of the tyrosine kinase Syk, abrogates TLR4 tyrosine phosphorylation. The kinetics of TLR4 tyrosine phosphorylation coincides with an early wave of Syk tyrosine phosphorylation.

dditionally, serine threonine kinase IL-1 receptor associated kinase 1 (IRAK-1) is transiently recruited to the complex containing adaptor molecule MyD88, TLR4 and Syk within 1 min of LPS engagement and dissociates by 30 min. Finally, the inhibition of Syk with piceatannol has no effect on LPS-mediated release of cytokines IL6, IL1-beta, TNF-alpha, neither on chemokines MIP1-alpha, MIP1-beta, MCP-1, IL8, Gro-alpha and RANTES. However, IL10 and IL12p40 release are significantly inhibited. Our findings implicate Syk as a novel modulator of LPS-mediated TLR4 responses in human monocytic cells and shed insight into the kinetics of early complex formation upon LPS engagement.

ABSTRACT 86

Nucleic Acid Sensing in the Cytosol

Evelyn Dixit, Tilmann Bürckstümmer, Gerhard Dürnberger, Melanie Planyavsky, Jacques Colinge, Keiryn L Bennett and Giulio Superti-Furga

CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences

Secretion of type 1 interferons (IFNα and IFNβ) is a central event of antiviral immunity. Viral infections are detected by cytosolic sensors such as RIG-I, MDA-5 and DAI or the endosomal TLRs 3, 7, 8 and 9 which detect extracellular nucleic acids that have reached the cell by endocytosis.

In order to identify novel components of the cytosolic nucleic acid sensing machinery, we combined proteomics with genomics, affording us the significant advantage of an unbiased approach. We identified 24 proteins among which five known components of virus-induced signaling pathways reside, namely RIG-I, MDA-5, IKK-i, PKR and 2'5'-Oligoadenylate Synthethase. The presence of these five proteins in our candidate list validates our approach and hints at the biological relevance of the remaining 19 proteins. The 19 candidates are functionally diverse and include kinases, helicases, DNA- and RNA-modifying enzymes and transcription factors. 15 out of 19 candidates have a more or less established link to immunological processes. Most interestingly, 9 out of 19 candidates are poorly annotated proteins.

In order to assess the functional relevance of each candidate for antiviral innate immunity, the effect of candidate silencing by RNAi on nucleic acid-stimulated IFNβ induction was analyzed. Currently, we focus on one particular candidate, the exonuclease Trex1 that seems to prevent immune responses to endogenous DNA and thus promotes autoimmunity when inactivated.

ABSTRACT 87

beta-PIX and Rac1 GTPase mediate trafficking and negative regulation of NOD2

Julia Eitel1, Matthias Krüll1, Andreas C. Hocke1, Norbert Suttorp1, and Bastian Opitz1
1Department of Internal Medicine/Infectious Diseases and Pulmonary Medicine, Charité - Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

The nucleotide-binding domain and leucine-rich repeat containing (NLR) protein NOD2 serves as a cytoplasmic pattern recognition molecule sensing bacterial muramyl dipeptide (MDP), whereas Toll-like receptor (TLR)-2 mediates cell surface recognition of bacterial lipopeptides. Here we show that NOD2 stimulation activated Rac1 in human THP-1 cells and primary human monocytes. Rac1 inhibition or knock-down, or actin cytoskeleton disruption increased MDP-stimulated IL-8 secretion and NF-kappaB activation, whereas TLR2-dependent cell activation was suppressed by Rac1 inhibition. beta-PIX plays a role in this negative regulation, because knock-down of beta-PIX also led to increased NOD2-mediated, but not TLR2-mediated IL-8 secretion. Coimmunoprecipitation experiments demonstrated that NOD2 interacted with beta-PIX as well as Rac1 upon MDP stimulation. Moreover, knock-down of beta-PIX or Rac1 abrogated membrane recruitment of NOD2, and interaction of NOD2 with its negative regulator Erbin. Overall, our data indicate that beta-PIX and Rac1 mediate trafficking and negative regulation of NOD2-dependent signaling which is different from Rac1´s positive regulatory role in TLR2 signaling.

ABSTRACT 88

INVOLVEMENT OF THE UBIQUITIN-LIKE DOMAIN OF TBK1 IN REGULATION OF INTERFERON-INDUCIBLE GENES

Fumiyo Ikeda1,2, Alexis Rozenknop1,3, Vladimir Rogov3, Volker Dötsch3, and Ivan Dikic1,2
1 Institute of Biochemistry II, Goethe-University Frankfurt am Main, Frankfurt, Germany, 2 Tumor Biology Program, Mediterranean Institute for Life Sciences, Split, Croatia, 3 Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance, Goethe-University Frankfurt am Main, Frankfurt, Germany

TANK binding kinase 1 (TBK1/NAK/T2K) plays an important role in the regulation of interferon-inducible genes in the innate immune system. Cell stimulation with ssRNA virus, dsDNA virus or gram-negative bacteria leads the activation of TBK1 or I-kappaB Kinase (IKK-i/IKK-epsilon), which in turn phosphorylates the transcription factors, interferon regulatory factor (IRF) 3 and IRF7. To understand the molecular basis of activation of TBK, we analyzed the sequence of TBK1 and identified a ubiquitin-like domain (ULD) localized adjacent to its kinase domain. Deletion or mutations of the ULD in TBK1 impaired kinase activity of TBK1, failed to induce IRF3 phosphorylation and nuclear localization and to activate IFN-beta or RANTES promoters. The ULD of TBK1 has a potency to interact with the TBK1 kinase domain and with IRF3 via distinct binding surfaces on the ULD. The importance of the ULD of TBK1 in LPS- or Poly(I)Poly(C)-stimulated IFN-beta production was demonstrated by reconstitution experiments in TBK1-IKK-i-deficient cells.

We propose that the ULD in TBK1 plays important roles in the regulation of the TBK1 kinase activity and downstream signaling pathways.

ABSTRACT 89

Role of Rab11 in signaling and intracellular trafficking Toll-like receptor 4

Harald Husebye1, Marie Hjelmseth1, Eivind Samstad1, Øyvind Halaas1, Eicke Latz2, Harald Stenmark1 and Terje Espevik1.

1) Norwegian University of Science and Technology, Trondheim, Norway.
2) 2) University of Massachusetts Medical School, Worcester, USA.

The immediate response to an invading organism is coordinated by the innate immune system. The pathogens are sensed by germ line-encoded receptors, including the Toll-like receptors (TLRs). Activation of TLRs results in production of pro-inflammatory cytokines important for the immune response. The signaling receptor for lipopolysaccaride (LPS) is TLR4 and MD-2 receiving LPS from CD14.

Rab11 (Rab for Ras related in brain) is a member of the Rab family protein family of small GTPases, controlling intracellular vesicle transport and fusion events. Like all GTPases of the Ras family, Rab11 functions as a molecular switch. Rab11 controls the recycling of a number of cellular receptors and is a marker of the peri-centriolar recycling compartment (ERC). Rab11 in relation to TLRs has been poorly studied.

TLR4 is localized on the plasma membrane, on endosomes and in a large peri-nuclear pool. We have evidence indicating that most of peri-nuclear TLR4 originates from recycling of the endocytosed receptor. High-resolution 3D-confocal microscopy revealed that peri-nuclear TLR4 was located in a compartment in vicinity to Golgi, where a prominent overlap with Rab11 was observed. The location of TLR4 in the ERC suggests Rab11 to play an important role in the intracellular trafficking of TLR4. Furthermore, targeting of Rab11 by siRNA had a marked effect on both TLR4 trafficking and signalling. Our data shows that Rab11 has a function in regulating both TLR4 signalling and transport of TLR4 to the phagocytic cup.

ABSTRACT 90

The transcription factor Ets 2 is activated by TLR ligation, translocates to the nucleus and regulates a subset of ETS dependent proinflammatory genes

T. Wilson, J. de Freitas, D.Xu, J.Gould and P.J. Hertzog

Centre for Innate Immunity and Infectious Diseases, Monash Institute of Medical Research, Monash University, Clayton Victoria, Australia.

Activation of TLRs triggers a complex intracellular network that involves adaptor proteins, protein kinases and ultimately activation of a number of transcription factors and induction of pro-inflammatory cytokines such as Interferons, TNF- , interleukins and chemokines. Analysis of TLR-signaling has identified the involvement of a number B, IRFs, STATs and AP1. Although it isκof transcription factors, including, NF clear that several MAP Kinases play important roles in TLR-signaling pathways, many of the factors downstream of these pathways have not been elucidated. Here, we show that the Ets transcription factor Ets2 is rapidly activated, translocates to the nucleus after TLR stimulation in both murine bone marrow macrophages (BMM) and the RAW264.7 macrophage-derived cell line. Furthermore, inhibition of JNK completely blocks Ets2 translocation to the nucleus and Ets2 is constitutively expressed in the cytoplasm and does not translocate to the nucleus in JNK null MEF. We demonstrate by Ets2 knockdown via RNAi, promoter-rporter assays and chIP that this factor regulates IL-6 production B and IRF activity. Furthermore, we have undertaken geneκindependently of NF- profiling analyses on Ets2-/- ES cell derived macrophages to identify the scope of ets regulation of important TLR driven proinflammatory genes. Taken together, these results identify for the first time that ETS 2 represents a primary activation pathway in TLR responses; it is phosphorylated by JNK translocates to the nucleus and regulates an important subset of proinflammatory genes.

ABSTRACT 91

BX795, a novel pharmacological inhibitor of TBK1 and IKKε

Kristopher Clark, Natalia Shpiro and Philip Cohen

MRC Protein Phosphorylation Unit, University of Dundee, Dundee, Scotland

TBK1 and IKKε play a critical role during innate immunity by regulating the activity of the transcription factor IRF-3 in response to pathogens. Normally, IRF-3 is present in the cytosol and upon phosphorylation by TBK1 and IKKε, IRF-3 dimerizes and translocates to the nucleus where it activates the transcription of Type I interferons. However, several agonists which activate TBK1 and IKKε fail to induce IRF-3-dependent gene transcription suggesting additional roles for these kinases. Notably, the kinase activity of TBK1 and IKKε is elevated in cancer cells which is required for cell transformation and proliferation. It is therefore of great interest to identify suitable pharmacological inhibitors to rapidly address the roles of TBK1 and IKKε in the immune system and cancer. Here, we identify BX795 as a potent and selective inhibitor of TBK1 and IKKε. A brief characterization of the effects of BX795 in mammalian cells will be presented.

ABSTRACT 92

TLR 9 engagement on distinct DC subsets differentially affects their ability to activate CD8 T cells.

Christelle de Brito, Martine Tomkowiak, Christophe Caux, Jacqueline Marvel and Yann Leverrier.
INSERM U851, Centre Léon Bérard

Immature dendritic cells (DC) sample the tissue microenvironment by internalizing exogenous material such as dead cells. Following their phagocytosis dead cells are delivered into endocytic compartments, degraded and the resulting peptides can be presented on MHC molecules to CD8 and CD4 T cells. If this process is associated with appropriate DC maturation signals it can induce an efficient T cell activation. Distinct DC subsets of distinct origin, localization and function have been described in vivo and can induce distinct types of immune responses. Therefore we have compared the ability of GM-CSF or Flt3-L derived DC to engulf EL4 necrotic cells expressing the model antigen NP (EL4-NP) and to activate transgenic NP specific CD8 T cells (F5 CD8 T cells) in vitro in response to the maturation signal provided by the TLR9 ligand CpG.
Our results show that immature GM-CSF derived DC are highly competent at phagocytosing necrotic EL4-NP cells and at cross-presenting the NP epitope to F5 CD8 T cells. As previously described, engagement of TLR9 prior to antigen uptake abrogates GM-CSF derived DC ability to activate CD8 T cells. In contrast, immature sorted conventional DC (cDC) or plasmacytoid DC (pDC) generated in Flt3-L cultures fail to efficiently internalize necrotic cells and to activate CD8 T cells. However pretreatment of cDC or pDC with CpG strongly enhances their ability to engulf entire necrotic cells and to uptake necrotic material through a process that resembles « nibbling ». In addition CpG increases the ability of Flt3-L derived DC to activate CD8 T cells by a cross-presentation mechanism. Interestingly in vitro activated CD8 T cells give rise to memory CD8 T cell when transfered in vivo.

Altogether our results show that the recognition of a TLR ligand by different DC subsets can have different immunological outcomes.

ABSTRACT 93

SPHINGOSINE 1-PHOSPHATE IS A NEGATIVE REGULATOR OF TLR2 SIGNALING

Ana I. Dueñas (1), Monica Aceves (1), Isabel Fernandez-Pisonero (1), Cristina Gómez (1), Antonio Orduña (2), Mariano Sánchez Crespo (1), Carmen García-Rodríguez (1)
(1) Instituto de Biología y Genética Molecular (CSIC-UVA), and (2) Hospital Clínico Universitario, Valladolid, Spain.

Increasing evidences link the Toll-like family of receptors (TLR) and the development of inflammatory diseases, including atherosclerosis. It has been reported that TLR can interact with minimally oxidized low-density lipoproteins and that specific phospholipid oxidation products inhibit TLR4 and TLR2 activation. These data prompted us to study the effect of several lipoprotein-carried lipids on TLR activation. In a model of TLR2-transfected 293 cells, TLR2-induced NF-kappa B activation was inhibited by sphingosine 1-phosphate, a bioactive lipid mediator associated to native lipoprotein particles that triggers many biological responses via a family of G-protein coupled receptors. No effect was observed when other lysophospholipids were tested, i.e lysophosphatidylcholine and lysophosphatidic acid. To address the physiological relevance of these results, experiments were also carried out in human peripheral blood monocytes and monocyte-derived macrophages. In both cell types, sphingosine 1-phosphate inhibited the activation of the NF-kappa B and ERK routes induced by two different TLR2 ligands, and the inhibitory effect was partially abrogated after pharmacological blockade of PI3K and Ras pathways. siRNA experiments demonstrated that the attenuation effect elicited by sphingosine 1-phosphate is mediated by sphingosine 1-phosphate receptors 1 and 2. Altogether, these findings disclose a complex mechanism of cross-talk between different components of lipoprotein particles and TLR ligands, in which engagement of sphingosine 1-phosphate receptors exert selective attenuation of TLR2-dependent NF-kappa B and ERK activation via PI3K and Ras signaling.

ABSTRACT 94

Cellular Detection of Cytoplasmic DNA

Katryn J. Stacey, Adi Idris, Jasmyn A. Dunn, Greg Kelly, Matthew J. Sweet, David A. Hume, Ian L. Ross, Tara L. Roberts.

Institute for Molecular Bioscience and CRC for Chronic Inflammatory Disease, University of Queensland, St Lucia Qld 4067, Australia

Double stranded (ds) DNA can be detected within the cytoplasm in a TLR9-independent manner. Recent published work suggests that distinct dsDNA recognition pathways lead to either IFN? induction or inflammasome and subsequent caspase 1 activation and IL-1□secretion. No specific sequence motif appears to be required, although DNAs vary in their potency. We have identified a candidate receptor for dsDNA by affinity chromatography and mass spectrometry. Both the endogenous and recombinant protein are specific for dsDNA and do not bind single stranded DNA. The receptor shows profound localisation to DNA introduced into cells within 5 minutes. In addition, it is bound stably to transfected DNA, and can be extracted from cells in a DNA-bound form. siRNA knockdown shows that the receptor is involved in induction of IFN? in response to transfected DNA and adenoviral infection, but does not mediate the rapid activation of caspase 3 observed in transfected primary macrophages. In summary, a novel receptor for cytoplasmic dsDNA has been found, distinct from the published DAI/ZBP-1. The nature of this receptor will be revealed!

ABSTRACT 95

USAGE OF ADAPTOR MOLECULES IN TLR2/1 VERSUS TLR2/6 SIGNALLING

Sandra Santos-Sierra, Julia Kalnitski, Douglas T. Golenbock* and Philipp Henneke
Centre for Pediatrics and Adolescent Medicine, University Medical Centre, Freiburg, Germany
*Dept. of Medicine, University of Massachusetts Medical Center, Worcester, MA, USA

Diacylated and triacylated proteins from a variety of bacteria have been shown to trigger cytokine production in a TLR2/6 or TLR2/1 dependent fashion. The specific contribution of TLR1 and 6 to downstream signaling events remains unclear. Here, we studied the differential engagement of Mal, MyD88 and PI3-kinase in response to the di- and triacylated TLR agonists MALP2 and Pam3CysK4. Using WT, TLR2, MyD88 and Mal knock-out mouse macrophages and fibroblasts, we identified specific functional clusters related to the activation of the TLR2/1 versus the TLR2/6 receptor multimer.

ABSTRACT 96

PISA (PYHIN PROTEIN STIMULATING ASC) TRIGGERS INFLAMMASOME ACTIVATION IN RESPONSE TO CYTOSOLIC B-DNA

Veit Hornung[1], Franz Bauernfeind[1], Marie Charrel-Dennis[1], Eicke Latz[1] and Kate A. Fitzgerald[1].

[1]Division of Infectious Diseases and Immunology, Department of Internal Medicine, University of Massachusetts Medical School, Worcester, MA, USA

The innate immune system senses non-self nucleic acids via a highly specialized system of pattern recognition receptors: Pathogen-derived RNA is sensed via the Toll-like recpeptors TLR3, TLR7, TLR8 and the cytoplasmic RNA helicases LGP2, MDA5 and RIG-I, whereas non-self DNA can be detected via TLR9 and an yet to be defined cytosolic DNA receptor. While most studies have focused on these receptors and their role in initiating transcription of pro-inflammatory genes, little is known about receptor systems and pathways that regulate the proteolytic activation of caspase-1 – a key switch in the maturation of the pro-cytokines IL-1b, IL-18 and IL-33. Here we identify PISA (**P**YH**I**N protein **s**timulating **A**SC), a member of the four human PYHIN proteins, as a receptor required for the activation of the ASC/caspase-1 axis in response to cytosolic B-DNA. PISA, but none of the other PYHIN proteins, interacts with ASC resulting in the activation of NF-kB and caspase-1. Binding of ASC is mediated by the pyrin domain of PISA, whereas its HIN domain binds to DNA. Knocking down the expression of PISA results in a downmodulation of inflammasome activation triggered by cytosolic B-DNA, whereas an inverse picture is seen for the B-DNA mediated interferon response. Altogether, we have identified PISA, a novel receptor that senses cytoslic double-stranded DNA and interacts with ASC leading to the activation of caspase-1.

ABSTRACT 97

Positive and negative regulation of TLR signalling by protein acetylation

Matthew J. Sweet1, Kate Schroder1, Melanie Andrews1, Katharine M. Irvine1, Larisa Labzin1, Maria Halili1, David A. Hume2 and David P. Fairlie1

1Institute for Molecular Bioscience, University of Queensland, Australia, 2Roslin Institute, University of Edinburgh, UK.

Although histone deacetylases (HDACs) were first identified for their role in modifying histone proteins and chromatin architecture, it is now apparent that they deacetylate lysine residues on an array of non-histone substrates. Consequently, protein acetylation/deacetylation controls diverse cellular processes including signal transduction, transcription factor activation, and protein trafficking/secretion. In mammalian cells, 18 HDACs, encoded by distinct genes, have been grouped into 4 classes: class I HDACs (HDAC1, 2, 3, 8), class II HDACs (HDAC4, 5, 6, 7, 9, 10), class III HDACs (SIRT1-7) and a single class IV HDAC (HDAC11). We have used expression profiling, chromatin immunoprecipitation, gene over-expression and knock-down, as well as novel HDAC inhibitors to define distinct and opposing roles for members of the class I and class II HDAC families in regulating TLR-mediated macrophage activation. Our data provide new insights into TLR signalling pathways, as well as a molecular basis for the anti-inflammatory effects of HDAC inhibitors in several inflammatory disease models. Lysine acetylation/deacetylation represents an alternative molecular switch to protein phosphorylation for the control of signal transduction and cellular activation.

ABSTRACT 98

Divergence in TLR4 responses between primary human and mouse macrophages

Kate Schroder1, Katharine M. Irvine1, Geoffrey Faulkner1, Harukazu Suzuki2, Yoshihide Hayashizaki2, David A. Hume3 and Matthew J. Sweet1

1Institute for Molecular Bioscience, University of Queensland, Australia, 2Omics Science Center, RIKEN, Japan, 3Roslin Institute, University of Edinburgh, UK.

The ability to mount an effective innate immune response is clearly under strong evolutionary selection pressure from pathogen challenge. We hypothesized that evolution in gene regulatory sequences, allowing for variation in gene expression, contributes to divergence in innate immune responses between mouse and human. We used microarrays to compare the transcriptional responses of primary human and mouse macrophages to the archetypal inflammatory stimulus, lipopolysaccharide (LPS). Although a large cluster of genes was similarly regulated, there was also considerable divergence in gene expression between the species. The divergent regulation of many genes with key roles in innate immunity was validated, including members of the TLR signalling pathway (e.g. Cd14), transcription factors (e.g. Stat4), cytokines (e.g. Ccl20) and chemokines (e.g. Cxcl13). Macrophage promoters were defined by genome-wide identification of transcription start sites by deep CAGE (Cap Analysis of Gene Expression) in both species. The impact of promoter evolution on transcriptional regulation on a genome-wide scale is a focus of current investigation. This study provides the first systematic comparison of mouse and human gene expression in innate immunity, and gives insight into immune system evolution and the limits of mice as model organisms for human infection and inflammatory disease.

ABSTRACT 99

BLIMP1-mediated suppression of Innate Immunity.

Martina Severa 1, Daniel R. Caffrey 2, Victor Boyartchuk 3 and Katherine A. Fitzgerald1
1 Division of Infectious Diseases and Immunology, Department of Medicine, The University of Massachusetts Medical School, Worcester, MA; 2 Pfizer Global Research and Development, Cambridge, MA and 3 Program in Gene Function and Expression, The University of Massachusetts Medical School, Worcester, MA.

BLIMP1 is a transcriptional repressor, which is a master regulator of B cell, T cell and keratinocyte cell differentiation. Although first identified as a repressor of Interferon beta gene transcription, the role of BLIMP1 in innate immunity has not been addressed further. Here, we show that viral and bacterial pathogens induce high levels of BLIMP1 in macrophages. The gram-positive bacterium Listeria monocytogenes induces up-regulation of BLIMP1 requiring signaling from both TLR2 and the IL1R. In contrast to its role in other lineages, BLIMP1 does not appear to control myeloid cell development, but instead functions to suppress innate defenses. Using a conditional knockout of BLIMP1 in myeloid cells (Blimp1 CKO), we show that Blimp1 CKO mice are protected from lethal infection induced by L. monocytogenes. Gene expression profiling and chromatin immunoprecipitation analysis revealed that BLIMP1 negatively regulates transcription of the chemokine CCL8, also called monocyte chemoattractant protein 2 (MCP-2), by directly binding its promoter. Indeed, BLIMP1-deficient macrophages express high levels of CCL8 and as a result BLIMP1 CKO mice have higher numbers of circulating neutrophils in the bone marrow and peripheral blood. Consistent with a role for elevated CCL8 in host-defense, administration of recombinant CCL8 i.v. led to reduced bacterial loads in livers and spleens of L. monocytogenes-infected wild type mice. Collectively, these data reveal an important role for CCL8 in host-defenses and unveil a novel function for BLIMP-1 in fine-tuning innate immunity by downregulating genes such as CCL8.

ABSTRACT 100

Endosomal Toll-like receptor signalling is dependent on cysteine proteases

Fernando Sepulveda, Renaud Colisson, Sophia Maschalidi, Lea Heslop and Bénédicte Manoury
Institut Curie

Toll-like receptors control host immune response against pathogens through recognition of molecular patterns uniquely present in micro organisms. Their signalling is essential for the development of innate and adaptative immune response. They can be divided in two families: 1) TLR1, 2, 4 and 6 which sense the presence of lipids and proteins from bacteria and are expressed at the plasma membrane; 2) TLR3, 7 and 9 which engage DNA and RNA and are localised in the endosomes. Recently, an ER resident protein, UNC93B, has been shown to be essential for endosomal TLR signalling. Asparagine endopeptidase (AEP), an asparagine specific endoprotease belonging to the cysteine protease family was shown to be required for antigen degradation in the MHC class II pathway. We now report that dendritic cells (DCs) from AEP deficient mice show a significant decrease in TNFa and IL-6 production after endosomal TLR stimulation. As expected, phosphorylation of ERK is impaired in AEP KO mice following TLR engagement. This defect seems to be due to an impaired trafficking of these endosomal TLRs in DCs lacking AEP activity. Using a subcellular fractionnation approach, we identified UNC93B specifically upregulated in AEP-/- phagosomes. Current experiments are addressing the role of TLRs and UNC93B together with AEP. Furthermore, TLRs signalling is also impaired in other cysteines proteases KO cells. Altogether, these results highlight the important role of cysteine proteases in TLRs signalling and thus in DCs function.

ABSTRACT 101

MS and mutational analysis of IRAK-4 reveals that phosphorylation at T342, T345, S346, and T352 is required for IL-1 signaling

Vikram R Rao, Wayne Stochaj, Marshall M. Siegel, Quentin Wright, Quing Yao, Elizabeth Fox, Robert Czerwinski, John W. Cuozzo, Jean-Baptiste Telliez, Lih-Ling Lin, Kerry Kelleher, and Mark Stahl

Departments of Inflammation Signaling and Chemical and Screening Sciences Wyeth Discovery Research, Cambridge MA and Pearl River New York

Interleukin-1 receptor-associated kinase 4 (IRAK-4) is a key regulatory kinase of the innate immune system. Mice and humans with deletions or mutations of IRAK-4 show severely impaired interlukin-1 and Toll-like receptor signaling and are sensitive to several types of pathogens. Here we describe the expression and characterization of the phosphorylation and activation states of the kinase domain (aa 154-460) of IRAK-4. The kinase domain was expressed in SF21 cells and purified to homogeneity. Different phosphorylated forms of the enzyme were separated by high-resolution anion exchange chromatography and then analyzed by LCMS. The predominant form of the enzyme purified from Sf21 cells was phosphorylated at amino acids T342, T345, S346 and T352, all of which reside in the activation loop of IRAK4. Incubation of de-phosphorylated IRAK4 in the presence of Mg+2-ATP also produces enzyme phosphorylated at same activation loop residues. Catalytic studies with the de-phosphorylated enzyme revealed a brief but reproducible "lag" phase in the initial rate when compared to studies with the phosphorylated enzyme. Phosphospecific antibodies recapitulate the results of the LCMS on the purified protein. Immunoblot analysis of IRAK-4 from IL-1 stimulated HeLa cells shows IL-1 dependent phosphorylation in the IRAK 4 kinase activation loop. In addition, mutation of these amino acids to alanine dramatically reduces the activity of the kinase and overexpression of these mutants in HeLa cells blocks IL-1 induced signaling. This study demonstrates the importance of activation loop auto-phosphorylation in the regulation of IRAK-4.

ABSTRACT 102

Mechanistic Understanding of TLR Adaptor Protein Mal And Its Interaction With TRAF6

Brett Verstak, Paul Hertzog, Ashley Mansell

Monash Institute of Medical Research - Centre for Functional Genomics and Human Disease, Monash University, Melbourne, VIC, Australia.

CRC for Chronic Inflammatory Diseases.

The innate immune system defends the body from microbial infection by initiating inflammation, the extreme form of which is sepsis or septic shock. A greater understanding of the signaling pathways regulating the pro-inflammatory response to microbial infection is of crucial importance to developing new therapeutics to treat such diseases.
The mechanistic understanding of TLR signaling, which regulates the innate immune response to microbial pathogens, is an area that still needs to be fully elucidated. The unique genes that are expressed upon TLR recognition, despite utilising a common signaling pathway to activate the prototypic inflammatory transcription factor NF-kB, are an important focal point in elucidating TLR responses. It is the involvement of adaptor proteins such as Mal, and the emergence of recently described family members, that we believe dictate these unique gene subsets and which are responsible for the control of the pro-inflammatory response. We recently described a novel interaction between Mal and TRAF6, independently of the canonical TLR signaling pathway, suggesting a novel role for Mal in regulating NF-kB-dependent gene transcription via activation of the MAP kinase pathway and p65 transactivation. Mutation of this TRAF6 binding motif site within Mal (MalE190A) has already shown to inhibit both TLR2 and TLR4 mediated NF-kB-dependent gene expression.
The work so far has involved utilising both in vivo and in vitro methods to fully describe and characterise the unique interaction between Mal and TRAF6 and to understand the mechanism that underpins the mediation of downstream signaling events emanating from association of these two proteins. A direct protein interaction of Mal/TRAF6 has been observed using both recombinant proteins, combined with endogenous immunoprecipitation techniques. This interaction is not dependent on any of the described post-translational modifications of Mal to mediate association with TRAF6. Mal has also been found to co-localise with TRAF6 at the plasma membrane of cells via confocal immunohistochemistry analysis, whereas MalE190A mutant failed to display similar co-localisation.

Overall, a greater mechanistic understanding of Mal/TRAF6 association will make a significant contribution to elucidating innate immune responses to pathogen challenge, providing possible therapeutics for use in controlling chronic inflammatory responses.

ABSTRACT 103

ABIN-3, TAX1BP1 AND A20, COOPERATING INHIBITORS OF NF-kappaB SIGNALING

Lynn Verstrepen (1,2) , Marja Kreike (1,2), Mira Haegman (1,2), and Rudi Beyaert (1,2)
1 Department of Molecular Biology, Ghent University, Technologiepark 927, B-9052 Gent, Belgium

2 Department for Molecular Biomedical Research, Unit of Molecular Signal Transduction in Inflammation, VIB, Technologiepark 927, B-9052 Gent, Belgium

Stimulation of TLRs leads to the initiation of several signaling cascades including the NF-kappaB pathway. As NF-kappaB dependent gene expression mediates chronic inflammation, a predisposing factor for the development of pathologies such as neoplasia, cardiomyopathies, autoimmune disorders or cancers, a tight regulation of the NF-kappaB signaling pathway is crucial. Ubiquitination of signaling intermediates plays a key role in both positive and negative regulation of the NF-kappaB pathway. We recently showed that TAX1BP1 recruits the ubiquitin-editing protein A20 to both TRAF6 and RIP1, leading to their de-ubiquitination. Consistent with these observations, TAX1BP1-/- MEF cells demonstrate increased NF-kappaB activation in response to LPS, IL-1 and TNF. Interestingly, we found that TAX1BP1 also interacts with ABIN-3, an A20-binding inhibitor of NF-kappaB. ABIN-3/TAX1BP1 binding depends on the first ubiquitin-binding zinc finger (UBZ1) of TAX1BP1 and the ABIN homology domain (AHD) 3 in ABIN-3. Deletion of AHD3 or mutation of UBZ1 partially disrupts the ability of respectively ABIN-3 or TAX1BP1 to inhibit NF-kappaB activation. These data suggest that the potential of ABIN-3 and TAX1BP1 to inhibit NF-kappaB activation in response to TNF, IL-1 or LPS is dependent on their mutual interaction.

ABSTRACT 104

The adenosine A3 receptor modulates TLR-mediated cytokine production

Melanie R Power1,2, Leighanne C Gallington1, John Kelly1, Palanivel Velupillai1, Khoa Nguyen3, Marlene Jacobson4, Bruce Cronstein3 and Ofer Levy1,2

1Medicine/Infectious Diseases, Children's Hospital Boston, Boston, MA, USA;

2Harvard Medical School, Boston, MA, USA;

3New York University, New York, NY, USA and

4Merck Research Laboratories, West Point, PA, USA

Newborns are susceptible to microbial infections, yet the underlying mechanisms of this susceptibility are incompletely defined. Skewed neonatal mononuclear cell cytokine responses to TLR2 agonists, characterized by low TNF but high IL-6 production, are mediated by increased neonatal mononuclear cell sensitivity to adenosine, an endogenous purine metabolite that acts via the adenosine A3 receptor (A3R) to increase cellular cAMP concentrations (Levy et al. J Immunol 177(3): 1956). To further characterize mechanisms by which the distinct neonatal adenosine system regulates TLR-mediated cytokine production, we studied human cord blood monocytes in vitro and a neonatal mouse model in vivo. Adenosine pretreatment (1-10μM) of human newborn monocytes inhibits TLR2-mediated TNF but not IL-6 production (ELISA). Human newborn monocytes exhibit >2fold higher basal expression of A3R mRNA than adult peripheral monocytes (real-time PCR), corresponding to greater basal A3R protein expression (western blot). Upon intraperitoneal administration of TLR agonists, A3R-deficient newborns produced higher levels of TNF after TLR2 stimulation when compared to wild type (WT) newborns whereas TLR4 agonist induced equivalent responses. Upon intravenous administration of TLR agonists, A3R-deficient newborn mice demonstrated equivalent TLR4-mediated TNF and IL-6 production, but impaired TLR2-mediated IL-6 production when compared to WT newborns. Thus, neonatal cord blood monocytes express relatively high amounts of adenosine A3R that plays tissue-specific roles in selectively blunting TLR4-mediated TNF and IL-6 production and enhancing TLR2-mediated IL-6 production in whole blood in vivo.

ABSTRACT 105

TOLL-LIKE RECEPTOR 2 SIGNALING IN RESPONSE TO LIPOTEICHOIC ACID PREFERENTIALLY OCCURS AT THE PLASMA MEMBRANE, INDEPENDENT OF LIGAND INTERNALIZATION, AND IS FACILITATED BY BOTH CD36 AND CD14

Nadra J. Nilsen 1, Susanne Deininger 2, Unni Nonstad 1, Frode Skjeldal 3, Harald Husebye 1, Dmitrii Rodionov 3, Sonja von Aulock 2, Thomas Hartung 2,4, Egil Lien 5, Oddmund Bakke 3 and Terje Espevik 1

1 Norwegian University of Science and Technology, Institute of Cancer Research and Molecular Medicine, N-7489 Trondheim, Norway

2 University of Konstanz, Biochemical Pharmacology, 78457 Konstanz, Germany.
3 University of Oslo, Department of Molecular Biosciences, N-0371 Oslo, Norway
4 European Commission of Joint Research Centre, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods, 21020 Ispra, Italy
5 University of Massachusetts Medical School, Department of Medicine, Division of Infectious Diseases and Immunology, LRB-311, 364 Plantation Street, Worcester, MA, 01605, USA

Lipoteichoic acid (LTA) is a central inducer of inflammatory responses caused by Gram- positive bacteria, such as Staphylococcus aureus (S. aureus), via activation of Toll-like receptor 2 (TLR2). Localization of TLR2 in relation to its co-receptors may be important for function. This study explores the signaling, uptake and trafficking pattern of LTA in relation to expression of TLR2 and its co-receptors CD36 and CD14 in human monocytes. We found TLR2 expressed in early endosomes, late endosomes/lysosomes and in Rab-11-positive compartments, but not in the Golgi apparatus or endoplasmic reticulum (ER). Rapid internalization of fluorescently labeled LTA was observed in human monocytes, colocalizing with markers for early and late endosomes, lysosomes, ER and Golgi network. Blocking CD14 and CD36 with antibodies inhibited LTA binding and LTA-induced TNF release from monocytes, emphasizing an important role for both molecules as co-receptors for TLR2. Importantly, blocking CD36 did not affect TNF release induced by Pam3CysSK4 or LPS. Expression of CD14 markedly enhanced LTA binding to the plasma membrane and also enhanced NF-κB activation. LTA internalization, but not NF-κB activation, was inhibited in Dynamin-I K44A dominant negative transfectants, suggesting that LTA is internalized by receptor-mediated endocytosis, but that internalization is not required for signaling. In fact, immobilizing LTA, and thereby inhibiting internalization, resulted in enhanced TNF release from monocytes. Our results suggest that LTA signaling preferentially occurs at the plasma membrane, is independent of internalization, and is facilitated by both CD36 and CD14 as co-receptors for TLR2.

ABSTRACT 106

UBC6e, a ubiquitin conjugating enzyme confined to the endoplasmic reticulum, is essential for TNF production in response to TLR engagement

Britta Mueller, You-Me Kim, Melanie Brinkmann, and Hidde Ploegh

Whitehead Institute for Biomedical Research, Cambridge, MA 02142

Ubiquitination plays important regulatory roles by controlling protein half-life, intracellular localization of proteins and protein-protein interactions. Ubiquitin is attached to target proteins through the concerted action of three enzymes, a ubiquitin activating enzyme (E1), a conjugating enzyme (E2), and a ubiquitin ligase (E3) (1). The membrane bound, endoplasmic reticulum (ER)-localized E2 enzyme UBC6e is essential for the retrotranslocation and degradation of class I MHC heavy chains (2). UBC6e is phosphorylated upon the induction of ER stress (3), but the significance of this modification, as well as other targets of UBC6e are not known. UBC6e is highly expressed in dendritic cells. We here describe a role for UBC6e in toll like receptor (TLR) signaling. UBC6e is a downstream target of TLR signaling, as demonstrated by rapid and near quantitative phosphorylation of UBC6e in response to TLR engagement. Phosphorylation is absent in dendritic cells derived from the "triple D" (3d) mice, that display a defect in the intracellular TLR 3, 7, and 9 pathways due a mutation in the protein Unc93B (4-6). When UBC6e levels are reduced, dendritic cells fail to produce inflammatory cytokines upon TLR stimulation. These findings may provide an interesting link between TLR signaling and retrotranslocation of proteins from the ER and underscore the role of the ER in TLR signaling, as evident also from the involvement of Unc93B.

1. Hershko, A. & Ciechanover, A. (1998) Annu Rev Biochem 67, 425-79.
2. Mueller, B., Klemm, E., et al. & Ploegh, H. (2008) PNAS in press
3. Oh, R. S., Bai, X. & Rommens, J. M. (2006) J Biol Chem 281, 21480-90.
4. Tabeta, K., Hoebe, K., et al. & Beutler, B. (2006) Nat Immunol 7, 156-64.
5. Brinkmann, M. M., Spooner, E., Hoebe, K., Beutler, B., Ploegh, H. L. & Kim, Y. M. (2007) J Cell Biol 177, 265-75.

6. Kim, Y. M., Brinkmann, M. M., Paquet, M. E. & Ploegh, H. L. (2008) Nature 452, 234-8.

ABSTRACT 107

The TLR2-MyD88-NOD2-RICK signaling axis regulates a balanced pro- and anti-inflammatory cytokine response to a complex microbial product

Lilian O. Moreira1,2, Karim C. El Kasmi1,5¶, Amber M. Smith1,2, David Finkelstein3, Sophie Fillon1,5¶, Yun-Gi Kim4, Gabriel Núñez4, Elaine Tuomanen1 and Peter J. Murray1,2
1Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN , USA

2 Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN, USA
3 Hartwell Center for Biotechnology and Bioinformatics, St. Jude Children's Research Hospital, Memphis, TN, USA

4 Department of Pathology, University of Michigan, Ann Arbor, MI, USA

5 ¶University of Colorado Health Sciences Center, Denver, CO, USA

Systemic infection with Streptococcus pneumoniae is associated with a pathogenic inflammatory response to cell wall fragments (PnCW), composed primarily of peptidoglycan and teichoic acids, that are shed during cell division and autolysis. We found that PnCW induced prodigious secretion of IL-10 from macrophages that was dependent on TLR2, and in part on NOD2, a cytoplasmic NLR protein. By contrast, inflammatory cytokine production was dependent on TLR2 but independent of NOD2. IL-10 secretion in response to cell wall fragments was also dependent on RICK (RIP2), a kinase associated with NOD2, and Myd88 but independent of the ERK pathway previously shown to be essential for zymosan-mediated production of IL-10 via TLR2. The reduction of IL-10 secretion by cell wall-activated NOD2-deficient cells translated into diminished downstream effects on IL-10-regulated autocrine-paracrine target gene expression. Since NOD2 is linked to aberrant immune responses in Crohn's Disease patients bearing mutations in NOD2, the temporal and quantitative effects of the TLR2-NOD2-RICK pathway on IL-10 secretion may affect homeostatic control of immune responses to gram-positive bacteria.

ABSTRACT 108

The dynamics and mechanisms of interleukin-1α and β nuclear import

Nadia M. Luheshi, Nancy J. Rothwell and David Brough

Faculty of Life Sciences, Michael Smith Building, University of Manchester, Oxford Road, Manchester, M13 9PT, U.K.

Pro-inflammatory members of the interleukin-1 (IL-1) family of cytokines (IL-1α and β) are important mediators of host defense responses to infection, but can also exacerbate the damaging inflammation that contributes to major human diseases. IL-1α and β are produced by cells of the innate immune system, such as macrophages, and act largely after their secretion by binding to the type I IL-1 receptor (IL-1R1) on responsive cells. There is evidence that IL-1α is also a nuclear protein that can act intracellularly. In this study we report that both IL-1α and β produced by microglia (CNS macrophages) in response to TLR4 stimulation are distributed between the cytosol and the nucleus. Using IL-1-β-galactosidase, and IL-1-GFP chimeras (analyzed by FRAP), we demonstrate that nuclear import of IL-1α is exclusively active, requiring a nuclear localization sequence and Ran, whilst IL-1β nuclear import is entirely passive. Despite utilizing different mechanisms of nuclear import, we have discovered that nuclear localization of both cytokines is regulated by local cell density. Inside the nucleus we present preliminary evidence indicating that IL-1α interacts with elements of the RNA splicing apparatus in an energy dependent fashion. These data provide valuable insights into the intranuclear actions of cytokines and the dynamic regulation of intracellular cytokine trafficking.

ABSTRACT 109

TLR-2 IS A NEGATIVE REGULATOR OF TH17 CELLS AND TISSUE PATHOLOGY IN A PULMONARY MODEL OF FUNGAL INFECTION

Loures, FV & Calich, VLG

Instituto de Ciências Biomédicas, USP, São Paulo, Brasil

The Paracoccidioidomycosis (PCM) is the most important deep mycosis in Latin America. The role of TLR2 in PCM was never investigated. The aim of our work was to characterize the involvement of TLR2 in murine PCM using TLR2-/- and C57BL/6 (WT) mice. In vitro cultivated macrophages (mo) were challenged with yeasts and fungicidal activity, cytokines and nitric oxide (NO) production assessed 48h later. Mice were infected by the intratracheal route with 1x106 yeasts. After 48h, 2 and 11 weeks, the mice were sacrificed and severity of infection was evaluated by CFU counts, presence of NO and cytokines in lung homogenates. Besides, flow cytometric analysis of lung infiltrating leucocytes. We verified that mo from TLR2-/- mice secreted lower levels of NO, MCP-1 and IL-10 and have a decreased ability to interact with fungus than those from WT mice resulting in decreased number of viable yeasts recovered 48h postinfection. In vivo, diminished fungal burdens and NO levels were detected in the lungs of TLR2-/- mice at all postinfection periods assayed. Augmented levels of IL-17 were detected in the lungs of TLR2-/- mice by 48h postinfection. At week 2, higher amounts of IL-17 and IL-23 associated with decreased levels of MCP-1 were found. At week 11, increased IL-23 was associated with decreased of MCP-1, IL-10 and IL-12. At both postinfection periods, increased numbers of PMN cells were observed in the lungs of TLR2−/−. By week 2 decreased number of mo associated with augmented numbers of lymphocytes were recovered from TLR2-/- mice relative to WT mice. Lungs of TLR2-/- mice presented decreased number of activated CD4+ and CD8+ T cells at weeks 2 and 11 although lower numbers of regulatory CD4CD25+FoxP3+ T cells were detected in this strain by week 11. Equivalent patterns of mortality and lung lesions were observed in both mouse strains. Conclusion: absence of TLR2 results in less severe infection but increased inflammatory response and lung pathology caused by PMN and TH17 cells.

ABSTRACT 110

Oxidized phospholipids inhibit phagocytosis via a PKA dependent mechanism

Ulrich Matt, Omar Sharif, Tanja Furtner, Karin Stich and Sylvia Knapp: Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria
& Dept of Internal Medicine 1, Division of Infectious Diseases and Tropical Medicine, Medical University Vienna, Austria

Phospholipids that are oxidized at sites of inflammation have been shown to interfere with the ability of LPS to bind to the TLR4 receptor complex, thereby protecting mice from LPS induced lethality. We could subsequently show that these oxidized lipids (OxPL) impaired outcome in gram negative sepsis caused by viable bacteria due to their ability to suppress phagocytosis by macrophages in vivo. Consequently we then aimed to characterize and analyse the molecular mechanism of this inhibition caused by OxPL.

OxPL consistently inhibited the phagocytic and macropinocytotic capacity of RAW 264.7 macrophages in a dose dependent and reversible manner. Internalization of pathogens as well as macropinocytosis requires the active modulation of the plasma membrane which is driven by actin polymerization. We found an actin dependent cell spread upon stimulation with OxPL in both primary peritoneal macrophages and RAW cells. Pharmacologic inhibitor studies disclosed that the OxPL induced actin spread and inhibiton of phagocytosis was independent of PI3kinase, MAPK and the smallGTPases Rho, Rac or Cdc42. Instead, using a dual approach that involved the use of specific Protein Kinase A (PKA) inhibitors and short hairpin RNAi (shRNAi) to the catalytic subunit of PKA, we revealed that the OxPL associated impairment in phagocytosis and alterations in cellular actin morphology could be prevented by reduction of PKA activity. However, although we observed OxPL to directly activate PKA in macrophages, we could not mimic these effects by increasing cellular cAMP levels nor by adding PKA-specific cAMP analogues.

Conclusively we found that OxPL potently inhibit phagocytosis via a PKA-specific mechanism, which can not be mimicked by increased cAMP levels in macrophages.

ABSTRACT 111

LIPOPOLYSACCHARIDE-INDUCED NEUTROPHIL MIGRATION IN TOLL LIKE RECEPTOR (TLR) 4-DEFICIENT MICE IS MEDIATED BY A CELLULAR MECHANISM AND NOT BY COMPLEMENT ACTIVATION

Esther B. Florsheim and Momtchilo Russo

Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, Brazil

Bacterial lipopolysaccharides (LPS) are potent inflammatory agents. It is known that LPS triggers immune responses through Toll-like receptor (TLR) 4 or via complement system activation and that both LPS-activated pathways induce neutrophil migration. We found that TLR4-deficient/mutant (C3H/HeJ) mice injected with i.p. LPS mounted an inflammatory response characterized by intense neutrophil migration. This data lead us to investigate the involvement of the complement system in TLR4-deficient mice. For this, animals were treated with cobra venom factor (CVF) to deplete complement components (C3-C9) by activation. Although C3 levels after CVF treatment were bellow 10%, CVF-treated animals exhibited a similar LPS-induced neutrophil migration to that obtained in untreated animals. We conclude that the LPS-induced neutrophil migration is complement-independent. Next, we examined whether LPS-primed peritoneal cells could induce neutrophil migration. TLR4 deficient mice received LPS i.v. and shortly after peritoneal cells were harvested and transferred to naïve donors. Peritoneal washings revealed that LPS-primed peritoneal cells induced a strong neutrophil migration. Our results indicate that the LPS-induced neutrophil migration in TLR4-deficient mice involves peritoneal cells but not the complement system. The peritoneal cell type responsible for LPS-induced neutrophil migration is under investigation.

ABSTRACT 112

Spatio-temporal regulation of TLR3 trafficking pathway towards the endosomes.

Alejandra Garcia Cattaneo and Philippe Benaroch.

INSERM U653, Transport Intracellulaire et Immunité, Institut Curie, Paris – France

Toll-Like receptors (TLRs) play an important role in the innate and adaptive immune response. They are involved in the recognition and processing of Pathogen-Associated Molecular Patterns (PAMPs) from virus, bacteria and other microbial agents. TLR3 recognizes double-stranded RNA (dsRNA), and is usually located in endosomes, although in some cells it is expressed at the plasma membrane. TLR3 has been shown to interact with its signalling partners in endosomal compartments and its activation is sensitive to Chloroquine, suggesting that signalling probably occurs in acidic compartments. TLR3 recruitment to endosomes may occur directly from the ER or it may require the secretory pathway, involving the Golgi apparatus, which might or not include trafficking via the plasma membrane.

Our aim is to study the spatio-temporal regulation of the TLR3 trafficking along the endocytic pathway.

We are carrying out two approaches to address this question. First, we developed amino and carboxyl tagged TLR3 quimeras and followed their subcellular localization after a stimulus with poly I:C, a synthetic double strand RNA analogue and we measured NF-kB translocations into the nucleus as a functional assay of TLR3. Second, we are evaluating the contribution of the secretory pathway in TLR3 trafficking by studying TLR3 signalling upon inactivation of the Golgi apparatus. This was performed by functional ablation of the Golgi in stably transfected cell lines carrying the Golgi protein Manosidase II coupled to HRP or using chemical inhibitors of this organelle, such as Brefeldin A. We are currently studying TLR3 activation in cells with non-functional Golgi.

ABSTRACT 113

Group A Streptococcus activates MyD88-dependent signalling and type I interferon production without involvement of TLR2, TLR4 and TLR9

Nina Gratz1, Maria Siller1, Barbara Schaljo1, Zaid A. Pirzada1, Irene Gattermeier1, Ivo Vojtek1, Carsten J. Kirschning2, Hermann Wagner2, Shizuo Akira3, Emmanuelle Charpentier1 and Pavel Kovarik1

1Max F. Perutz Laboratories, University of Vienna, Dr. Bohr-Gasse 9, 1030 Vienna, Austria 2Technical University of Munich, 81675 Munich, Germany

3Department of Host Defense, Osaka University, Osaka 565-0871, Japan

Bacterial pathogens are recognized by the innate immune system through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs). Engagement of TLRs triggers signalling cascades that launch innate immune responses. Activation of MAPKs and NF-kB, elements of the major signalling pathways induced by TLRs, depends in most cases on the adaptor molecule MyD88. In addition, Gram-negative or intracellular bacteria elicit MyD88-independent signalling that results in production of type I interferon (IFN). Here we show that in mouse macrophages the activation of MyD88-dependent signalling by the extracellular Gram-positive human pathogen Group A Streptococcus (GAS; Streptococcus pyogenes) does not require TLR2, a receptor implicated in sensing of Gram-positive bacteria, or TLR4 and TLR9. Redundant engagement of either of these TLR molecules was excluded by using TLR2/4/9 triple-deficient macrophages. We further demonstrate that infection of macrophages by GAS causes interferon regulatory factor 3 (IRF3)-dependent, MyD88-independent production of type I interferon (IFN). Surprisingly, IFN is also induced by GAS lacking SLO and SLS, the genes encoding cytolysins that were shown to be required for IFN production in response to other Gram-positive bacteria. Our data indicate that (i) GAS is recognized by a MyD88-dependent receptor other than any of those typically used by bacteria, and (ii) the IFN induction by GAS resembles IFN induction by bacteria requiring cytoplasmic escape yet it is independent of the GAS cytolysins.

ABSTRACT 114

Toll like receptor 7 signaling in various species

H Brinkmann, A Kjerrstrom, V Richard, C Bryant

Cambridge University

The toll-like receptor (TLR) signaling pathway is known to be one of the front-line subsystems against invasive microorganisms for both innate and adaptive immunity. In humans there are 10 different TLRs (TLR1-10) whereas in rodents TLRs 11, 12, and 13 have also been identified. A number of pathogen associated products have been demonstrated to be ligands for TLRs 1-11. The TLRs and their signaling pathways have been evolutionarily well conserved in both invertebrates and vertebrates. There are differences, however, in the cytokine and anti-viral genes induced after activation of TLRs 7 and 8. There are also differences in the ligands that activate TLR 7 and 8 between rodents and humans.

There is a considerable body of data on human TLR signalling, but there are still considerable gaps in our knowledge about TLR activation and subsequent signalling cascades in species like rodents or monkeys. In this study we used two different specific ligands for TLR 7 and compared their activity in human, mouse, rat and rhesus monkey PBMC assays. In rodents comparative analysis was performed with two different mouse and rat strains (Balb/c + C57Bl/6 and SD+Wistar respectively). We compared the cytokine expression and antiviral gene expression in response to TLR7 activation in these species.

ABSTRACT 115

The C-Terminal Regulatory Domain Is the RNA 5′-Triphosphate Sensor of RIG-I

Sheng Cui, Katharina Eisenächer, Axel Kirchhofer, Krzysztof Brzózka, Karl-Klaus Conzelmann, Anne Krug, and Karl-Peter Hopfner

Gene Center, Ludwig-Maximilians-University of Munich, Feodor-Lynen-Strasse 25, 81377 Munich, Germany

The ATPase RIG-I senses viral RNAs that contain 5′-triphosphates in the cytoplasm. It initiates a signaling cascade that activates innate immune response by interferon and cytokine production, providing essential antiviral protection for the host. The mode of RNA 5′-triphosphate sensing by RIG-I remains elusive. We show that the C-terminal regulatory domain RD of RIG-I binds viral RNA in a 5′-triphosphate-dependent manner and activates the RIG-I ATPase by RNA-dependent dimerization. The crystal structure of RD reveals a zinc-binding domain that is structurally related to GDP/GTP exchange factors of Rab-like GTPases. The zinc coordination site is essential for RIG-I signaling and is also conserved in MDA5 and LGP2, suggesting related RD domains in all three enzymes. Structure-guided mutagenesis identifies a positively charged groove as likely 5′-triphosphate-binding site of RIG-I. This groove is distinct in MDA5 and LGP2, raising the possibility that RD confers ligand specificity.

Inflammatory processes in health and disease

ABSTRACT 116

TLR-mediated inflammatory responses of the intestine

Maria Lawrenz, Sonja Dullat, Alexander Visekruna and Ulrich Steinhoff

Max-Planck Institute for Infection Biology, Charitéplatz 1, 10117 Berlin

The two major forms of inflammatory bowel diseases (IBD), ulcerative colitis (UC) and Crohn´s disease (CD) are distinguished by their inflammatory mechanisms. We have recently shown, that in contrast to UC, inflamed intestinal tissue of CD patients is characterized by enhanced levels of NF-kappa B. As UC and CD patients reveal enhanced reactivity against microbial products of the endogenous flora, we wondered whether this is reflected by activation of Mitogen activated protein kinases (MAPK). We thus compared the expression of P-ERK in the epithelium and cells of the lamina propria. We found that CD but not UC patients express high levels of P-ERK. Although we assumed that microbial patterns of the endogenous flora are responsible for P-ERK induction, TNF-alpha but not LPS triggered MAPK in the epithelial cell line HT-29. In contrast, in bone-marrow derived macrophages (BMM), P-ERK was induced by LPS after prestimulation with IFN-gamma. Further, activation of ERK in BMM was TPL-2 dependent as treatment of cells with proteasome inhibitors abrogated P-ERK induction. We are currently analyzing the effects of various TLR-ligands to induce MAPK via the apical or basal site of polarized epithelial cells. Further, we are investigating the proteasome as therapeutic target in TLR-driven inflammatory responses.

ABSTRACT 117

Commensal microbiota is fundamental for the development of inflammatory pain

Flavio A. Amaral1, Daniela Sachs1, Vivian V. Costa1, Caio T. Fagundes1, Daniel Cisalpino1, Thiago M. Cunha2, Sergio H. Ferreira2, Fernando Q. Cunha2, Tarcilia A. Silva3, Jacques R. Nicoli4, Leda Q. Vieira1, Danielle G. Souza4, and Mauro M. Teixeira1
Departamento de 1Bioquımica e Imunologia e 4Microbiologia, ICB-UFMG;

Departmento de Patologia Oral, FO-UFMG, 2Departamento de Farmacologia, FMRP-USP – Brazil

The ability of an individual to sense pain is fundamental for its capacity to adapt to its environment and to avoid damage. The sensation of pain can be enhanced by acute or chronic inflammation. In the present study, we have investigated whether inflammatory pain, as measured by hypernociceptive responses, was modified in the absence of the microbiota. To this end, we evaluated mechanical nociceptive responses induced by a range of inflammatory stimuli in germ-free and conventional mice. Our experiments show that inflammatory hypernociception induced by carrageenan, lipopolysaccharide, TNF-α, IL-1β, and the chemokine CXCL1 was reduced in germfree mice. In contrast, hypernociception induced by prostaglandins and dopamine was similar in germ-free or conventional mice. Reduction of hypernociception induced by carrageenan was associated with reduced tissue inflammation and could be reversed by reposition of the microbiota or systemic administration of lipopolysaccharide. Significantly, decreased hypernociception in germ-free mice was accompanied by enhanced IL-10 expression upon stimulation and could be reversed by treatment with an anti-IL-10 antibody. Therefore, these results show that contact with commensal microbiota is necessary for mice to develop inflammatory hypernociception. These findings implicate an important role of the interaction between the commensal microbiota and the host in favoring adaptation to environmental stresses, including those that cause pain.

ABSTRACT 118

Regulation of TLR-mediated eicosanoid production in macrophages by phospholipases A2

Alma M. Astudillo, Violeta Ruipérez, María A. Balboa and Jesús Balsinde

Institute of Molecular Biology and Genetics, Spanish National Research Council and University of Valladolid School of Medicine, Valladolid, Spain

Arachidonic acid is released in macrophage-like cell lines activated through Toll-like receptors (TLRs) involving the action of phospholipases A2, leading to eicosanoid generation. However, the signaling mechanisms of distinct TLR-dependent activation of phospholipases A2 remain unknown. We previously reported that cPLA2α regulates group V PLA2 expression, which in turn induces COX-2 production in TLR4-stimulated P388D1 macrophage-like cells. In the present study, we have used 10 different agonists of Toll-like receptors to define the role of PLA2 in arachidonic acid release and eicosanoid generation. Treatment of P388D1 and Raw 264.7 cells with the selective inhibitor of cPLA2α, pyrrophenone, and the cell-permeable inhibitor of sPLA2, scalaradial, showed that the contribution of these PLA2 to arachidonic acid release depended on which TLR was activated. No changes in the level of arachidonic acid release were observed when the iPLA2 inhibitor BEL was used. In this study, we identify that phosphorylation of ERK1/2 and cPLA2 was blocked by scalaradial depending on the TLR stimulated which provides a TLR-dependent regulation mechanism of group V sPLA2 and group IV cPLA2. Moreover, ERK1/2 phosphorylation was also blunted by antisense oligonucleotide designed to specifically inhibit expression of group V PLA2. Collectively, the results suggest a model whereby sPLA2-V contributes to arachidonic acid release by amplifying cPLA2 activation through phosphorylation of ERK1/2.

ABSTRACT 119

The Nalp3 inflammasome is essential for the development of silicosis

Suzanne L. Cassel1, Stephanie C. Eisenbarth2, Shankar S. Iyer1, Jeffrey J. Sadler1,

Richard A. Flavell2, and Fayyaz S. Sutterwala1

1Department of Internal Medicine, University of Iowa, Iowa City, IA, USA, 2Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA.

Inhalation of crystalline silica and asbestos is known to cause the progressive pulmonary fibrotic disorders silicosis and asbestosis respectively. Although alveolar macrophages are believed to initiate these inflammatory responses, the mechanism by which this occurs has been unclear. Here we show that the inflammatory response and subsequent development of pulmonary fibrosis following inhalation of silica is dependent on the Nalp3 inflammasome. Stimulation of macrophages with silica results in the activation of caspase-1 in a Nalp3-dependent manner. Macrophages deficient in components of the Nalp3 inflammasome were incapable of secreting the proinflammatory cytokines interleukin (IL)-1beta and IL-18 in response to silica. Similarly, asbestos was capable of activating caspase-1 in a Nalp3 dependent manner. Activation of the Nalp3 inflammasome by silica required both an efflux of intracellular potassium as well as the generation of reactive oxygen species. This study demonstrates a key role for the Nalp3 inflammasome in the pathogenesis of pneumoconiosis.

ABSTRACT 120

The B Cell Receptor governs the subcellular location of TLR9 leading to hyperresponses

Akanksha Chaturvedi, Dr. Susan Pierce

NIAID/NIH

B cell receptor (BCR) and Toll-like receptor 9 (TLR9) has been shown to synergize in response to DNA-containing antigens. Synergistic signaling between the two has been implicated to play a central role in many systemic autoimmune diseases. Recently we described a novel mechanism underlying the synergistic signaling between the BCR and TLR9 in response to DNA-containing antigens that required the active recruitment of TLR9-containing endocytic vesicles to the multivesicular, multilamellar autophagosomes. We showed that although the BCR initiates signaling at the B cell surface in response to DNA-containing antigens, it continues to signal as it is internalized from the cell surface into the autophagosomes. The internalized BCR signaled through a phospholipase D-dependent pathway to recruit TLR9-containing endosomes to autophagosome via microtubular network. This recruitment of was necessary for hyper-activation of MAP kinases. We now extend these observations providing molecular details to the role of autophagy in TLR9 recruitment to compartments positive for both LAMP1 and LC3. We also show that TLR9 recruitment is independent of TLR9 signaling but dependent on the initiation of autophagy. These results provide evidence for a new mechanism underlying a fundamental property of the immune system, namely the synergistic interaction between receptors of the innate and adaptive immunity.

ABSTRACT 121

Role of CD14 in the recognition of smooth and rough LPS in the lung

A.F. de Vos, A. Anas, J.W. Hovius and T. van der Poll

Center for Experimental & Molecular Medicine, Acadmic Medical Center, Amsterdam, The Netherlands

Introduction: CD14 is essential for activation of the TLR4-MD2 complex in vitro by the smooth form but not rough form of LPS. The aim of our study was to investigate the role of CD14 in the distinction of different LPS chemotypes in vivo during lung inflammation.
Methods: CD14 knockout (KO) and wild-type (WT) mice were intranasally inoculated with smooth or rough LPS (0.1, 1.0 and 10 μg). Lung inflammation was quantified by determining differential cell counts and TNF-α concentrations in bronchoalveolar lavage fluid obtained 6h later.

Results: Lung inflammation was attenuated in CD14 KO mice treated with 0.1 μg smooth LPS as indicated by reduced neutrophil influx and lower TNF-α concentrations. Surprisingly, CD14 KO mice demonstrated enhanced lung inflammation upon administration of 10 μg smooth LPS. Lung inflammation induced by rough LPS was also partly CD14 dependent: whereas CD14 KO mice displayed less neutrophil influx upon inoculation with 0.1 μg LPS, these mice showed enhanced TNF-α release after administration of 1.0 and 10 μg LPS. Intranasal administration of soluble CD14 to CD14 KO mice treated with 0.1 μg LPS partially reversed the inflammatory response towards the response observed in WT mice.

Conclusions: Membrane-bound CD14 facilitates the effects of low dose LPS in the lung in vivo, whereas this receptor inhibits the effects of higher LPS doses. The ability of CD14 to discriminate between smooth and rough LPS in vitro is less clear in vivo during lung inflammation.

ABSTRACT 122

Toll-like receptor 7 stimulation activates directly Natural Killer T cells: implications in allergic asthma

Françoise Grela, Emilie Bardel, Aude Aumeunier, Michel Dy, André Herbelin, Nathalie Thieblemont
CNRS UMR 8147, Université Paris Descartes, Faculté de Médecine, Hôpital Necker, Paris, France

According to the hygiene hypothesis, allergic diseases could be prevented by infections. Experimental studies have validated that Toll-like receptor (TLR) stimulation may prevent allergic asthma. We show here that triggering TLR7 with R848 activates the invariant Natural Killer T (iNKT) cells to produce in vivo both IL-4 and IFN-γ. We validate the relevance of this observation by showing, in an experimental allergic model, that R848-activated iNKT cells drives to asthma protection. Mechanistically, we provide evidence that iNKT cells, which express TLR7, can directly respond to R848 in the presence of IL-12 in vitro by producing high levels of IFN-γ without TCR engagement and in the absence of dendritic cells. This work provides the demonstration of a new mechanism of activation of iNKT cells in response to a TLR agonist.

ABSTRACT 123

MICROVESICLES ACTIVATE TOLL-LIKE RECEPTORS AND CONTRIBUTE TO INFLAMMATORY PROCESSES

Mateja Manček-Keber1, Mojca Frank2, Blaz Rozman2, Roman Jerala1

1National Institute of Chemistry, Department of Biotechnology, Ljubljana, Slovenia
2Department of Rheumatology, University Medical Centre Ljubljana, Ljubljana, Slovenia

Microvesicles (MVs) shed from the cell plasma membrane of healthy and damaged cells under normal physiological conditions, such as cell activation or growth and when submitted to stress conditions, such as hypoxia or oxidative injury. Their level in peripheral blood is elevated in patients with chronic inflammatory diseases (atherosclerosis, thromboembolic events) or suffering from infection. Toll-like receptors (TLRs) are involved in innate immunity as receptors, which recognize microbial substances. Activation of TLRs proceeds through dimerization of receptors leading to proinflammatory cytokine and type I IFN expression. Lately TLRs are being also recognized as receptors involved in sterile inflammation, such as atherosclerosis, rheumatoid arthritis, but a mechanism of action and endogen ligands are still mostly unknown. Our results show MVs, isolated from human plasma of patients with certain inflammatory diseases, are able to induce TLR dependent NF-κB activation and IL-8 production, on the other hand MVs from healthy patients had only minor effect. The difference in activation was not due to the number of MVs as their concentration was determined using flow cytometer. We believe the composition and origin of phospholipids, which constitute MVs, as well their oxidative status determine their activity, as preliminary results show that only MVs from certain diseases and with increased content of peroxidized phospholipids support this phenomenon.

ABSTRACT 124

Nasal CpG Administration Induces Local Airway Inflammation

Anne Månsson and Lars Olaf Cardell

Laboratory of Clinical and Experimental Allergy Research, Department of Otorhinolaryngology, Malmö University Hospital, Lund University, Malmö, Sweden
Department of Otorhinolaryngology, Karolinska Institutet, Huddinge, Sweden

Allergic rhinitis (AR) is an inflammatory reaction not restricted to a single local compartment, but rather involving both the upper and lower airways. Allergens are not always the sole trigger of inflammations of this sort as respiratory infections might also cause exacerbations of AR. CpG motifs are present at high frequency in bacterial and viral DNA and induce innate immunity via TLR9. The aim of this study was to examine the effects of intranasal CpG exposure, as a mimic of upper airway infection, in healthy subjects, patients with AR, and in AR patients treated with allergen-specific immunotherapy. Leukocytes in nasal lavage (NAL) and nasal airway resistance were assayed 30 min, 6, 24 and 48 h after CpG provocation along with measurements of nasal and pulmonary nitric oxide (NO) levels. 30 min after CpG challenge, increased nasal resistance and nasal NO production could be recorded, reaching a maximum after 6 h. NAL leukocytes were found to be increased 24-48 h after provocation. In addition, IL-8 levels in NAL were altered in response to CpG administration. Lastly, it appeared as if the various patient groups reacted differently to nasal CpG administration, with the strongest response in the allergic subjects. In conclusion, the present study demonstrates that nasal CpG exposure induces an upper airway inflammation. This suggests that viral or bacterial respiratory infections might aggravate the disease condition in patients with AR.

ABSTRACT 125

Anti-inflammatory roles for TBK1 in innate and adaptive immunity

Marchlik, E?, S Marusic?, P Thakker?, M Senices?, L Lowe?, N Stedman?, V Roberts?, J Pelker?, N Goutagny*, W Kuang§, GR Askew§, T Simon¶, R Pfeifer?, D Young?, KA Fitzgerald*, L-L Lin?, and JP Hall?

From the Departments of ?Inflammation, §Biological Technologies, ?Exploratory Drug Safety, and ¶BioResources, Wyeth Research, Cambridge, Massachusetts, and the *Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, Massachusetts

TBK1 is an IKK homologue that is critical for immunity against viruses and other microbial pathogens that activate TLR3- and TLR4-dependent signaling pathways. The activation of these receptors culminates in phosphorylation of the IRF3/7 transcription factors by TBK1, an event that is required for IRF dimerization and nuclear entry and the subsequent induction of type I interferon expression. To address the role of TBK1 in inflammatory diseases, mice were generated that harbor two copies of a hypomorphic Tbk1 allele. This allele encodes a TBK1 protein that is both catalytically inactive and expressed at very low levels. As expected, TBK1-deficient macrophages exhibit reduced LPS- (TLR4) and poly(I:C)- (TLR3) induced IFN-beta and RANTES production. Naïve TBK1-deficient mice have increased circulating lymphocytes and monocytes, increased inflammatory cell infiltrates in multiple organs, slight to mild splenic lymphoid hyperplasia, increased splenic extramedullary hematopoiesis, and abnormal hair coats. In an EAE model disease developed earlier and with greater severity in TBK1-deficient animals compared to controls, and a greater proliferation of TBK1-deficient lymph node cells was observed upon ex vivo re-stimulation with antigen. In a model of LPS-induced shock/death TBK1-deficient mice succumbed much more rapidly than their wild-type counterparts. We are investigating the cytokines in our TBK1-deficient mice that might mediate these disease phenotypes. Our data reveal novel anti-inflammatory roles for TBK1 in innate and adaptive immunity.

ABSTRACT 126

TLR-9 senses human fetal DNA

Andrea S. Nugent, MB,BCH*#, Sean Daly,MD *, John O'Leary, PhD* and Luke A.J. O'Neill# PhD

#School of Biochemistry and Immunology, Trinity College Dublin, *The Coombe Women and Infants University Hospital, Dublin, Ireland

Toll-like receptor 9 (TLR9) senses CpG motifs in DNA. These are more common in bacterial and viral DNA and TLR9 has been shown to have an important role in the sensing of various pathogens during host defence. Recent studies have suggested that abnormal epigenetic changes in CpG-rich islands in fetal mammalian DNA might contribute to the high rate of early pregnancy loss. It is also well known that higher concentrations of free fetal DNA are found in mothers who deliver prematurely. We therefore wished to test the hypothesis that fetal DNA might be sensed by TLR9, and might provoke an inflammatory response, which in turn could lead to preterm labour and early pregnancy loss.

Our investigations have shown that fetal DNA added to the Namalwa B cell line (which expresses high levels of TLR9) or PMBCs could rapidly activate NF-kappaB (as measured by increased I-kappaB degradation) and also p38 activation, both of which are typical TLR9 signals. We also found increased production of the pro-inflammatory cytokines IL6 and TNF. The effects of fetal DNA were more potent than either synthetic CpG -containing oligonucleotides, or adult DNA. We also found that chloroquin, which has been shown to inhibit TLR9 signaling, blocked the effect. Finally, we have found that the effect of fetal DNA on cytokine induction is significantly reduced in TLR9-deficient bone marrow-derived macrophages. We have therefore made the novel observation that TLR-9 senses fetal DNA and facilitates an inflammatory reaction. This may then contribute to preterm labour or early pregnancy loss

ABSTRACT 127

Prolonged Protein Kinase C phosphorylation following macrophage stimulation in Crohn's Disease - a possible link to apoptosis?

Palmer CD, Rahman FZ, Hayee BH, Bloom SL*, Smith AM & Segal AW
Centre for Molecular Medicine, University College London and *Department of Gastroenterology, UCL Hospitals NHS Foundation Trust, London, UK

Crohn's Disease (CD) is characterised by chronic granulomatous inflammation of the gastrointestinal tract. Although the aetiology of CD remains unknown, strong evidence suggests that microbial components are involved in its pathogenesis. Several hypotheses propose an exacerbated immune response in the onset/perpetuation of the disease. Our laboratory has previously shown a failure of acute inflammation in CD that operates at the level of the macrophage. We have further evidence showing that this predisposes CD patients to ineffective clearance of bacteria. Subsequent persistence of antigenic material could secondarily induce the T cell-driven granulomatous chronic inflammation that is characteristic of active CD. Microbial antigens stimulate inflammatory cytokine release via pattern recognition receptors such as the Toll like receptors (TLRs). We show here that CD macrophages stimulated with bacteria or TLR ligands exhibit an abnormal inflammatory response. Protein kinase C (PKC) isoforms are critically important in both TLR and apoptotic signalling pathways. Activation via TLRs resulted in prolonged phosphorylation of PKC in CD macrophages. PMA stimulation also resulted in increased PKC phosporylation and in addition identified a significantly impaired apoptotic response in CD macrophages. Taken together, our data shows that CD macrophages are severely functionally impaired, as they display abnormal TLR-induced inflammatory responses and are resistant to PKC-induced apoptotic cell death compared to healthy controls.

ABSTRACT 128

TLR-4 IS INVOLVED IN TUBULO-INTERSITIAL INJURY FOLLOWING UNILATERAL URETHER OBSTRUCTION

W.P.C. Pulskens1, G.J.D. Teske1, L.M. Butter1, T. van der Poll2, S. Florquin1, J.C. Leemans1 Department of Pathology and 2CEMM, AMC, Amsterdam, The Netherlands.

Tubulo-interstitial injury is a common finding in the chronically injured kidney that can be characterized by a cascade of events including enhanced inflammation. TLRs can initiate an inflammatory response upon activation by endogenous danger ligands released during tissue injury. Interestingly, TLR4 is constitutively expressed in the kidney. However, the role of this organ-specific expression in progressive renal injury is yet unknown.

We used C57Bl/6 wild type and TLR4-/- mice (n=8/group) that were subjected to unilateral urether obstruction (UUO) by a permanent double ligation of the right urether. The contralateral kidney served as an internal control. Mice were subsequently sacrificed after one and 14 days to determine renal morphology, (myo) fibroblast accumulation and renal cytokine levels.

As assessed by real time RT-PCR, we found a strong increase in TLR4 mRNA levels 7 and 14 days after UUO induction, when compared with sham-operated mice. Interestingly, TLR4-/- mice developed more tubular damage in the obstructed kidneys than wild type mice after one day of UUO whereas after 14 days of UUO the level of injury was similar in both groups. In addition, we found that TLR4 deficiency increased the level of interstitial myofibroblasts after one day of UUO, whereas after 14 days of UUO a tendency towards lower levels of myofibroblasts was seen in obstructed kidneys of TLR4-/- mice compared with wild type mice. In contrast, no differences were found in renal levels of HGF and active or total TGFβ between obstructed kidneys of wild type and TLR4-/- mice one and 14 days after UUO.

In conclusion, TLR4 is markedly upregulated in the kidney after UUO-injury and mediates myofibroblast accumulation and tubulo-interstitial damage in experimental obstructive nephropathy.

ABSTRACT 129

Negative regulation of TLR2-mediated pro-inflammatory responses by soluble TLR2

A.-C. Raby1, E. Le Bouder1, C. Colmont1, J.E. Rey-Nores2, J. Davies1, P. Richards1, S.A. Jones1, P. Brennan1, N. Topley1, and M.O. Labéta1

Department of Medical Biochemistry & Immunology, School of Medicine, Cardiff University1. School of Biosciences, University of Wales Institute, Cardiff2, U. K.

We have described the existence of a natural soluble form of TLR2 (sTLR2), and here show the ability of sTLR2 to regulate pro-inflammatory responses in vitro and in vivo and demonstrate the mechanism underlying sTLR2 negative regulatory capacity. Cells overexpressing sTLR2, or stimulated in the presence of sTLR2 protein, are TLR2 hyposensitive. Negative regulation was TLR2-specific, affected NF-κB activation, phagocytosis and superoxide production. Natural sTLR2-depleted serum rendered leukocytes hypersensitive to TLR2-mediated stimulation. Mice administered sTLR2 with either Gram +ve bacteria-derived components or live Gram +ve bacteria showed lower peritoneal levels of the neutrophil (PMN) chemoatractant, KC; lower PMN numbers and late apoptotic PMN. Mononuclear cell recruitment was not affected, and endogenous peritoneal sTLR2 increased. Notably, the anti-inflammatory effect of sTLR2 did not compromise the capacity of mice to clear the infection. sTLR2 interfered with the mobilization of TLR2 to lipid rafts, acted as a decoy receptor, and disrupted the CD14-TLR2 interaction by associating with CD14. In order to identify the region(s) of sTLR2 involved in the interaction with CD14, the leucine-rich repeats (LRRs) of TLR2 were point mutated and the ability of these mutants to affect CD14-dependent signalling was evaluated. Peptides of the selected LRRs were generated and tested for their capacity to modulate TLR2 triggering. These findings establish sTLR2 as a negative regulator of TLR2-mediated inflammatory responses, capable of blunting immune responses without abrogating microbial recognition, and will inform the design of novel therapeutics against acute and chronic inflammatory conditions.

ABSTRACT 130

Impaired bacterial clearance in Crohn's Disease results from attenuated macrophage pro-inflammatory cytokine release following TLR stimulation

Rahman, Farooq1,2, Hayee, Bu'Hussain1,2, Bloom, Stuart2, Smith, Andrew1, Segal, Anthony1
1 Department of Medicine, UCL, London, UK.

2 Department of Gastroenterology, UCLH NHS Foundation Trust, London, UK.

Our research has demonstrated gross defects in the innate immune response to E.coli in Crohn's disease (CD), with the primary defect appearing to operate at the level of macrophage. We show here that the acute inflammatory response of primary macrophages in CD is grossly abnormal following stimulation with E.coli and TLR ligands. Pro-inflammatory cytokine release was significantly reduced in CD, with significant differences in cytokine profiles in ileal and colonic CD. Interestingly the levels of the anti-inflammatory cytokines IL-10 and IL-1ra were similar to healthy controls. Similar results were seen following stimulation with TLR2 and TLR4 agonists. In vivo studies of bacterial challenge in CD demonstrate a marked reduction in acute inflammation and bacterial clearance that was most pronounced in colonic CD. Results were independent of age, gender, smoking status and drug treatment. Despite the therapeutic efficacy of immunosuppressant and anti-TNF therapy, macrophages from CD patients have a grossly impaired cytokine response to TLR agonists. Importantly the marked difference in cytokine profiles in ileal and colonic CD suggest alternative defective mechanisms in the different CD phenotypes. It is entirely feasible that the attenuated macrophage response to bacteria in CD results in persistence of antigenic material that periodically breaches the bowel wall, secondarily driving the T cell-mediated chronic granulomatous inflammation that is the hallmark of this disease.

ABSTRACT 131

INVOLVEMENT OF ADHESION IN MACROPHAGE PRODUCTION OF NEUTROPHIL INFLAMMATORY CHEMOKINES, KC AND MIP-2

K. De Filippo, R. Henderson, M. Laschinger, N. Hogg

Leukocyte Adhesion Lab, Cancer Research UK, Lincoln's Inn Fields. London

Neutrophils are the first immune cells to migrate into infected tissue sites. Therefore an important step in the initiation of an immune response is the synthesis of the neutrophil-recruiting chemokines. In this in vivo study, we show that resident tissue macrophages are the source of the major neutrophil chemoattractants, KC and MIP-2. Synthesis of these chemokines is rapidly regulated at the transcriptional level by signalling through the toll-like receptors (TLRs) initiated by the adaptor molecules MyD88 and TRIF. KC and MIP-2 are both produced by signalling through MyD88. However MIP-2, but not KC, is also synthesized through the TRIF adaptor protein, identifying it as a new product of this alternative pathway. Use of both pathways by TLR4 ensures maximal levels of KC and MIP-2 that lead to robust neutrophil recruitment.

A question is whether adhesion through integrins co-operate with these TLR-adaptor molecules in the synthesis of chemokines KC and MIP-2 in tissue macrophages. There is evidence in the literature suggesting that the adaptor MyD88 requires the help of Mac-1 (CD11b/CD18) for chemokine (IL-6) synthesis. Although we have data suggesting that adhesion is required for KC synthesis, we have had difficulty reproducing a role for Mac-1. In vitro and in vivo studies making use of various CD11/CD18 deficient macrophages are presently under analysis.

ABSTRACT 132

Functional TLR9 is Involved in the Systemic Inflammatory Response to Injury.

Roop Gill, David J. Kaczorowski, Steven C. Gribar, Patricia Loughran, David J. Hackam, Timothy R. Billiar

University of Pittsburgh Medical Center, Pittsburgh, P.A., U.S.A.

Background: Trauma and shock induce a systemic inflammatory response that is associated with end-organ damage and immune dysregulation. We and others have shown that TLR4, but not TLR2, is central to this response, possibly through the recognition of endogenous "danger signal". TLR9 may also recognize endogenous molecules released during injury (DNA). Therefore we tested the hypothesis that TLR9 is also involved in the activation of inflammatory pathways following trauma.

Methods: Mice deficient in TLR9 response and wild-type C57/Bl-6 mice (n=6/group) underwent bilateral femur fracture, soft tissue crush injury, and 1.5 hours shock to a mean BP of 25 mmHg followed by resuscitation for 4.5 hours with Lactated Ringers. Sham groups underwent femoral artery cannulation only. In some groups, 1mg/kg CpG DNA was administered intra-peritoneally simultaneous to trauma. Blood and organs were obtained to measure IL-6 and IL-10 by ELISA, AST and ALT by serology, and end-organ injury by histology and TUNEL staining (reviewed by a blinded examiner).

Results: The systemic inflammatory response and end-organ injury following trauma was attenuated in mice deficient in functional TLR9 as seen by significantly lower serum levels of IL-6, IL-10, ALT, and AST ($p<0.05$). TUNEL staining and histology found a significant attenuation of lung and hepatic injury in the absence of functional TLR9. Damage to the intestinal mucosa however was not reduced by the absence of functional TLR9. Mice given CpG DNA, a stimulator of TLR9, showed an enhanced inflammatory response to trauma.

Conclusion: The findings demonstrate that TLR9 is central to the injury-induced inflammatory response and end-organ injury seen in trauma, and implicate the release of endogenous DNA in post-injury induced inflammation.

ABSTRACT 133

THE TOLL-LIKE RECEPTOR 4 IS INVOLVED IN INFLAMMATORY REACTIONS (OVERGROWTH) AGAINST ALGINATE CAPSULES.

Anne Mari Rokstad 1, Bjørg Steinkjer1, Egil Lien1,2, Terje Espevik1

1Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Norway. 2Department of Medicine, Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Massachusetts.

Alginate capsules are currently investigated as bioreactors for delivering therapeutic molecules like insulin and cancer inhibiting proteins. One main problem with the concepts is that the capsules tend to be overgrown by inflammatory cells, with a variation depending on the capsule type. In this study we evaluated the role of Toll-like receptor (TLR) 2 and 4 in the overgrowth reactions against implanted alginate capsules by using transgenic mice lacking TLR2 and TLR4. The F10 generations of KO mice backcrossed in C57Bl/6J phenotype were used and compared with wild type mice. Alginate beads and alginate-poly-L-lysine-alginate (APA) capsules were implanted intra peritoneal in male and female mice (n=5 each group). Animals were sacrificed and the amount of free floating capsules was estimated. In addition, the degree of overgrowth of the free floating capsules was evaluated on a 0-5 score scale. Our data showed that TLR4 KO mice gave significant higher retrieval of implanted APA capsules than wild type in both sexes, while a significant difference was achieved in females for implanted alginate beads. Trends towards higher retrieval rate were seen in TLR2 KO mice, but significant difference to wild type was only seen in male given APA capsules. In TLR4 KO mice, a lower amount of cells were attached to the free floating fraction of alginate beads. These results suggest that TLR4 is a key component in the inflammatory reactions against implanted alginate bioreactors.

ABSTRACT 134

Group V phospholipase A2-derived lysophosphatidylcholine mediates cyclooxygenase-2 induction in TLR4-stimulated macrophages

Violeta Ruipérez, Javier Casas, María A. Balboa and Jesús Balsinde

Institute of Molecular Biology and Genetics, Spanish National Research Council and University of Valladolid School of Medicine, Valladolid, Spain

Activation of macrophages and macrophage cell lines by bacterial LPS, an agonist of Toll-like receptor 4, elicits a delayed phase of PG biosynthesis that appears to be entirely mediated by cyclooxygenase-2 (COX-2). In previous work, we found that a catalytically active group V secreted phospholipase A2 (sPLA2-V) was required for COX-2 induction, but the nature of the sPLA2-V metabolite involved was not defined. In this study, we identify lysophosphatidylcholine (lysoPC) as the sPLA2-V downstream mediator involved in COX-2 induction in TLR4-stimulated macrophages. Inhibition of sPLA2-V by RNA interference or by the cell-permeable compound scalaradial blocked LPS-induced COX-2 expression, and this inhibition was overcome by incubating the cells with a nonhydrolyzable lysoPC analog, but not by arachidonic acid or oleic acid. Moreover, inhibition of sPLA2-V by scalaradial also prevented the activation of the transcription factor c-Rel, and such an inhibition was also selectively overcome by the lysoPC analog. Collectively, these results support a model whereby sPLA2-V hydrolysis of phospholipids upon TLR4 stimulation results in lysoPC generation, which in turn regulates COX-2 expression by a mechanism involving the transcriptional activity of c-Rel.

ABSTRACT 135

NETWORK OF CYTOKINES AND GROWTH FACTORS OF RELEVANCE TO HEALING OF ACUTE WOUNDS

Øystein Sandanger, Øystein Grimstad, Liv Ryan, Brita Pukstad, and Terje Espevik. Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

Healing of wounds is a complex biological event that involves intimate interplay between resident and infiltrating cell types. The healing process consists of overlapping phases such as coagulation, inflammation, proliferation, angiogenesis and resolution, which are controlled by cytokines and growth factors. Endogenous and exogenous TLR ligands have essential roles in the cytokine- and growth factor responses required for proper wound healing. Studies of cytokines and growth factors involved in acute wound healing are few and conflicting. In this study we performed a multiplex analysis of 27 cytokines (TNF-alfa, IFN-gamma, IL-1 beta, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL15, IL-17, Eotaxin, RANTES, MCP-1, IP-10, MIP-1 alfa, MIP-1 beta, G-CSF, GM-CSF, FGF, PDGF, VEGF) in drainage fluids from human sterile surgical wounds. In order to get a better understanding of the kinetics of production and cytokine network operating in acute wounds, we also performed multiplex analysis of 27 cytokines in supernatants from human neutrophiles, monocytes, endothelial cells, keratinocytes and fibroblasts stimulated with proinflammatory cytokines (IL-1 beta, TNF-alfa, IL-6), anti inflammatory cytokine (IL-10) and TLR ligands (Pam3Cys, Poly I:C and LPS). The results from the sterile acute wounds suggest that proinflammatory cytokines (IL-6, IL-1 beta, TNF-alfa and IFN-gamma) had a more rapid kinetics of production compared to IL-1RA, IP-10, VEGF and IL-10. Furthermore, multiplex data from the various cell types revealed new and unexpected interactions between cytokines and growth factors that may be important for understanding the wound healing process.

ABSTRACT 136

TLR AGONISTS INDUCE INFLAMMATION AND CELL DEATH IN A MODEL OF HEAD AND NECK SQUAMOUS CELL CARCINOMAS

Camilla Rydberg1, Anne Månsson2, Rolf Uddman2 and Lars Olaf Cardell1
1Division of ENT diseases Huddinge, Karolinska Institutet, Stockholm, Sweden
2Laboratory of Clinical and Experimental Allergy Research, Department of Otorhinolaryngology, Malmö University Hospital, Lund University, Sweden

Several studies suggest a role for Toll-like receptors (TLRs) in the pathogenesis of various cancers. Therefore, the present study was designed to investigate differences in TLR responsiveness in healthy and tumorigenic airway epithelial cells. To this end, the human pharyngeal epithelial cell line Detroit was used and compared to the healthy bronchial epithelial cell line NL-20 along with primary human nasal epithelial cells (HNECs). Real-time RT-PCR, flow cytometry and ELISA were used to determine expression of TLRs, ICAM-1, IL-8 secretion, cell viability and signaling pathways. Detroit was found to express high levels of TLR2, -3 and -5, whereas NL-20 expressed only low levels of TLR3. HNECs had the same TLR profile as Detroit, but displayed different expression levels. The TLR agonists Pam3CSK4 (TLR2), poly(I:C) (TLR3) and flagellin (TLR5) were powerful inducers of ICAM-1 expression and IL-8 secretion in Detroit. Pam3CSK4 and poly(I:C) also decreased cell viability, and poly(I:C) affected migration of Detroit. In contrast, NL-20 only responded with a small IL-8 secretion upon poly(I:C) stimulation. Moreover, the signaling pathways differed between the different ligands, but also between the different cell lines. Detroit activated the NF-kappaB signaling cascade when stimulated by poly(I:C), whereas activation of NL-20 was mediated via p38. This study shows that TLR activation of the tumorigenic cells induces an inflammatory response, cell death and an altered migratory behavior, whereas the healthy cells are almost unresponsive. The present study highlights the importance of the TLR system in HNSCC.

ABSTRACT 137

Altered inflammatory response in farmers chronically exposed to organic material

Karin Sahlander 1, Kjell Larsson 1, Lena Palmberg 1

1Lung and Allergy Research, National Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Background: Exposure in a pig barn, an environment with high levels of organic compounds, leads to increased levels of pro-inflammatory cytokines and neutrophilic granulocytes the airways and in blood in previously unexposed healthy individuals. However, in farmers who are continuously exposed in pig barns the inflammatory response to acute exposure is attenuated as compared to healthy subjects. Objective: To investigate if the expression of TLR2 and TLR4 on blood monocytes and the concentration of soluble ST2 (sST2) in serum is altered after acute exposure in a pig barn or LPS challenge and if there is a difference between continuously exposed farmers and previously unexposed individuals in these respect.

Methods: Eleven non-smoking pig farmers and 12 non-smoking, previously unexposed healthy controls underwent 3 hours exposure in a pig barn and LPS challenge on separate days, at least 3 weeks apart, in a randomized order. Blood was drawn 2 weeks before and 7 h after the exposures started. TLR expression was measured with flow cytometry and sST2 with ELISA.

Results: Farmers showed reduced expression of TLR2 on blood monocytes compared to controls ($p<0.01$) but no difference between the groups regarding TLR4 was observed. However, there was no alteration in TLR2 or TLR4 expression due to exposure neither within nor between groups. There was no difference in the basal level of sST2 in serum between farmers and controls. Pig barn exposure led to an increase of serum sST2 in the control group ($p<0.01$), but not in the pig farmers ($p<0.01$ between groups). There was a slight increase in sST2 in the controls after LPS challenge ($p=0.067$) which was not observed in farmers.

Conclusion: The reduced TLR2 expression and the attenuated ST2 response to acute exposure in a pig barn observed in farmers is most likely a consequence of repeated, on a daily basis, exposure to organic material.

ABSTRACT 138

TLR-4/MD2 has a critical role in intraocular cytokines secretion during Endotoxin-Induced Uveitis (EIU)

E. V. Salazar, G. Bernal, L. Baute, B. E. Brito.

Cellular and Molecular Pathology Laboratory, Venezuelan Institute for Scientific Research (IVIC), Caracas, Venezuela.

Purpose: To study if the activation of TLR-4/MD2 mediates intraocular cytokines secretion during EIU. Methods: C3H/HeN mice, 8 weeks old, were injected intravitreally (i.vt.) with 20 µg/ml of anti-TLR-4/MD2 (MTS510). Control animals were injected with saline or irrelevant antibody (IAb). Six hours later, EIU was induced by intraperitoneal (i.p.) injection of 2 mg/ml LPS and controls were injected with apyrogenic saline. Twenty four hours later, aqueous humor (AH) were obtained and tested by bioassay for TNF and IL-6 secretion. For RT-PCR analysis, iris/ciliary body were obtained 3 hours after i.p. LPS challenge and mRNA was amplified for both cytokines. Iris/ciliary body explant cultures were challenge with LPS for 24 hours and supernatants were tested for TNF and IL-6 secretion. Results were expressed as mean±SEM. Mann-Whitney U-test was used for statistical analysis. Results: Twenty four hours after LPS injection, cytokines levels increased in AH of mice treated with saline i.vt./LPS i.p. (TNF: 4,961±628 pg/ml; IL-6: 9,501±2,174 pg/ml); IAb i.vt./LPS i.p. (TNF: 4,943±494; IL-6: 9,972±1,821 pg/ml) while anti-TLR-4/MD2 i.vt./LPS i.p. a significant inhibition of 89% in TNF (537.2±68.3 pg/m; p=0.0001) and 72% in IL-6 (2,649±406 pg/ml; p=0.001). TNF-alpha and IL-6 mRNA expression was detected in the iris/ciliary body from saline i.vt./LPS i.p. and IAb i.vt./LPS i.p. treated mice, being absent in those pre-treated with anti-TLR-4/MD2. In iris/ciliary body explant an inhibition of 71% in TNF (74±6.17 pg/ml) and 30% in IL-6 (146±41.2 pg/ml) was observed with anti-TLR-4/MD2 pre-incubation, compared to explant challenge with LPS (TNF: 255±45.2 pg/ml; IL-6: 207±3.71pg/ml). Conclusion: Anti-TLR-4/MD2 significantly inhibit TNF and IL-6 secretion, and also blocks TNF and IL-6 mRNA expression, suggesting that TLR-4/MD2 has a determinant role during EIU development.

ABSTRACT 139

Defective CD14-dependent TLR4 signalling results in abnormal inflammatory response in ulcerative colitis

Andrew M Smith1, Farooq Z Rahman1, Bu'Hussain Hayee1, Stuart L Bloom2, Anthony W Segal1

1 Department of Medicine, University College London

2 Department of Gastroenterology, UCL Hospitals NHS Foundation Trust, London

Discrimination between bacterial and viral infection is paramount in generating an appropriate immune response. Here we show that this ability is defective in ulcerative colitis (UC), due to the inability in turning off the anti-viral response during a bacterial challenge. This defect has severe consequences in vivo resulting in delayed resolution and induction of T cell driven chronic inflammation. Subcutaneous injection of heat killed E.coli resulted in a significantly protracted inflammatory response and an elevation in systemic CXCL10 levels in UC patients. Abnormalities in bacterial recognition in vitro by macrophages isolated from UC patients resulted in the increased CXCL10 release we observed in vivo. Further analysis showed that signalling through the TRIF-dependent pathway downstream of the CD14/TLR4 complex resulted in increased interferon (IFN) levels which fed back via the IFN receptor producing the elevation in CXCL10. The regulatory pathway which dampens TRIF-dependent signalling in healthy subjects is defective in UC macrophages, resulting in an abnormally high level of IFN release, upregulation of co stimulatory (CD80/86) and antigen processing molecules (HSP70, HSP60). These events are usually associated with T cell activation and an anti-viral response. These findings identify a basic immunological defect in bacterial recognition and TLR signalling which provides a mechanism to explain the increased CXCL10 levels associated with UC.

ABSTRACT 140

IRF3 HAS A CRITICAL ROLE IN ALCOHOLIC LIVER DISEASE

G Szabo, I Hritz, A Velayudham, B Nath, D Catalano, K Kodys, E Kurt-Jones, P Mandrekar
University of Massachusetts Medical School, Worcester, MA, USA

Kupffer cell (KC) activation by lipopolysaccharide (LPS) is central in the pathogenesis of alcoholic liver disease (ALD). LPS recognition by Toll-like receptor 4 (TLR4) activates the MyD88-dependent pathway leading to rapid NFkB activation while recruitment of TRIF activates both NFkB and IRF3, resulting in inflammatory cytokine and type I IFN induction. The aim of this study was to evaluate the role of TLR4-induced pathways in the pathogenesis of ALD. Wild-type (WT), TLR4-, MyD88- or IRF3-deficient (KO) mice received Lieber-de-Carli diet with 4.5 v/v% ethanol (EtOH) or isocaloric liquid control (control) diet for 3-5 weeks. EtoH significantly increased serum ALT indicating liver damage in WT ($p<0.05$) and MyD88-KO ($p<0.03$) but not in TLR4- or IRF3-KO mice. Liver steatosis and triglyceride were increased in EtOH WT ($p<0.03$) and MyD88-KO mice ($p<0.02$) compared to controls. TLR4 or IRF3 deficiency attenuated alcohol-induced steatosis and triglyceride deposition in the liver. Expression of TNFa and IL-6 mRNA was significantly higher in EtOH WT ($p<0.03$), and MyD88-KO mouse livers ($p<0.05$) compared to controls while there was no increase in IRF3- or TLR4-KO mice. Consistent with the role of reactive oxygen radicals produced by the NADPH oxidase complex in ALD, mRNA of p47phox, p22phox, p67phox, gp91phox were increased in EtOH WT and MyD88-KO ($p<0.03$) but not in TLR4- and IRF3-KO mice. These results suggested that TLR4-induced, IRF3-dependent pathways play a role in ALD. Indeed, activation of IRF3 was suggested by increased expression of ISG56 and IRF7 in EtOH livers in WT mice. Upregulation of the IRF3 target gene, IRF7, occurred in isolated KC after alcohol feeding. Our results suggest that activation of the IRF3-mediated, TLR4-dependent signaling pathways are critical in development of alcoholic liver damage, steatosis, and inflammation. Furthermore, IRF3 activation occurs in the KC. These findings suggest a novel role for IRF3 and activation of the Type I IFN pathways in ALD.

ABSTRACT 141

TOLL-LIKE RECEPTOR 4 AGONISTS ADSORBED TO ALUM ATTENUATE ALLERGIC AIRWAY DISEASE VIA MYD88 ADAPTOR MOLECULE AND IL-12/IFN-γ AXIS.

Juliana Bortolatto1, Érica Borducchi1, Dunia Rodriguez1, Alexandre C. Keller1, Eliana Faquim-Mauro2, Karina Bortoluci1, Daniel Mucida1, Eliane Gomes1, Ana Christ1, Silvia Schnyder-Candrian3, Bruno Schnyder3, Bernhard Ryffel3 and Momtchilo Russo1
1Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, Brazil
2Laboratory of Immunopathology, Institute Butantan, São Paulo, Brazil
3Molecular Immunology and Embryology, Centre National de la Recherche Scientifique, Orléans, France.

Epidemiological and experimental data suggest that bacterial lipopolysaccharides (LPS) can either protect or exacerbate allergic asthma. LPS triggers immune responses through Toll-like receptor (TLR) 4 that in turn activates two major signaling pathways via either MyD88 or TRIF adaptor proteins. LPS is a pro-Th1 adjuvant while aluminum hydroxide (alum) is a strong Th2 adjuvant, but the effect of mixing both adjuvants on development of lung allergy have not been investigated.
We determined whether natural (LPS) or synthetic (ER-803022) TLR4 agonists adsorbed onto alum adjuvant affect allergen sensitization and development of airway allergic disease. To dissect LPS-induced molecular pathways we used TLR4-, MyD88-, TRIF-, or IL-12/IFN-γ deficient mice. Sensitization with OVA plus LPS co-adsorbed onto alum impaired in dose-dependent manner OVA-induced Th2-mediated allergic responses such as airway eosinophilia, airway hyperreactivity, mucus hyper production and serum levels of IgE antibodies. LPS impaired the development of Th2 immunity signaling via TLR4 and MyD88 molecules and via IL-12/IFN-γ axis but not through TRIF pathway. Moreover, the synthetic TLR4 agonists that proved to have a less systemic inflammatory response than LPS also protected against allergic asthma development.
Our work indicates that TLRs agonists plus allergens co-adsorbed to alum might represent a novel strategy for down modulating allergic responses.

ABSTRACT 142

Lyn kinase is necessary for establishment of LPS tolerance in mast cells

Alejandro Martin Avila[1,2], Jaciel Medina Tamayo[1], Iris Madera Salcedo[1] and Claudia González Espinosa[1]

[1]Pharmacobiology Department, Center for Research and Advanced Studies (CINVESTAV), South Campus. Mexico City, Mexico.

[2]Biomedical Sciences PhD Program, National Autonomous University of Mexico (UNAM), Mexico City, Mexico.

Mast cells are able to respond to stimuli coming from innate and adaptive immunity. They express, among others, the high affinity IgE receptor (FcεRI) and the Toll-like receptor 4 (TLR4). After their stimulation, via immunoglobulin E (IgE)-antigen complexes or bacterial lipopolisaccharide (LPS), unique signal transduction systems are activated leading to a selective release of preformed or *de novo* synthesized mediators. A complex process of integration of signals from TLR4 and FcεRI receptors occurs in mast cells resident in tissues in contact with bacterial compounds and allergens. Description of the molecular cross-talk between FcεRI and TLR4 -activated signaling cascades is a requisite to understand the influence of bacterial infection on allergy. Prolonged stimulation with LPS causes mast cell tolerance and upregulation of the SH2-containing inositol phosphatase 1 (SHIP1) is associated to transient unresponsiveness to LPS. Since after FcεRI activation, SHIP1 is recruited to the membrane in a tyrosine kinase-dependent fashion, we decided to analyze if the tyrosine kinase Lyn (strongly activated by FcεRI crosslinking) could be involved in the establishment of LPS tolerance using mast cells derived from Lyn KO mice. We found that Lyn is essential in the establishment of LPS tolerance, due to LPS-dependent SHIP induction was severely affected in Lyn KO cells although they show an increased synthesis of IL-2 and IL-4 mRNAs after TLR4 stimulation. *In vivo* experiments shown that Lyn is a negative regulator of LPS-induced TNF-α secretion in murine peritoneal cavity, so, it is possible that as in the FcεRI cascade, Lyn could be able to activate positive and negative signals on cytokine synthesis after TLR4 activation.

ABSTRACT 143

Toll-like receptor 9 (TLR9) activation attenuates TLR4 signaling in enterocytes via IRAK-M and attenuates the severity of intestinal inflammation.

Steven C. Gribar, Ward M. Richardson, Jeffrey W. Kohler, Maria F. Branca, Theresa D. Dubowski, Jun Li, Chhinder P. Sodhi, David J. Hackam

Introduction: Necrotizing enterocolitis (NEC) is characterized by intestinal inflammation and systemic endotoxemia and its pathogenesis has recently established to require toll-like receptor 4 (TLR4) signaling. As enterocytes are constantly exposed to multiple bacterial by-products, including LPS and bacterial DNA (CpG-DNA), we now hypothesize that activation of TLR9, which recognizes CpG-DNA, attenuates enterocyte TLR4-mediated signaling and attenuates the severity of intestinal inflammation in NEC.

Methods: TLR expression were assessed by qRT-PCR, SDS-PAGE, and confocal microscopy. MAP-Kinase phosphorylation was assessed by SDS-PAGE, NF-□B activation was determined using confocal microscopy and IL-6 induction was measured using qRT-PCR. Systemic IL-6 was quantified by ELISA. Experimental endotoxemia was induced by ip injection of LPS. NEC was induced by formula gavage and intermittent hypoxia. IRAK-M, IRAK-1 and TRAF-6 distribution were assessed by confocal microscopy and immunoprecipitation.

Results: Cultured (IEC-6) enterocytes as well as murine and human intestinal mucosa were shown to expressed TLR4 and TLR9. In experimental and human NEC, an increase in TLR4 expression and decrease in TLR9 expression was found. Signaling cross-talk between TLR4 and TLR9 was suggested as LPS-mediated MAP-Kinase phosphorylation, NF-□B activation and IL-6 induction were attenuated by CpG-DNA in cultured enterocytes and was reversed in enterocytes where TLR9 expression was knocked-down using siRNA. Similarly, CpG-DNA attenuated intestinal mucosal IL-6 induction and systemic IL-6 release in mice subjected to experimental endotoxemia, an effect that did not occur in TLR9 mutant mice. In support of a mechanism mediating this effect, CpG-DNA led to a re-distribution of IRAK-M in enterocytes, and a disruption of IRAK-1 and TRAF-6 interactions, while inhibition of IRAK-M prevented the effects of CpG-DNA on TLR4 signaling. The protective effect of TLR9 activation in enterocytes was further demonstrated as administration of CpG-DNA attenuated experimental NEC severity by gross and histologic analysis.

Sepsis and Immune Responses to Bacteria

ABSTRACT 144

SEPSIS-INDUCED SUPPRESSION OF LUNG HOST DEFENSE IS MEDIATED BY ST2

Jacobien J. Hoogerwerf1,2, Masja Leendertse1,2, Andrew N.J. McKenzie3, Tom van der Poll1,2

1Center for Infection and Immunity Amsterdam, 2Center of Experimental and Molecular Medicine, University of Amsterdam, The Netherlands, 3MRC Laboratory of Molecular Biology, Cambridge, UK.

Patients with sepsis display - after surviving the initial hyperinflammatory phase - features consistent with immunosuppression, which renders the host susceptible to nosocomial infections, in particular bacterial pneumonia. We here sought to determine the role of ST2 - a TLR inhibitor - in modulating host defense in the lung during sepsis using a murine model of cecal ligation and puncture (CLP)-induced sepsis followed by secondary challenge with intranasal P. aeruginosa. For this CLP / sham was performed on BALB/c wild-type (WT) and ST2 knock out (KO) mice, 24 hrs after which animals were challenged with either 108 live or heat-killed P. aeruginosa (HKPA); 0, 6 and 24 hours thereafter mice were sacrificed and lungs were harvested. Septic mice demonstrated impaired clearance of Pseudomonas and reduced levels of IL-6 and TNF-α as compared with sham mice. ST2 expression on the surface of alveolar macrophages and CD4+ cells was upregulated in both sham and CLP mice as compared with naïve mice; in contrast, ST2 expression on CD8+ cells was only upregulated in CLP mice. Following sepsis and secondary pneumonia, ST2KO mice had higher survival rates and improved bacterial clearance in lungs when compared with WT mice. Although ST2KO mice showed reduced levels of IL-6 and TNF-α 24 hrs after induction of pneumonia, increased levels of these cytokines were found in lungs 6 hrs after challenge with HKPA as compared with WT mice. Furthermore, ST2KO mice demonstrated reduced levels of anti-inflammatory cytokines IL-10 and IL-4 0 and 24 hrs after induction of secondary pneumonia. These findings indicate that ST2 contributes to the immunocompromised state during sepsis and the ensuing disturbed homeostasis of lung host defense.

ABSTRACT 145

The role of Matrix Metalloproteinase-8 in endotoxemia and immunity

Dejonckheere Eline1,2, Van Lint Philippe1,2, Vanlaere Ineke1,2, De Colvenaer Veerle3, Leclercq Georges3 and Libert Claude1,2

1Department for Molecular Biomedical Research, VIB, Ghent, Belgium, 2Department of Molecular Biology, Ghent University, Ghent, Belgium, 3Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, Ghent, Belgium

Sepsis arises when the immune system is unable to eradicate an infection, leading to an exaggerated and often fatal systemic inflammation. Despite intensive research, still over 750 000 cases of sepsis a year are reported in the USA.

Systemic administration of LPS to laboratory animals leads to endotoxemia and mimics sepsis. Treating mice with a broad-spectrum MMP inhibitor (MMPi) completely protected against endotoxemia. By administering a lethal dose of LPS to different MMP KO mice, MMP8 KOs were found to show a prominent resistance.

To elucidate the role of MMP8 in endotoxemia we focused on differences in cytokine profiles. Studies showed that MMP8 KOs have a profound defect in IFNg induction after LPS challenge, which affected all IFNg producing cell types. This was not caused by defective production of IL12, or impaired reaction to IL12 and IL18. However, we observed that MMP8 KOs have lower levels of IL1b and IL18 in circulation following endotoxemia. This appeared not to be due to direct proteolytic maturation of pro-IL1b or pro-IL18 by MMP8, nor to a caspase-1 dependent event. We found that treating caspase-1 KOs with an MMPi further increased resistance to LPS significantly. Furthermore, MMPi treatment of caspase-1 KOs resulted in a significant drop in residual IL1b and IFNg, which implies that MMPs function in a caspase-1 independent manner. Since MMP8 acts rather late in endotoxemia, we believe it mediates the release of extracellular matrix components which in turn lead to caspase-1 independent IL1b and IL18 driven IFNg production.

Further study will be needed to clarify whether MMP8 inhibition can be beneficial in treating clinically relevant pathologies, such as sepsis, trauma or Th1-driven pathologies.

ABSTRACT 146

Tir8/Sigirr prevents murine lupus by suppressing the immunostimulatory effects of lupus autoantigens.

Maciej Lech1, Onkar P. Kulkarni1, Stephanie Pfeiffer1, Emina Savarese2, Anne Krug2, Cecilia Garlanda3, Alberto Mantovani3, Hans-Joachim Anders1

1 Medical Policlinic, University of Munich, Munich, Germany

2 Department of Medicine, Technical University of Munich, Munich, Germany

3 Istituto Clinico Humanitas and Fondazione Humanitas per la Ricerca, Rozzano, Italy

Background: Linkage analyses identified unknown lupus susceptibility gene at the p15 region of chromosome 11. The Sigirr gene (also known as TIR8) is located exactly at 11p15.5 and encodes for an orphan receptor of the TLR/IL-1 receptor family. We hypothesised that SIGIRR can inhibit the activation of dendritic cells and B cells upon exposure to the lupus immuncomplexes and that mutations in this gene may associate with the lupus disease. Results: Sigirr does inhibit the activation of dendritic cells and B cells upon exposure to the lupus autoantigen and TLR7 ligand U1snRNP. Generated Sigirr-deficient C57BL/6lpr/lpr mice developed a progressive lymphoproliferative syndrome followed by severe autoimmune lung disease and lupus nephritis as compared to the minor abnomalities observed in C57BL/6lpr/lpr mice. Lack of Sigirr was associated with enhanced activation of dendritic cells, the expression of type I interferons, multiple proinflammatory and anti-apoptotic mediators as well as the expansion of CD4 T cells and reduction of regulatory CD4+CD25+ T cells. Furthermore, lack of Sigirr enhanced the activation and proliferation of B cells including the production of autoantibodies against multiple nuclear lupus autoantigens. Moreover, lupus autoantibodies in Sigirr deficient mice results in renal immune complex deposition complement activation and tissue injury.

Conclusion: Sigirr inhibits dendritic cells and B cells signalling upon stimulation with lupus immuncomplexes. The mutation in Sigirr gene might be involved in the aggravation of autoimmunity and manifestation of lupus disease. These data identify Sigirr as a novel SLE susceptibility gene in mice.

ABSTRACT 147

Protection from lethal Gram-negative septic shock by anti-Toll-like receptor 4 antibodies

Thierry Roger, Didier Le Roy, Céline Froidevaux, Davide Mauri1, Kim Burns2, Shizuo Akira3 and Thierry Calandra

Infectious Diseases Service, CHUV & University of Lausanne, Lausanne, 1Apotech Biochemicals, Epalinges, 2Department of Biochemistry, University of Lausanne, Lausanne, Switzerland; 3Department of Host Defense, Osaka University, Osaka, Japan

Background: TLR4 plays a critical role in the sensing of endotoxin and therefore in innate immune defenses against Gram-negative (GN) bacteria. Here we studied the effect of anti-TLR4 antibodies in an experimental mouse model of Gram-negative sepsis.

Methods: Anti-mouse TLR4 IgG were raised in rabbits immunized with a soluble chimeric mouse TLR4-human IgG1 fusion protein. Wild-type (WT), MyD88-/-, TLR2-/- and TLR4-/- mice were subjected to endotoxemia (n=8) and E. coli O18 sepsis (2.10e5-2.10e9 CFU i.p. followed by antibiotics; n=10-12) with or without treatment with 40-200 mg/kg of anti-TLR4 or control IgG.

Results: In vitro, anti-mTLR4 IgG inhibited LPS-induced NF-kB and ERK1/2 activation and TNF production by macrophages. As a proof of principle of anti-TLR4 targeted therapies, TLR4-/- and MyD88-/- mice were found to be fully resistant to lethal endotoxemia or E. coli septic shock, while WT and TLR2-/- mice rapidly succumbed in both models. In agreement with these findings, anti-TLR4 IgG inhibited TNF and IL-6 production and protected animals from lethal endotoxemia ($p=0.02$). Furthermore, both prophylactic and therapeutic (up to 13h after sepsis onset) administration of anti-TLR4 IgG reduced TNF and IL-6 production and increased survival of mice inoculated with live E. coli (survival of control vs anti-TLR4: 0% vs 80%, $P<0.005$ and 30% vs 73%, $P=0.03$ for prophylactic and therapeutic treatment, respectively).

Conclusions: These results provide strong support to the concept of neutralization of the TLR4 pathway as an adjunctive therapy for human Gram-negative sepsis, which is being currently investigated in phase 3 clinical trials with anti-TLR4 sepsis drugs (E5564 and TAK-242).

ABSTRACT 148

In sepsis host protection via MyD88-mediated inflammation is opposed by S. aureus lipoproteins, which enable bacterial growth via iron uptake

M. Schmaler[1], F. Götz[2] and R. Landmann[1]

[1]Dept. Biomedicine, University Hospital, Basel, Switzerland

[2]Microbial genetics, University Tübingen, Germany

Pure Lipoproteins are ligands of TLR2; physiologically they are part of the bacterial cell envelope, in Staphylococcus aureus (SA wt) they mediate cytokine induction in vitro. The effect of lipoproteins (LP) for SA in TLR2-MyD88 mediated systemic infection is unknown. Two SA strains deficient in the enzyme LP-diacylglyceryl transferase (Δlgt), which attaches the lipid anchor to prolipoproteins, were used to study benefits and costs of lipoproteins for SA. First, SA was shown to require LP, TLR2 and MyD88 for early and strong cytokine induction in peritoneal macrophages. Second, LP endowed SA to cause severe sepsis with more organ inflammation and higher bacterial load than Δlgt, both in C57BL/6 and TLR2-/- mice. Third, in MyD88 deficiency LP allowed SA strong growth, while Δlgt remained avirulent. These findings indicate that LP gave a growth advantage, which was more marked in the absence of inflammation. Supporting this hypothesis, SA wt was shown to require LP for growth under iron-restricted conditions and for iron uptake in vitro and ex vivo. Furthermore, in MyD88-/- but not C57BL/6 mice, iron loading allowed Δlgt growth to the same level as SA wt. The results illustrate that iron usage by SA LP is counteracted by inflammation. However in the liver SA even profited from inflammation to hide in granulomas, a feature lacking in Δlgt and in MyD88-/- mice. In summary while SA benefits from LP for growth via iron acquisition, it pays the price of inflammation via MyD88 except for the liver, where it utilizes inflammation for survival.

ABSTRACT 149

The Toll-like receptor 4 signaling pathway and Salmonella infections: a complex relationship

Talbot, S., Tötemeyer, S., Yamamoto, M.1, Akira, S.1, Mastroeni, P., Maskell, D.J. and Bryant, C.E.

Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, UK; 1Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka, Japan

Toll-like receptor 4 (TLR4) is essential for initiating the immune response against systemic Salmonella enterica serovar Typhimurium infection. In susceptible mice infection with sub-lethal doses of S. Typhimurium triggers an initial innate immune response which controls the bacterial growth until the antigen specific immune response clears the infection.

Our results show that whilst TLR4 and MyD88 are essential for the innate resistance phase, their co-receptor, Mal, is not. However, bone marrow derived macrophages from Mal deficient mice have a reduced response to the TLR4 ligand, lipopolysaccharide, but behave similarly to wild type macrophages when infected with S. Typhimurium in vitro. Mice lacking the MyD88-independent adaptor TRIF are able to limit the bacterial growth of S. Typhimurium in vivo, albeit reaching higher bacterial burdens than their wild type controls. This is surprising as bone marrow derived macrophages from these mice, when infected with S. Typhimurium, showed little production of cytokines critically involved in the innate immune response.
In conclusion this study highlights a necessity for a MyD88-dependent, yet Mal-independent, TLR4 pathway in the innate immune response to S. Typhimurium infection. The TRIF dependent pathway is also required for a full innate immune response to S. Typhimurium in vivo and in vitro.

ABSTRACT 150

TLRs-dependent release of IL-8 from human neutrophils H. pylori-infected

Lourdes Alvarez-Arellano1, Javier Torres1, and Carmen Maldonado-Bernal1.

1Infectious Diseases Medical Research Unit, IMSS. Mexico City, Mexico.

H. pylori (Hp) is a bacterium that colonizes the human gastric mucosa. Hp infection is associated with a marked infiltration of gastric epithelium and lamina propia by neutrophils. Neutrophils are the first line of defence against bacterial infection. Their excessive accumulation, activation, and prolonged survival also contribute to tissue damage. The activation of neutrophils leads to the expression of pro-inflammatory mediators such as IL-1β, TNF-α and IL-8. Recently, we reported neutrophils activation by TLRs 2 and 4 post-Hp infections, but the role of TLRs 1, 5 and 6 in that activation and expression of cytokines is unknown. We studied the role of TLRs 1, 5 and 6 in the induction of IL-8 in human neutrophils infected with Hp. Methods. Neutrophils were isolated from human peripheral blood by gradient centrifugation and were infected with Hp for 1, 3, 6 and 24 h, subsequently the production of IL-8, TNF-α, IL-1β and IL-10 was measured by ELISA. For determined the role of TLR1, TLR5 and TLR6 in induction of IL-8 we employed TLRs-neutralizing antibodies, neutrophils were pre-treated with anti-human IgG antibody and after they were treated with anti- TLRs 1, 5 or 6 antibodies followed by 24 h Hp infection. Cell-free, supernatants were collected and IL-8 production was measured by ELISA.

At 3 h after infection we observed a significant increase in the release of IL-8 and at 6 h after infection, a significant increase in the release of TNF-α and IL-1β. The release of IL-10 was significant incremented at 24 h. Blocking experiments revealed that the anti-TLR1, anti-TLR5 and anti-TLR6 decreased about 40%, 20% and 45% respectively in IL-8 secretion in the response to Hp-infection, this revealed that the TLR1, TLR5 and TLR6 participate in IL-8 secretion in response to Hp- infection.

In this study we demonstrated the participation of TLR1, TLR5 and TLR6 in the activation of human neutrophils by H. pylori-infection and TLRs-dependent release of IL-8.

ABSTRACT 151

The response of intracellular Salmonella enterica to TLR4 modulation of the macrophage environment

John Wright, Sabine Totemeyer, Jay Hinton*, Duncan Maskell and Clare E Bryant
Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES.
*Molecular Microbiology Group, Institute of Food Research, Norwich, NR4 7UA

Toll-like receptor 4 (TLR4) is essential to drive the macrophage innate immune response against Salmonella enterica serovar Typhimurium. TLR4 is critical for controlling S. Typhimurium growth in the murine host and plays a key role in regulating host gene expression in response to Salmonella infection. It is unknown, however, whether activation of TLR4, by changing the host phagosomal environment, induces changes in bacterial gene expression. We report the use of microarray analysis of S. Typhimurium gene expression during infection of bone-marrow derived macrophages from wild-type and TLR4-/- mice. Comparative analysis of the bacterial transcriptome reveals 21 genes that are more highly expressed in wild-type macrophages than TLR4-/- cells. The majority of these genes have putative functions in oxidative stress resistance, metal ion transport and acid resistance. In addition, 3 genes of a putative fimbrial operon, yehE, stcA and stcB, are upregulated in wild-type macrophages. Additional array data indicate that these genes are induced in conditions of low pH, oxidative stress and low metal ion concentration, conditions that are present in the phagosome. Deletion of this operon, but not stcA alone, results in reduced spleen and liver colonisation by S. Typhimurium in a mouse typhoid model. Our work is currently focused on defining the individual roles of these genes in infection both in vitro and in vivo and to determine how TLR4 activity influences the expression of these bacterial genes.

ABSTRACT 152

Role of IRF3 and IRF7 in Chlamydophila pneumoniae-mediated IFN-beta response and control of bacterial replication in endothelial cells

Claudia Buß, Juliane Lippmann, Vincent van Laak, Norbert Suttorp, Bastian Opitz and Julia Eitel

Department of Internal Medicine/Infectious Diseases and Pulmonary Medicine, Charité - Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

The innate immune system serves as the first line of defense against invading pathogens. It detects microbial components by pattern recognition receptors (PRRs), some of which are able to regulate type I interferon production (e.g. IFN-beta) via IRF transcription factors. IFN-beta was originally identified as an important factor in antiviral immunity, however, recent studies also indicate a role of this cytokine in immune response against (intracellular) bacteria. The gram-negative, obligate intracellular bacterium Chlamydophila pneumoniae is a common cause of upper and lower respiratory tract diseases and has also been associated with the development of vascular lesions and atherosclerosis.

Here we show that C. pneumoniae induced IFN-beta production in human endothelial cells. Moreover, C. pneumoniae infection led to IRF3 and IRF7 nuclear translocation in HUVECs. RNAi-experiments show that IRF3 and IRF7 as well as the IFN-beta-promoter stimulator-1 (IPS-1) are essential for the induction of IFN-beta. Finally, replication experiments indicate a decrease of bacterial replication in the presence of recombinant human IFN-beta and reveal an increase of the bacterial load in IRF3- and IRF7-siRNA treated HUVECs.

In conclusion, our data indicate a critical role of IPS-1, IRF3, IRF7 and IFN-beta in Chlamydophila pneumoniae-infected human endothelial cells.

ABSTRACT 153

Expression of Toll-Like Receptors in gastric mucosa of children infected with Helicobacter pylori.

Margarita Camorlinga-Ponce1, Sara Huerta-Yepes2, Hugo Lagunas-Servin, Leopoldo Muñoz-Perez1, Armando Madrazo3, Guillermo Ramon4, Javier Torres1, and Carmen Maldonado-Bernal1. 1Unidad de Investigación en Enfermedades Infecciosas, Hospital de Pediatría. Centro Médico Nacional SXXI, IMSS, Mexico City. MEXICO. 2Unidad de Enfermedades Oncológicas, Hospital Infantil de Mexico, Mexico City. 3 Servicio de Gastroenterologia . Hospital de Pediatría. Centro Médico Nacional SXXI, IMSS, Mexico City,4 Laboratorio de patología, Hospital de Pediatría. Centro Médico Nacional SXXI, IMSS, Mexico City. MEXICO

Helicobacter pylori infection is associated with gastric and duodenal ulcer, mucosa-associated gastric lymphoma, and gastric adenocarcinoma. The acquisition of infection usually occurs in childhood. Even with the induction of strong local immune response in infected hosts, H. pylori is capable to establishing chronic infection that can lead in some cases to gastric cancer.

Toll-Like receptors (TLRs) are expressed by many distinct cell types throughout the gastrointestinal tract, to play an important role in the recognition of H. pylori. So far, the expression of TLRs has been extensively studied in vitro, only a few studies have been conducted in vivo. This study analyzed TLRs expression in gastric mucosa in children with and without H. pylori infection.

We analyzed by immunohistochemistry the expression of TLRs 2, 4, 5 and 9 in gastric biopsies. Twenty children were studied, 10 H. pylori infected (mean age 7±1.4 years; 4 males) and 10 without H. pylori infection (mean age 4.5 years; 4 males).
We observed significantly higher expression of H. pylori uninfected-children for TLR2 (3435 ± 1777; 1843 ± 1021 p=0.036) and TLR4 (3878 ± 3700; 886 ± 970 p=0.027), than H. pylori infected-children respectively. There was significantly higher TLR5 expression in H. pylori infected children than uninfected (1864 ± 1574; 4507 ± 3658 p=0.05 respectively), but there was no such difference in the expression of TLR9 (1872± 1718; 2628 ± 2657). Inflammatory cell infiltration was normal to mild in uninfected children and mild to moderate in H. pylori infected children. The results propose that H. pylori in gastric mucosa of children down-regulate TLRs 2 and 4 expression and up-regulate TLR5.

ABSTRACT 154

NOD1 PROTECTS NON-MYELOID CELLS FROM SHIGELLA FLEXNERI-INDUCED CELL DEATH BY PREVENTING MITOCHONDRIAL DAMAGE

L.A. Carneiro1, L.H. Travassos2, J.G. Magalhães2, I. Tattoli1, D.J. Philpott2 and S.E. Girardin1
1Department of Laboratory Medicine and Pathobiology and 2Department of Immunology, University of Toronto, Toronto, ON, Canada

The fate of cells infected by a bacterial pathogen is a complex question that is likely dependent on the nature of both the pathogen and the infected cell. We show here that Shigella flexneri induces a necrotic type of cell death in non myeloid cell through a mechanism that is distinct from all those that have been described in myeloid cells. Importantly, in contrast with the notion of necrosis as an accidental and uncontrolled degeneration, we show that Shigella-induced necrosis in non-myeloid cells is a tightly regulated process that has mitochondrial dysfunction as its central feature. Finally, Nod1, a cytosolic pattern-recognition molecule, plays a crucial role in promoting non-myeloid cells survival by inducing NF-κB activation and preventing mitochondrial damage.

ABSTRACT 155

TLR4 is of primary importance in host defense against Gram-negative infection with Klebsiella pneumoniae but TLR2 "help" is needed when bacterial numbers are high

Catharina W. Wieland1, Arjan J. Hogendijk1, and Tom van der Poll1

1Center of Experimental and Molecular Medicine, Academic Medical Center Amsterdam, The Netherlands

Klebsiella (K.) species are opportunistic pathogens that can give rise to severe diseases such as pneumonia, sepsis, urinary tract infections and soft tissue infections. Typically, Klebsiella infections are nosocomial and mainly caused by the Gram-negative K. pneumoniae, the medically most important species of the genus. We set out to validate and extend our previous data using C3H/HeJ mice that demonstrated an important role for TLR4 in K. pneumoniae pneumonia. By using TLR2 and -4 single and TLR2x4 double knock-out (KO) mice on a C57BL/6 background, the roles of TLR2 and TLR4 were investigated independently and together. We intranasally inoculated C57BL/6 wild-type (WT) and KO mice with K. pneumoniae (4x10E3 Colony Forming Units (CFU) per mouse) and studied host defense. Shortly after infection, both TLR4 and TLR2x4 KO mice showed an attenuated pro-inflammatory response in the lungs. This was associated with higher bacterial counts 24 h after infection in lungs, liver and spleens of both TLR4 and TLR2x4 KO animals. Interestingly, although no differences in antibacterial host defense of TLR2 KO animals were observed, TLR2x4 KO animals were more susceptible to K. pneumoniae infection than the single KO mice: After 44h of infection, 0/8 WT, 0/8 TLR2 KO mice, 5/8 TLR4 KO mice and 8/8 TLR2x4 KO mice had succumbed. Moreover, when infecting all strains with a high dose of K. pneumoniae (10E4 CFU), no differences in outgrowth were detected between WT, TLR2 and TLR4 KO animals, whereas double KO animals suffered from higher bacterial burdens in lungs, liver, spleen and blood. These data confirm our previous research that during low dose infections, TLR4 is of primary importance in host defense against K. pneumoniae. Nevertheless, when high numbers of bacteria are present, TLR2 acts together with TLR4 to orchestrate the immune response.

ABSTRACT 156

Synergistic effects of sex hormone on the expression and function of TLRs in an immortalized fallopian tube cell line

R. Aflatoonian1, W. Aboussahoud1, K.F. Lee2, W.S.B. Yeung2, S.W. Tsao3, and A. Fazeli1
1Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Sheffield, United Kingdom. 2 Department of Obstetrics and Gynaecology, The University of Hong Kong, Pokfulam, Hong Kong.3 Department of Anatomy, The University of Hong Kong, Pokfulam, Hong Kong.

Toll Like Receptors recognise pathogen-associated molecular patterns and constitute a major part of the innate immune system. Little has been done to identify TLRs expression and function under influence of sex hormone in the female reproductive tract, particularly in the fallopian tubes. The aims of this study were to test the synergistic effects of sex hormones on the expression and function of TLRs in an immortalised human fallopian tube epithelial cell line (OE-E6/E7).The OE-E6/E7cell line was treated by estradiol and progesterone and were divided into four groups; control (without any additional treatment of sex hormone), Menstruation (1nM progesterone and 0.1nM estradiol), Pre-ovulation (6.5nM progesterone and1.5nM estradiol) and window of implantation (35nM progesterone and 1nM estradiol). Relative TLRs 1-6 expression quantities were compared between these groups using real time quantitative PCR. In addition the synergistic effect of sex hormones was investigated on the function of TLR3 in OE-E6/E7 cells by treating these cells with poly I:C (25 μg/ml for 24 hours) in these four groups and measuring IL-6 production using ELISA. The expression of TLRs 1-6 was altered in OE-E6/E7 with different concentrations of sex hormones. The highest expression of all the TLR genes was in window of implantation group, compared to all other groups. IL-6 production was significantly increased in the presence of poly (I:C) in OE-E6/E7 cells in all groups. Although presence of increasing levels of sex hormones enhanced TLR3 response to its specific ligand (poly (I:C)) in OE-E6/E7 cells. Further experiments are in progress to elucidate the regulatory mechanism behind this novel effect of sex hormones in modulating innate immunity in the human female reproductive tract.

ABSTRACT 157

Differential TLR binding among porins from pathogenic and non-pathogenic Neisseriae

Paola Massari, Xiuping Liu, Lee M. Wetzler

Department of Medicine, Division of Infectious Diseases, Boston University School of Medicine

PorB porin from N. meningitidis has been shown to directly bind to TLR2 on the surface of transformed HEK 293 cells expressing TLR2. Furthermore, TLR1 is required for cell activation, making Nme PorB a TLR2/TLR1 ligand. A strong correlation between immune cell stimulation via TLRs and immune adjuvant activity has been proposed for several TLR ligands [i.e. CpG DNA (TLR9 ligand), Nme PorB (TLR2/1 ligand)]. We have recently demonstrated that purified porin PorB from the commensal bacterium N. lactamica also acts as a powerful immune adjuvant, similar to porins from pathogenic Neisseriae, and hypothesize a potential role for TLR2 (and possibly other TLRs) on immune cell activation by Nlac PorB.

Using 293 HEK cells stably transfected with various TLR constructs and using fluorescently labeled Nlac PorB, we demonstrate a specific, dose-dependent binding to 293-TLR2 cells by FACS, similarly to Nme PorB. Binding competition experiments using unlabeled Nlac PorB were consistent with a specific binding of the porin to TLR2, as the fluorescence associated with the cells was decreased by excess amount of inhibitor. However, when we used unlabeled Nme PorB as inhibitor, this failed to block binding of Nlac PorB to the surface of 293-TLR2 cells. This could be explained by differences in in co-receptor selectivity or by differences in TLR binding site among these porins. To address the first hypothesis, we used 293-TLR2/TLR1 cells and 293-TLR2/TLR6 cells and determined that Nlac PorB also binds preferentially to the TLR2/TLR1 dimer. To address the second hypothesis, we used Nlac PorB as inhibitor for Nme PorB binding and determined that Nme PorB binding to 293-TLR2 cells was not inhibited by Nlac PorB. Interestingly, when we used Pam3CSK4, a TLR2/TLR1 ligand capable of blocking Nme PorB binding, this also failed to block Nlac PorB binding, suggesting that different binding sites on TLR2 might be engaged by Nlac PorB and Nme PorB. As the immune adjuvant activity of Neisserial porins depends on interaction and signaling via TLRs, we examined whether binding to naive murine lymphocytes could be detected by FACS using fluorescently labeled Nme PorB. B cells and macrophages from C57Bl/6 WT mice and TLR2 KO mice were examined and, surprisingly, Nme PorB was able to bind to the surface of B cells and macrophages from both WT and TLR2 KO mice. This could be explained by the fact that, as opposite to 293-HEK cells, B cells and macrophages normally express a whole range of TLRs, some of which might compensate for the ablation of TLR2. Nme PorB binding was inhibited by excess amounts of both Nme PorB and Nlac PorB, again suggesting that the contribution of other TLRs can not be ruled out, although TLR2 may or may not be expressed on the cell surface. similarly to B cells, binding of Nme PorB to WT macrophages was also detected.

ABSTRACT 158

THE MACROPHAGE INDUCIBLE C-TYPE LECTIN, MINCLE, IS AN ESSENTIAL COMPONENT OF THE INNATE IMMUNE RESPONSE TO CANDIDA ALBICANS

C.A.Wells and R.B. Ashman

Griffith University; The University of QLD

The recognition of carbohydrate moieties by cells of the innate immune system is emerging as an essential element in anti-fungal immunity - likewise increasing attention is directed to the C-type lectin family of pattern recognition receptors. Mincle, a novel c-type lectin, is here shown to play an important role in macrophage responses to the yeast Candida albicans. Gene profiling demonstrated a shared pattern of expression between Mincle and Tlr2 in primary mouse macrophages exposed to live C. albicans. Co-regulation of Mincle and Tlr2 implied a functional role for Mincle in the response of macrophages to Candida infection. Immunofluorescence identified endogenous Mincle on the cell surface, and during yeast ingestion Mincle was found concentrated at the nascent phagocytic cup. Pre-incubation of RAW264.7 cells with the Mincle antibody resulted in dose-dependent inhibition of TNF in response to live Candida. The most prominent reduction occurred at a 1:300 secretion.αdilution of original IgG antibody with up to 60% reduction in TNF- Likewise macrophages isolated from KO mice consistently produced significantly than isogenic controls in response to Candida stimulation,αless TNF- & in some experiments the yeast-stimulated KO BMM produced no TNF at all. Loss of functional Mincle had no effect on the number of yeast particles phagocytosed nor on the number of macrophages clearing yeast. These data demonstrated that production by Mincle was not dependent on yeast uptake,αthe regulation of TNF- but rather indicated that Mincle may mediate inflammatory signalling pathways. After systemic challenge with Candida, the magnitude of the fungal burden in the kidneys of KO mice was significantly greater than in the controls. This observation provides compelling evidence for an essential role for Mincle in innate immune recognition and clearance of the yeast infection in vivo.

ABSTRACT 159

The role of Staphylococcal Panton-Valentine Leukocidin during lung inflammation in vivo

Ana Zivkovic 1,2, Karin Stich 2, Omar Sharif 1,2, Ulrich Matt 1,2, Isabella Haslinger 2, Tanja Furtner 1,2, Sylvia Knapp 1,2

1 Research Center for Molecular Medicine (CeMM) of the Austrian Academy of Sciences , Vienna

2 Department of Medicine I, Div. of Infectious Diseases and Trop. Medicine, Medical University Vienna

Panton-Valentine Leukocidin (PVL) positive Staphylococcus aureus (S. aureus) is an emerging pathogen associated with highly lethal necrotizing pneumonia. PVL is beta-barrel pore-forming toxin that binds to polymorphonuclear cells causing cell death. However, the precise mechanisms leading to necrotizing pneumonia are not fully understood and the role of PVL herein is controversial.

We therefore aim to investigate the molecular mechanisms underlying PVL-associated lung inflammation and necrosis and to understand the synergistic potential with well-known S. aureus ligands such as lipoteichoic acid (LTA). We produced PVL and investigated the biological properties by assessing the cytotoxic and proinflammatory potential. Human neutrophils proved to be highly susceptible to our purified PVL, which was quantified by LDH release. Monocytes seemed less susceptible to PVL alone, whereas the simultaneous addition of LTA greatly enhanced this effect. Being primarily interested in PVL-associated respiratory tract infections we investigated the sensitivity of alveolar macrophages but found these cells able to resist the cytotoxic properties of PVL. However, when administering PVL to mouse lungs we found the toxin to elicit a modest inflammatory response, indicated by the rapid induction of cytokines and cell influx. This inflammatory response was greatly enhanced in the presence of the Toll-like receptor 2 ligand LTA, which itself is known to importantly induce lung inflammation in vivo. Thus far our in vivo data demonstrate that PVL exerts cytotoxic and inflammatory properties in the presence of additional S. aureus ligands. Further in vivo studies should help clarify the detailed biological mechanisms of this synergistic effect.

ABSTRACT 160

MyD88 dependent nitric oxide, but not reactive oxygen species are required for clearance of streptococcal DNA by macrophages

Sachin D. Deshmuk, Bernhard Kremer, Kristina Brückner, Douglas T. Golenbock* and Philipp Henneke

Centre for Pediatrics and Adolescent Medicine, University Medical Centre, Freiburg, Germany
*Dept. of Medicine, University of Massachusetts Medical Center, Worcester, MA, USA

Pattern recognition receptors for bacterial substructures are expressed in distinct subcellular compartments of macrophages. Accordingly, it seems pivotal that during the sequence of cell-to-cell contact, phagocytosis and endosomal maturation bacterial substructures are generated in order to make them accessible for these receptors. In this study we aimed to unravel mechanisms which underlay degradation of bacterial DNA, which represents an important microbial pattern for Toll-like receptors (TLRs) and cytosolic receptors like DAI. We found that expression of MyD88 was essential for the digestion of DNA from group B streptococcus (GBS). Among three putative mediators of DNA degradation, only DNASe IIa and of nitric oxide (NO), but not reactive oxygen species (ROS) were induced in a MyD88-dependent fashion. Furthermore, macrophages from mice with a targeted deletion of the NADPH oxidase gp91phox, which are completely devoid of ROS, showed enhanced degradation of bacterial DNA and nitric oxide formation. In conclusion, these data highlight the potential role of MyD88 and NO, but not ROS in the degradation of streptococcal DNA. It remains to be established, whether this process propagates or terminates receptor-based inflammatory signaling.

ABSTRACT 161

RESISTANCE OF MACROPHAGES TO LEGIONELLA PNEUMOPHILA REQUIRES A CONCERTED ACTION OF THE TRANSCRIPTIONAL REGULATORS IRF1/IRF8 AND THE NOD-LIKE RECEPTOR NAIP5

Anne Fortier 1, 2 and Philippe Gros 1, 2

1 Department of Biochemistry and 2 Center for the Study of Host Resistance, McGill University, Canada

Intracellular replication of Legionella pneumophila in murine macrophages is genetically controlled by two genes members of the Nod-like receptors family, Naip5 and Nlrc4. A model has been proposed in which recognition of Flagellin by Naip5 and processing and Nlrc4 leads to inflammasome assembly, resulting in IL-1b caspase-1-dependent cell death. However, this model can not fully explain some of the early effects of Naip5 and Nlrc4 on L. pneumophila-containing phagosomes maturation detected 1 hour post infection. In order to identify genes necessary to prevent intracellular replication of L. pneumophila, and which activity is regulated by early Naip5 signaling, we used transcript profiling of total RNA from Naip5-deficient and Naip5-sufficient macrophages, prior to and 4 hours following infection with L. pneumophila. Irf1 was found to be up-regulated by L. pneumophila infection and its induction further modulated by Naip5 status. Here, we show that macrophages bearing a loss-of-function mutation at either Irf1 or at its binding partner Irf8 are permissive to L. pneumophila replication. Deficiency in Irf1 and Irf8 does not result in a generalized defect in bactericidal activity as Irf1-/- and Irf8R294C macrophages are capable of efficient killing of an avirulent dotA mutant. Although Irf1/Irf8 targets are highly expressed in Naip5-deficient mice in)(IL-12p40, iNOS, and IFN-y response to L. pneumophila, they are not sufficient for resistance, a finding supported by the resistance of IL-12p40-/- macrophages to L. pneumophila infection. These results suggest a link between L. pneumophila sensing by the NLR protein Naip5 and Irf1/Irf8 transcriptional activation of a number of effector genes outside the IL-12/IFN-y loop but that play a critical role in immune response to Legionella pneumophila.

ABSTRACT 162

Impact of TLR2 on non-oxidative and oxidative killing mechanisms of PMN against Staphylococcus aureus

N. Jann[1], S. Kristian[2], A. Peschel[3], R. Gallo□, R. Landmann[1]

[1]Dept. Biomedicine, University Hospital Basel, Switzerland

[2]Dept. Pediatrics, University of California, San Diego, USA

[3]Dept. Medical Microbiology and Hygiene, University of Tubingen, Germany

□Dept. Medicine, University of California, San Diego, USA

PMN kill engulfed bacteria by antimicrobial peptides (AMPs), reactive oxygen species (ROS) and formation of neutrophil extracellular traps (NETs) by NADPH oxidase. Staphylococcus aureus (SA) is resistant to AMPs, i.e. CRAMP (cathelin-related antimicrobial peptide) in mice, due to alanylation of teichoic acids (TA) and to ROS by antioxidative enzymes. In a local infection model in C57BL/6 mice, SA wt persisted while a mutant with dealanylated TA (ΔdltA) was cleared. In TLR2-/- mice, wt and ΔdltA proliferated better, but wt more than ΔdltA. Thus, we postulated a TLR2-regulation of PMN killing mechanisms. Here we show, that SA wt and ΔdltA grew similar in CRAMP-/- mice in vivo and in the presence of CRAMP-/- PMN in vitro. Involvement of TLR2 in regulation of CRAMP was investigated by studying CRAMP expression in TLR2-/- PMN. We found that CRAMP was similarly expressed and colocalized with phagocytosed SA in C57BL/6 and TLR2-/- PMN. These findings excluded a role of TLR2 in non-oxidative killing. Further, its function in oxidative and NET-dependent killing was studied. TLR2-/- and gp91phox-/- PMN (gp91phox: NADPH oxidase subunit) showed similar killing of SA but reduced compared to C57BL/6 PMN. In TLR2-/- PMN, superoxide production induced by SA was delayed compared to C57BL/6 PMN. Induction of NETs by SA was independent of TLR2. Summarized, TLR2 enhances oxidative killing of SA possibly regulating NADPH oxidase assembly.

ABSTRACT 163

NOD2 is essential for induction of IL-17 production by Borrelia burgdorferi sensu lato.

Frank L. van de Veerdonk, Bart-Jan Kullberg, Jos W. M. van der Meer, Mihai G. Netea, and Leo A.B. Joosten.

Department of Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Borreliosis is an infectious disease caused by pathogenic spirochetes of the Borrelia burgdorferi (Bb) sensu lato. Chronic borreliosis is associated with arthritis, acrodermatitis chronica atrophicans and neurological involvement dependent on the subspecies of B.b. In the present study we investigated which members of the PRR family is involved in the B.b.induced cytokine production. Human PBMC's as well as murine peritoneal macrophages and splenocytes were exposed to B.b. or B. afzelii (B.a.) Cytokine production of macrophages and splenocytes from TLR2-/- or TLR4-/- mice were examined. In addition, PBMC's isolated from healthy controls and individuals homozygous for the frameshift mutation 3020insC Nod2 (Nod2fs) were exposed to Borrelia species. Here we showed that production cytokines after recognition of B.b. or B.a., such as TNF and IL-1 is partly dependent on TLR2. In contrast, TLR4 is not involved in the production of these cytokines. Of high interest, PBMC's from individuals bearing Nod2fs mutation exposed to Borrelia species showed impaired cytokine production, especially IL-17. These data indicted that apart from TLR2, Nod2 is also involved in the Borrelia-induced cytokine production. Since several polymorphisms are known for both TLR2 and Nod2, it is tempting to speculate that an impaired function of either TLR2 or Nod2 may lead to disturb innate immune response to Borrelia species. However, these mutations may have a protective effect in the autoimmune processes of chronic borreliosis due to the lack of IL-17 production.

ABSTRACT 164

Stachybotrys chartarum and macrocyclic trichothecene mycotoxins activate inflammatory response in human macrophages

Päivi Kankkunen, Johanna Rintahaka, Annika Aalto, Marina Leino, Marja-Leena Majuri, Harri Alenius, Henrik Wolff, and Sampsa Matikainen

Finnish Institute of Occupational Health

Stachybotrys chartarum is a well-known damp building mold which is associated to wide variety of health problems. Macrocyclic trichothecene mycotoxins produced by S. chartarum have been proposed to be the main reason for the symptoms. In this report we have studied the effect of S. chartarum and macrocyclic trichothecene mycotoxins, satratoxins and roridin A, on pro-inflammatory cytokine response in human macrophages. S. chartarum activated IL-1β and IL-18 mRNA expression in human macrophages. Furthermore, satratoxin positive S. chartarum and LPS synergistically enhanced IL-1β and IL-18 mRNA expression. This co-stimulation also synergistically enhanced secretion of these cytokines. Furthermore, satratoxin positive S. chartarum, but not satratoxin negative S. chartarum, activated caspase-1, which is needed for proteolytic processing of IL-1β and IL-18 before they are secreted. Similarly, only a satratoxin positive strain of S. chartarum activated caspase-3 which is an effector caspase of apoptosis. In line with these results, roridin A, a mycotoxin related to satratoxin, activated caspase-1 and caspase-3. Roridin A also strongly enhanced LPS-dependent secretion of IL-1β and IL-18. In conclusion, our results suggest that satratoxins and roridin A together with LPS activate inflammasome-associated caspase-1 and secretion of IL-1β and IL-18 in human macrophages. This synergistic effect of mycotoxins and LPS may explain some of the adverse health effects associated to damp building syndromes.

ABSTRACT 165

Role of Nod1/2 proteins in the autophagic response against intracellular bacteria

Leonardo H. Travassos1, Seamus Hussey1, Linda Yuan1, Leticia de A.M Carneiro3, João G. Magalhaes1, Lionel Le Bourhis1, Kauru Geddes1, Nicola Jones2, Stephen E. Girardin3, and Dana J. Philpott1

1 Department of Immunology and 3 Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, and 2 Hospital for Sick Kids, Toronto, Ontario

Autophagy is a degradation pathway that plays a major role in keeping cells free of long-lived proteins and organelles. During the induction of autophagy parts of the cytoplasm are engulfed by double- or multiple-membrane structures called autophagosomes that will eventually fuse with lysosomes. In the past few years, several studies have highlighted a new protective role for autophagy in the clearance of intracellular bacteria that reach the cytosol, but how they are specifically targeted by autophagy is not clear. Nod2 (and possibly Nod1) are two susceptibility genes for instestinal bowel disease (IBD) that are involved in intracellular bacterial detection. Here, we investigated the putative role of Nod1 and Nod2 in the autophagic response triggered by intracellular bacteria. Through the analysis of the cellular distribution of the autophagy marker GFP-LC3 by fluorescence microscopy and the convertion of LC3-I to LC3-II by western blotting, we present evidence that while Nod1 and Nod2 are not required for basal and stress-induced autophagy, Nod1-deficient mouse embryonic fibroblasts display a decreased autophagic response upon infection with Shigella flexneri or Listeria monocytogenes. In addition, we also observed that in Nod1-deficient cells the number of bacteria present in autophagosomes is significantly reduced in comparison with wild-type cells. Our results identify, for the first time, a link between intracellular detection of bacteria by Nod-like receptors and the induction of autophag.

ABSTRACT 166

Expression of Toll-like receptors 2, 4 and 5 in childrens infected with Helicobater pylori, quantification of cytokines and characterization of the strains

Lagunes HE1, Maldonado C.1, Madrazo A2, Torres J 1, Camorlinga M1

1Laboratory of Bacteriology. Hospital of Pediatrics CMNS XXI.

Service of Gastroenterology. Hospital of Pediatrics CMNS XXI. México, D.F.

We collected blood and gastric biopsies from 32 children with chronic abdominal pain, 13 Hp+ (5 Males; mean age 7 years (range 3-16) and 19 Hp- (8 Males; mean age 4.5 years (range 2 - 15). Mononuclear cells were separated from blood. Expression analysis was performed by quantitative real-time PCR with Taqman (Applied). TLR2 (Hp- 3.50±1.68 vs Hp+ 2.75±1.93) and TLR4 (Hp- 4.76±2.48 vs Hp+3.70±2.41), TLR5 (Hp- 3.42± 1.63 vs Hp+ 3.76±1.54). Cytokines in the plasma of both groups were quantified by ELISA, and did not show significant differences: IL-8 (10.98±12.3 vs 3.35±0.5 pg/ml); IL-10 (24.9±9.6 vs 20.5±6.6 pg/ml); TNF-α 18.74±1.8 vs 14.8±3.0 pg/ml). The level of expression of TLR2 and 4 in Hp- children was lower than Hp+, TLR5 showed a higher expression level in Hp+ children.

ABSTRACT 167

Helicobacter flagellins evade physical binding to TLR5, but bind to epithelial cells and may influence cellular signalling

Anna Leibo1, Sae Kyung Lee2, Daniela Goeppel1, Fang Ye1, Elena Katzowitsch2, Oliver Dittrich-Breiholz5, Michael Kracht5, Torsten Sterzenbach1,2, Lena Alexopoulou4, Shin Ichi Aizawa3, Sebastian Suerbaum1,2, Christine Josenhans1,2§*
1) Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School, 30625 Hannover, Germany

2) Institute for Hygiene and Microbiology, University of Wuerzburg, Wuerzburg, Germany
3) Prefectural University of Hiroshima, Shobara, Japan

4) Centre d'Immunologie de Marseille-Luminy, Marseille, France

5) Institute for Pharmacology, Hannover Medical School, Hannover, Germany

Flagellins of the chronic gastric pathogen Helicobacter pylori and related bacteria have been shown to possess only low potential to activate proinflammatory signalling in gastric and intestinal epithelial cells, which possess active TOLL-like receptor 5 (TLR5) (Lee et al., 2003; Gewirtz et al., 2004, Andersen-Nissen et al., 2005). Flagellins of numerous other pathogenic and non-pathogenic bacterial species are potent activators of the human innate immune system and of proinflammatory responses by binding to TLR5. Evasion of TLR5-mediated responses appears to be a potent strategy for persistent bacteria such as H. pylori to evade the human innate and adaptive immune responses. The mechanism of the flagellar immune evasion by H. pylori was not known.We show here by co-immunoprecipitation and binding assays that both H. pylori flagellins, FlaA and FlaB, which both have low activity to induce NF-kB activation and IL-8 release via TLR5, do evade stable physical binding to TLR5, but still bind to human and mouse cells. Moreover, H. pylori flagellins can induce signalling in human gastric epithelial cells as determined by microarray hybridisation and RT PCR. In order to assess the contribution of TLR5 to these signalling events, primary mouse embryonal fibroblasts (MEF) of TLR5-/- mice were employed (Feuillet et al., 2006). Activation assays of primary MEF and comparative microarray hybridisations suggested that regulation events are induced by H. pylori flagellins, largely independently of TLR5.

Lee et al., 2003, Mic. Infect. 5(15):1345-56.
Gewirtz et al., 2004, J. Infect Dis. 189(10):1914-20.
Andersen-Nissen et al., 2005, Proc. Natl. Acad. Sci. U.S.A. 102(26):9247-52.
Feuillet et al., 2006, Proc. Natl. Acad. Sci. U.S.A. 103(33):12487-92.

ABSTRACT 168

Role of human ZBP1 (DLM-1/DAI) in IFNb responses induced by intracellular bacteria or cytosolic DNA

Juliane Lippmann[1], Stefan Rothenburg[2], Nikolaus Deigendesch[3], Julia Eitel[1], Karolin Meixenberger[1], Vincent van Laak[1], Norbert Suttorp[1] and Bastian Opitz[1]

[1]Department of Internal Medicine/Infectious Diseases and Pulmonary Medicine, Charité Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany; [2]National Institutes of Health, National Institute of Child & Human Development, Bethesda Md 20892-2427, USA [3]Institute of Immunology, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Intracellular bacteria and cytosolic stimulation with DNA activate type I IFN responses independent of Toll-like receptors, most Nod-like receptors and RIG-like receptors. A recent study suggested that murine ZBP1 (DLM-1/DAI) represents the long anticipated pattern-recognition receptor (PRR) which mediates IFN□□ responses to cytosolic DNA.
Here we show that infection with *Legionella pneumophila* or *Listeria monocytogenes*, and intracellular challenge with poly(dA-dT), but not with poly(dG-dC), induced expression of IFN□, full-length ZBP1 and a prominent splice variant lacking the first Z□ domain (ZBP1□Z□). Overexpression of ZBP1 or ZBP1□Z□ did not activate IRF3, NF□B or IFN□ luciferase reporters, and only slightly amplified poly(dA-dT)-stimulated IFN□ reporter activation in HEK293 cells. Ectopic expression of ZBP1 or ZBP1□Z□ in A549 cells had no effect on IFN□ and IL-8 production induced by bacteria or poly(dA-dT). Multiple ZBP1 siRNAs had little effect on IFN□ or IL-8 expression induced by poly(dA-dT) or bacterial infection in epithelial or monocytic cells, while IRF3 siRNA strongly impaired the IFN□ responses.
In conclusion, our data do not support an essential role of ZBP1 in IFNβ responses to intracellular bacteria and cytosolic poly(dA-dT).

ABSTRACT 169

Suppression of cell-mediated immunity by toll signaling from avirulent Listeria monocytogenes

Nicole Meyer-Morse, Keith S. Bahjat, Edward E. Lemmens, Thomas W. Dubensky Jr., Dirk G. Brockstedt and Daniel A. Portnoy

University of California at Berkeley; Anza Therapeutics

Listeria monocytogenes is a Gram-positive facultative intracellular pathogen that is capable of inducing a robust CD8 T cell response during infections. Bacterial entry into the cytosol is required to induce this immune response. We found that increasing the percentage of vacuolar-trapped bacteria in the immunizing inoculum resulted in a concomitant decrease in pro-inflammtory cytokines as well as a decrease in L. monocytogenes specific T cells. Further, vacuolar trapped bacteria actively blocked the immune response to cytosolic bacteria through MyD88 signaling resulting in IL10 expression. Eliminating MyD88 signaling or specifically blocking the IL10 receptor restored immunity in mice immunized with the combination of vacolar and cyosolic bacteria. Memory T cell proliferation was also restored to wild type levels, in the absence of IL10 receptor signaling. This suppression was not limited to vacuolar L. monocytogenes, as we were able to reproduce these results when mice were immunized with cytosolic bacteria in combination with Bacilis subtilis, a non-pathogenic Gram-positive bacterium. These data suggest that MyD88 dependent IL10 induction is a dominant negative signal eminating from the vacuole that blocks the immunizing capability of the cytosolic bacteria. We propose that cells use compartmentalization (vacuolar versus cytosolic) as a signal to determine when to mount an immune response to a potential pathogen.

ABSTRACT 170

Primary murine bone marrow macrophages can be reinfected by Salmonella enterica serovar Typhimurium in vitro

Murcia A.1*, McKinley T.J.1, Restif O.1, Wood J.L.N.1, Maskell D.J.1, Gog J.R.2, Bryant C.E.1

1Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES; 2Department of Applied Mathematics and Theoretical Physics, University of Cambridge,Wilberforce Road, Cambridge CB3 0WA (ceb27@cam.ac.uk)

The aim of this study was to investigate whether murine macrophages already infected with Salmonella enterica serovar Typhimurium could be reinfected. S. Typhimurium (SL1344) was labelled with two different fluorescent proteins, either GFP or DsRed. We defined reinfection events as internalization of differently labelled bacteria in the same macrophage at separated times. Single (e.g. infected with one labelled bacteria type) or sequential (e.g. infected with one labelled bacteria type for a given time before the second labelled bacteria was added) infection experiments in vitro were performed and visualised. Observation by direct microscopy showed that a significant proportion of macrophages were co-infected with red and green-labelled bacteria in sequential infections, which was statistically higher than expected by chance. However, the possibility that multiple, differentially labelled bacteria were internalized simultaneously cannot be excluded. Under the null hypothesis that reinfection events do not occur, the proportion of cells infected with the first bacteria should not decrease when the second one is added. Our experiments showed a statistically significant decrease in the percentage of cells infected with the first labelled bacteria upon addition of the second bacteria in sequential infections with $p=0.0193$. Our experimental data show that the decrease in the percentage of cells infected with the first labelled bacteria occurs upon addition of a second labelled bacteria, suggesting that macrophages already infected by Salmonella can accept a second bacterium and thus providing strong evidence that reinfection events do occur.

ABSTRACT 171

Killed Propionibacterium acnes modulation on TLRs, co-stimulatory, MHCII molecules expression and cytokines synthesis of B1 lymphocytes subsets from mice peritoneal exudate cells

Juliana S. Mussalem, Tatiana M. Yendo, Carla C. Squaiella, Ieda M. Longo-Maugéri
Departamento de Micro, Imuno e Parasitologia, Universidade Federal de São Paulo, SP, Brasil

B1 cells are a subset of B lymphocytes that express macrophage and lymphocyte markers on membrane. Among B1 cells, the major subset is B1b. Enriched B1b culture can be obtained from mice exudate peritoneal adherent cells supernatant on 5th culture day. When this subset is re-cultivated, it differentiates to lymphophage. The modulation of B1 lymphocytes by biological adjuvants is not much explored, leading us to analyze B1 cells from mice treated with killed Propionibacterium acnes (P. acnes) or its soluble polysaccharide (PS), once bacteria suspension acts as adjuvant on macrophage and lymphocyte function. So, we evaluated macrophages and B1 cells in vivo, analyzing the activation status of these cells by TLR, co-stimulatory molecules and MHCII expression and cytokines synthesis, and how these populations would behave in a B1b enriched culture, concerning TLR expression. P. acnes elevated all B1 lymphocytes in vivo and B1b remained the major subset, so our focus was mainly on this subset. Bacteria up-regulated B1b cell number, expression of co-stimulatory molecules, intracellular cytokines, TLR2, 4 and 9, but diminished B1b cells expressing MHCII. PS is involved in cell activation, elevating the number of TLR2, 4 and co-stimulatory molecules on B1b surface. In vitro the B1b number decreased, and TLR2, 4 and 9 expression was higher than control. Our results indicate that P. acnes modulated in vivo B1b, and the cell activation state is kept in vitro.

Support: FAPESP, CNPq, CAPES

ABSTRACT 172

Dendritic cells restrict intracellular Legionella pneumophila replication by activating caspase-3-dependent apoptosis

Catarina Nogueira 1,2 and Craig Roy1

1 Section of Microbial Pathogenesis, Yale University School of Medicine, New Haven, Connecticut 06536, USA

2 Instituto de Ciencias Biomedicas Dr Abel Salazar, Universidade do Porto, Porto, Portugal

Dendritic cells (DCs) play a crucial role in pathogen detection and induction of subsequent innate and adaptive immune responses to eliminate pathogens from the infected host. Intracellular pathogens present a unique challenge to DCs, as they are capable of infecting these cells and manipulating host cell functions. To understand how immune responses are generated against intracellular pathogens that can infect DCs, we examined the interactions between DCs and the intracellular bacterial pathogen Legionella pneumophila. We have previously shown that although L. pneumophila can replicate inside macrophages, L. pneumophila fails to replicate within DCs. The mechanism underlying how DCs restrict bacterial replication is unknown. Here, we find that upon L. pneumophila infection, DCs activate caspase-3-dependent apoptosis in response to virulent L. pneumophila that use a functional type IV secretion system (TFSS) to inject effector proteins into the host cytosol but not avirulent mutants lacking a TFSS. The absence of caspase-3 renders DCs permissive to L. pneumophila replication. Given that DCs restrict the replication of many intracellular pathogens, this may be a common strategy used by DCs to control bacterial dissemination.

ABSTRACT 173

LPS primes Dictyostelium discoideum amoeboid cells for bacterial clearance

Walk, P. Srisawangvong, D. Cassilly, D. Samaroo, and M. Snyder

Dept. of Biological Sciences, Towson University, Towson, MD

Mammalian innate immune cells detect pathogen-associated molecular patterns (PAMPs) using evolutionarily-conserved pattern-recognition receptors that can be studied using a variety of model organisms. Dictyostelium discoideum is a unique model organism that exists for part of its lifecycle as single-celled amoebae that phagocytize bacteria for nutrient uptake. It has not been appreciated whether D. discoideum use pattern-recognition receptors to detect bacterial prey. Here we show that D. discoideum amoeboid cells indeed respond to known PAMPs, as exposure to the bacterial cell wall product LPS increases the rate of clearance of phagocytized bacteria from D. discoideum cells. The increased killing of bacteria may be related to the induction of reactive oxygen species in D. discoideum cells by the bacterial cell wall products LPS and peptidoglycan. In addition, as measured by qRT-PCR, exposure of D. discoideum amoeboid cells to LPS and peptidoglycan upregulates the expression of genes homologous to those known to function in microbial recognition. Upregulated genes include a putative scavenger receptor and a gene containing the toll/interleukin-1 receptor domain. We are currently creating D. discoideum cells that overexpress or are deficient for these gene products. The characterization of such genes should provide insight into conserved molecular mechanisms underlying microbial detection.

ABSTRACT 174

Naip and Ipaf control Legionella pneumophila replication in human cells

Vinzing M, Eitel J, Lippmann J, Hocke AC, Zahlten J, Slevogt H, N`Guessan PD, Günther S, Schmeck B, Hippenstiel S, Flieger A, Suttorp N, Opitz B
Charité University Medicine Berlin

In mice, different alleles of the mNAIP5 (murine neuronal apoptosis inhibitory protein-5)/mBirc1e gene determine whether macrophages restrict or support intracellular replication of Legionella pneumophila, and whether a mouse is resistant or (moderately) susceptible to Legionella infection. In the resistant mice strains, the nucleotide-binding oligomerization domain (Nod)-like receptor (NLR) family member mNAIP5/mBirc1e, as well as the NLR protein mIpaf (murine ICE protease-activating factor), are involved in recognition of Legionella flagellin and in restriction of bacterial replication. Human macrophages and lung epithelial cells support L. pneumophila growth, and humans can develop severe pneumonia (Legionnaires disease) after Legionella infection. The role of human orthologs to mNAIP5/mBirc1e and mIpaf in this bacterial infection has not been elucidated. Herein we demonstrate that flagellin-deficient L. pneumophila replicate more efficiently in human THP-1 macrophages, primary monocyte-derived macrophages, and alveolar macrophages, and in A549 lung epithelial cells compared with wild-type bacteria. Additionally, we note expression of the mNAIP5 ortholog hNAIP in all cell types examined, and expression of hIpaf in human macrophages. Gene silencing of hNAIP or hIpaf in macrophages or of hNAIP in lung epithelial cells leads to an enhanced bacterial growth, and overexpression of both molecules strongly reduces Legionella replication. In contrast to experiments with wild-type L. pneumophila, hNAIP or hIpaf knock-down affects the (enhanced) replication of flagellin-deficient Legionella only marginally. In conclusion, hNAIP and hIpaf mediate innate intracellular defense against flagellated Legionella in human cells.

ABSTRACT 175

Unexpected role for PTEN in the inflammatory response to Streptococcus pneumoniae induced pneumonia in mice

Gernot Schabbauer, Ulrich Matt, Philipp Günzl, Tanja Furtner, Thomas Perkmann, Barbara Sokolikova, Bernd Binder and Sylvia Knapp

Medical University Vienna, Vienna, Austria

The role of the Phosphoinositide-3-Kinase/PTEN pathway in acute inflammatory disease is controversial. PI3-Kinase is described as essential signaling component for chemotactic influx of inflammatory cells, required to fend off pathogens. On the contrary, PI3-Kinase exerts immune-modulatory properties limiting the amplitude of the acute inflammatory response to infection, thereby preventing host tissue damage.
We hypothesized that ablation of the endogenous PI3-Kinase antagonist PTEN modulates the inflammatory response in pneumococcal pneumonia. To investigate the role of this signaling pathway in Streptococcus pneumoniae induced pneumonia, we analyzed the immune response of infected mice carrying a monocyte/macrophage/granulocyte specific pten gene deletion.

In vitro, we observed sustained activation of the PI3-Kinase/AKT/GSK3ß signaling axis in response to Streptococcus pneumoniae in PTEN deficient macrophages. Simultaneously, expression of pro-inflammatory mediators was significantly reduced. Notably, phagocytosis of Streptococci in PTEN deficient macrophages was enhanced.
In vivo, PTEN deficient mice exhibited a diminished immune response to Streptococcus pneumoniae infection. Unexpectedly, we found reduced neutrophil influx and low levels of pro-inflammatory molecules in the broncho-alveolar space. PTEN deficiency led to reduced inflammation in the broncho-alveolar compartment, as measured by myeloperoxidase expression and cytokine release. Ablation of PTEN in neutrophils and macrophages led to improved clinical scores and delayed significantly the detrimental course of the disease.

PI3K and PTEN play a surprising role in the immune response to pneumococcal lung infection. PTEN deficiency, which results in constitutively elevated PI3K activity, reduces inflammatory damage and improves survival.

ABSTRACT 176

NOD/RIP2 SIGNALING IN C. PNEUMONIAE LUNG INFECTION

Kenichi Shimada1, Shuang Chen1, Paul W. Dempsey2, Anatoly V. Slepenkin3, Randa Alsabeh1, Ellena Peterson3, Rosalinda Sorrentino1, Timothy Crother1, Moshe Arditi1.
1Cedars-Sinai Medical Center and 2UCLA and 3UCI, Los Angeles, CA 90048, USA.
Background: Cytosolic Nod proteins and the adaptor (Rip2) participate in host immune responses. We investigated the importance of NODs/Rip2 signaling in host responses to C. pneumoniae (Cpn) lung infection.

Methods: We infected 8-wk old WT or Rip2-/- mice intratracheally with Cpn (1x106 IFU) and sacrificed them at day 3, 5, 14, and 35. Bronchoalveolar lavage (BAL) was collected to measure cell count and inflammatory cytokine/chemokine release (ELISA). Lungs were analyzed for histopathology and lung homogenates were used for cytokine/chemokine levels, quantitative bacterial culture and to identify infiltrating cells by FACS. Bone marrow (BM) chimeras were generated to investigate the relative contribution of BM derived hematopoietic cells vs. non-BM derived stromal cells in host responses against Cpn.

Results: Rip2-/- mice infected with Cpn exhibited γdelayed neutrophil recruitment to the lungs early on (day 3). IL-6 and IFN- level as well as KC and MIP-2 in BAL were lower in Rip2-/- mice compared to WT mice at day3. Rip2-/- mice were unable to clear the bacteria from their lungs at day 5 and 14, had increased lung inflammation compared to WT mice and even at day 35 they continued to exhibit severe lung inflammation thath led to increased mortality ($p < 0.05$). WT mice cleared the bacteria and recovered. We examined the localization of Cpn in Lungs after infection by FACS. Cpn mostly localized in macrophages and neutrophils. Quantitative bacterial lung cultures in infected bone marrow chimeric mice revealed that Rip2 in BM-derived cells rather than non-hematopoietic stromal cells play a role in bacterial host responses to clear the bacteria in lungs.

Conclusion: The Nod/Rip2 pathway contributes to the recognition of C. pneumoniae infection and host immune responses that lead to the clearance of the bacterium from infected lungs.

ABSTRACT 177

Immune detection of type IV secretion activates MAP kinases to discriminate between virulent and avirulent bacteria

Sunny Shin1, Christopher L. Case1, Kristina A. Archer1,3, Koichi S. Kobayashi2, Richard A. Flavell3,4, Craig R. Roy1, and Dario S. Zamboni1,5

1 Section of Microbial Pathogenesis, Yale University School of Medicine, New Haven, Connecticut 06536, USA, 2 Section of Cancer Immunology and AIDS, Dana-Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115, USA, 3 Department of Immunobiology, Yale University School of Medicine, New Haven, Connecticut 06520, USA, 4 Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06520, USA, 5 Present address: Department of Cell Biology, University of São Paulo, Medical School Ribeirão Preto, 14049-900 Ribeirão Preto, SP, Brazil

How the immune system distinguishes pathogenic from nonpathogenic microbes and initiates an appropriate response remains poorly understood. Infection with the pathogen Legionella pneumophila, which utilizes a type IV secretion system (T4SS) to deliver bacterial proteins into host cells, caused an increased pro-inflammatory response compared to avirulent mutants in which the T4SS was inactivated. This enhanced response involved NF-κB activation by TLR-dependent detection of Legionella and TLR-independent detection of the T4SS by Nod1 and Nod2. A TLR- and RIP2-independent pathway leading to p38 and SAPK/JNK MAP kinase activation was also found to play an important role in the immune response to virulent Legionella. Activation of this MAP kinase pathway was T4SS-dependent and coordinated with TLR signaling to mount a robust response to virulent Legionella. These findings demonstrate that coincident detection of multiple bacterial determinants enables immune discrimination of virulent from avirulent bacteria and define a previously uncharacterized immune response to bacterial type IV secretion that activates MAPK signaling.

ABSTRACT 178

Modulation of the type I hypersensitivity to OVA by killed-Propionibacterium acnes regulating the expression of TLRs and

co-stimulatory molecules on APCs

Carla C. Squaiella, Juliana S. Mussalem, Tatiana M. Yendo, Ieda M. Longo-Maugéri
Departamento de Micro, Imuno e Parasitologia, Universidade Federal de São Paulo, SP, Brasil

Heat-killed Propionibacterium acnes (P. acnes) can modulate the immune response to several antigens. One important compound of P. acnes is its soluble polysaccharide (PS). P. acnes and PS can potentiate or suppress the type I hypersensitivity to OVA in mice, depending on the treatment protocol. The mechanisms of these effects probably involve the modulation of antigen presenting cells (APCs). Herein, we determined the activation status of spleen APCs from P. acnes- or PS-treated mice submitted to the type I hypersensitivity reaction, by analyzing the expression of TLRs and co-stimulatory molecules. When the reaction was potentiated by P. acnes or PS treatment, there was an increase in the number of B cells, macrophages and dendritic cells expressing TLR2, 4, 9, CD80 or CD86. But when the reaction was suppressed, there was an increase of APCs TLR2+ and TLR9+, but not TLR4+, and lower numbers of APCs expressing CD80 or CD86. The expression of TLRs, CD80 and CD86 on APCs was also modified by treatments. This suggests that the differences in the expression of TLRs and co-stimulatory molecules on APCs and in the number of these cells could be involved with potentiation or suppression of the reaction by the bacterium or its compound. Our data indicate that the modulation of type I hypersensitivity reaction by P. acnes or PS depends on the activation status of APCs, mainly the expression of TLRs and co-stimulatory molecules.

Support: FAPESP, CNPq, CAPES.

ABSTRACT 179

Staphylococcus epidermidis activates innate immunity via TLR2 and modulates TLR2 expression

Tobias Strunk*1, Melanie Coombs*2,3, David Burgner1, Andrew Currie1, Victoria Philbin2,3, Douglas Golenbock4, Michael Otto5, Karen Simmer1,6, Peter Richmond1, and Ofer Levy2,3

* These authors contributed equally to this study.

1School of Paediatrics and Child Health, University of Western Australia, Perth, Australia, 2Division Infectious Diseases Children's Hospital Boston and 3Harvard Medical School, Boston, MA, 4University of Massachusetts Medical School, Worcester, MA, 5Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT, 6 School of Women's and Infants Health, University of Western Australia, Perth, Australia.

Staphylococcus epidermidis (SE) is a nosocomial pathogen causing infection in immunocompromised populations, including patients at the extremes of age and those with congenital or acquired immunodeficiency. The mechanisms by which SE engages the host innate immune system are incompletely defined, and we therefore undertook the current study. Live, heat killed (HK) and ethanol killed (EK)-SE strain 1457 activated a TLR transcriptome in primary human mononuclear cells (MC) and induced production of early response cytokines, including TNF, IL-6 and IL-10. Transfection of human embryonic kidney cells with TLR2 conferred responsiveness to whole SE. TLR2-deficient murine monocytic macrophage cells demonstrated impaired SE-induced TNF production. Neutralizing polyclonal antibodies directed against TLR2 and TLR6 inhibited SE-induced IL6 production from adult MCs in vitro. SE selectively suppressed MC expression of CD14, TLR2, TLR4 and TLR6. In a murine model, intravenous injection of SE induced cytokine production, which was greatly diminished in TLR2-deficient mice. We conclude that SE interacts with the innate immune system via TLR2.

ABSTRACT 180

Uropathogenic *Escherichia coli* Block MyD88-Dependent and Activate MyD88-Independent Signaling Pathways in Rat Testicular Cells

Sudhanshu Bhushan*, Svetlin Tchatalbachev†, Joerg Klug*, Monika Fijak*, Charles Pineau‡, Trinad Chakraborty†, and Andreas Meinhardt*

*Department of Anatomy and Cell Biology, Unit of Reproductive Biology and †Department of Medical Microbiology, Justus-Liebig-University Giessen, Giessen, Germany; and ‡Institut National de la Sante´ et de la Recherche Me´dicale, U625, Groupe d'e´tude de la reproduction chez l'homme et les mammife`res, Institut Fe´de´ratif de Recherche 140, Campus de Beaulieu, Rennes, France

Uropathogenic *Escherichia coli* (UPEC) is the most common etiological cause of urogenital tract infections and represents a considerable cause of immunological male infertility. We examined TLR 1–11 expression profiles in testicular cells and the functional response to infection with UPEC. All testicular cell types expressed mRNAs for at least two TLRs and, in particular, synthesis of TLR4 was induced in testicular macrophages (TM), Sertoli cells (SC), peritubular cells (PTC), and peritoneal macrophages (PM) after UPEC exposure. Even though MyD88-dependent pathways were activated as exemplified by phosphorylation of mitogen-activated protein kinases in TM, SC, PTC, and PM and by the degradation of IκBα and the nuclear translocation of NF-κB in PTC and PM, treatment with UPEC did not result in secretion of the proinflammatory cytokines IL-1α, IL-6, and TNF-α in any of the investigated cells. Moreover, stimulated production of these cytokines by nonpathogenic commensal *E. coli* or LPS in PM was completely abolished after coincubation with UPEC. Instead, in SC, PTC, TM, and PM, UPEC exposure resulted in activation of MyD88-independent signaling as documented by nuclear transfer of IFN-related factor-3 and elevated expression of type I IFNs α and β, IFN-γ inducible protein 10, MCP-1, and RANTES. We conclude that in this in vitro model UPEC can actively suppress MyD88-dependent signaling at different levels to prevent proinflammatory cytokine secretion by testicular cells. Thus, testicular innate immune defense is shifted to an antiviral-like MyD88-independent response. The Journal of Immunology, 2008, 180: 5537–5547.

ABSTRACT 181

Candida albicans stimulation of IL-1beta bypasses inflammasome activation in human primary monocytes

Frank van de Veerdonk1, Claudia Nold2, Marcel Nold2, L

eo Joosten1, Jonathan van der Meer2, Bart-Jan Kullberg1, Jos W. M. Van der Meer1, Charles A. Dinarello2, Mihai G. Netea1,2

1Radboud University Nijmegen, The Netherlands, and 2University of Colorado Health Sciences Center, Denver, Colorado

The release of IL-1beta has been postulated to depend on a protein complex called the inflammasome that activates caspase-1. Controversy has surrounded the capacity of fungal pathogens to induce production of IL-1beta, as no inflammasome activators are expressed by fungi. In the present study we demonstrate that human monocytes respond with release of IL-1beta after stimulation with TLR ligands or the fungal pathogen C. albicans. IL-1beta stimulation by C. albicans is modulated at the transcription level, through interaction with mannose receptor and dectin-1/TLR2 pathways. Western-blots of demonstrate both the constitutive activation of caspase-1 in monocytes, and the spontaneous release of ATP necessary for IL-1beta secretion. No activation of the inflammasome by fungal PAMPs are necessary for the stimulation of IL-1beta. siRNA experiments demonstrated that the constitutive activation of caspase-1 depends on ASC and NALP3. In contrast, caspase-1 is not active in macrophages, and macrophages respond with IL-1beta release only after two-hit stimulations with TLR ligands and inflammasome activators such as ATP. This dichotomy of IL-1beta processing and release underlines the functional differences between primary human monocytes and macrophages, two cell types that are present in different body compartments and have different functional specializations.

ABSTRACT 182

CONTRIBUTION OF CD44 TO HOST DEFENSE AND RESOLUTION OF INFLAMMATION DURING BACTERIAL PNEUMONIA

G.J.W. van der Windt (1,2), S. Florquin (3) and T. van der Poll (1,2)
(1) Centre for Infection and Immunity Amsterdam (CINIMA), (2) Centre for Experimental and Molecular Medicine and (3) Department of Pathology, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands.

Pneumonia is a major health problem and the most frequent cause of sepsis. CD44 is a glycoprotein involved in the induction and in the resolution of non-infectious inflammation. The aim of our study was to determine the contribution of CD44 to the immune response and to the resolution of inflammation during Gram-negative and Gram-positive pneumonia. Therefore, wild type C57BL/6J mice (WT) and CD44 gene deficient (CD44-/-) mice were intranasally inoculated with the common respiratory pathogens Klebsiella pneumoniae or Streptococcus pneumoniae. CD44-/- mice displayed significantly reduced bacterial loads in lung, spleen, and blood at 2 days after infection of either pathogen. At 5 days after S. pneumoniae infection, however, no bacteria could be detected in WT mice, whereas abundant numbers of bacteria were still found in blood, lung and spleen of CD44-/- mice. At this time-point, similar bacterial loads were found in both mouse strains after K. pneumoniae infection. At 10 days after infection, both WT and CD44-/- mice were able to clear either pathogen. Furthermore, CD44-/- mice displayed attenuated resolution of lung inflammation as reflected by the prolonged presence of inflammatory cells and prolonged inflammation of the interstitium, bronchii, blood vessels and pleura at 5 and 10 days after infection. Taken together, the absence of CD44 results in a more efficient anti-bacterial response early after the induction of Gram-negative and Gram-positive pneumonia, however, in the later phase of infection CD44 deficiency impairs the resolution of pulmonary inflammation which is accompanied by the prolonged presence of bacteria.

ABSTRACT 183

DELINEATION OF THE ROLES OF TRIF/MYD88 AND TLR2/TLR4 IN THE PATHOGENISIS OF E. coli PERITONITIS

Cornelis van 't Veer, Petra S. van den Pangaart, Daniëlle W.M. Kruijswijk, Alex F. de Vos, and Tom van der Poll

Center for Experimental and Molecular Medicine, University of Amsterdam, Amsterdam, The Netherlands

The role of Toll-like receptors in the pathogenesis of E. coli peritonitis was investigated in a murine model. The initial resistance and response to E. coli peritonitis is solely driven by TLR4. The TLR4 adaptors Trif and MyD88 were found to be both partially and equally important for the TLR4 mediated bacterial resistance. TLR2 plays a role in advanced disease depending on the presence of TLR4. In TLR4+/+ mice TLR2 signaling events are detrimental to the host response exemplified by lower bacterial numbers in TLR2-/- mice in late stage peritonitis. In TLR4-/- mice TLR2 is crucial to the residual TLR mediated resistance as indicated by elevated bacterial outgrowth and sudden early death of double deficient TLR2-/-TLR4-/- mice, the latter does not occur in TLR4-/- mice. TLR4-/- mice appear compensated during late stage E. coli peritonitis by increased TNFα and IFN-γ production compared to WT. Trif mediated processes become detrimental to the host at a later stage of E. coli peritonitis when bacterial numbers have increased. Unexpectedly, Trif mediated IRF3 phosphorylation and IFN-β production is absent in the early stage of E. coli peritonitis. A rapid induction was observed of the Trif inhibitor A20 which is driven by a noted NF-κB response. Fierce IFN-β expression in late stage E. coli peritonitis occurs completely independent of Trif and TLR4. Importantly, Trif-deficient mice show enhanced leukocyte attraction to the peritoneal cavity at a late stage of E. coli peritonitis and display significantly delayed mortality. This late beneficial Trif-deficient phenotype is associated with specific absence of the chemokine LIX. The data indicate that detrimental signaling by Trif during advanced stage E. coli peritonitis is not associated with IFN-β production but with deranged leukocyte attraction probably by over production of chemokines.

ABSTRACT 184

TREM-1 improves the inflammatory response and outcome to *Streptococcus pneumoniae* infection by reducing levels of negative regulators in the lung.

Omar Sharif, Heimo Lager, Isabella Haslinger, Ulrich Matt, Karin Stich, Tanja Furtner, Katharina Schmidt and Sylvia Knapp.

Center for Molecular Medicine (CeMM) of the Austrian Academy of Sciences, Vienna, Austria; Department of Internal Medicine 1, Division of Infectious Diseases and Tropical Medicine and Dept. of Pathology, Medical University Vienna, Austria.

Background: Triggering receptor expressed on myeloid cells (TREM-1) is a cell surface receptor present on both monocytes/macrophages and neutrophils that has been shown to amplify cytokine and chemokine production in response to bacterial Toll like receptor (TLR) ligands. Blocking studies revealed TREM-1 as a valuable target to prevent overwhelming inflammation during sepsis. At the same time, TREM-1 is highly expressed within the pulmonary compartment with soluble TREM-1 being a valuable marker indicating the presence of pneumonia in humans. The biological role of TREM-1 during community acquired pneumonia, such as pneumococcal pneumonia is not known. The aim of the present study was to determine the function of TREM-1 in the inflammatory response to *Streptococcus pneumoniae* infection *in vivo*, the mechanism where by this occurred, and the outcome of this on infection.

Methods: C57BL/6 mice were intranasally infected with S. pneumoniae followed by i.p. treatment with PBS, isotype Ab or agonistic TREM-1 mAb. Bacterial counts, lung histology, neutrophil influx, chemokine/cytokine responses and signaling mediators as well as survival were evaluated *in vivo* in a time dependent manner. *In vitro*, immortalized lung alveolar macrophages (MHS) and respiratory epithelial cells were utilized to study the response to S. *pneumoniae* treatment.

Results: Mice pre-treated with agonistic TREM-1 displayed a significantly amplified cytokine and chemokine responses (TNF, MIP-2 and IL-6) as well as neutrophil influx to the lungs 6h after induction of pneumonia. This enhanced early inflammation was associated with lower numbers of bacteria in the lungs and reduced pulmonary inflammation at 48hr, resulting in improved survival. *In vitro* studies corroborated these results at the early time point. A reduction of negative regulators of TLR signaling in lungs was observed in mice pre-treated with TREM-1 *in vivo*.

Conclusion: TREM-1 boosts the early inflammatory response during S. *pneumoniae* infection *in vivo* through lowering levels of negative regulators in the lung, which results in accelerated bacterial clearance and ultimately improved survival.

ABSTRACT 185

Silencing IRAK-4, restoring balance in pneumonia?

A. J. Hoogendijk, T. van der Poll, and C. W. Wieland

Center for Experimental and Molecular Medicine, Academic Medical Center, Amsterdam, The Netherlands

Pneumonia remains a major cause of sepsis. The Gram-positive bacterium Streptococcus pneumoniae (S. pneumoniae) is the most frequent pathogen causing community acquired pneumonia. After entering the body, the innate immune system senses lipoteichoic acid (LTA), a prominent cell wall component of S. pneumoniae, via Toll-like receptor 2 (TLR2). TLR2 signaling is dependent on the adaptor protein MyD88; MyD88 activation leads to NF-kB activation utilizing, amongst others, interleukin-1 receptor associated kinase 4 (IRAK-4). Subsequent induction of inflammation is important for an appropriate host defense against pneumonia. However, the inability to regulate the inflammatory response within the lung during ongoing infection can induce substantial organ damage. Modulating these dynamics might prove beneficial to the outcome of pneumonia.

Airway epithelial cells are important players in the innate immune response against S. pneumoniae and we set out to reduce IRAK-4 in these cells by short interfering (si)RNA. We have been able to reduce IRAK-4 by 40%, in RAW 264.7 cells, in comparison to moque siRNA transfected cells (confirmed by semiquantitative PCR and Westernblot). Moreover, in transfected mouse lung epithelial cells (MLE-12 cells) after stimulation with the TLR2 ligand LTA, significantly less KC was released in the IRAK-4 siRNA treated cells indicating a functional effect of reducing IRAK-4 by siRNA. In future experiments we want to partially to silence IRAK-4 in vivo by intranasal inoculation of siRNA in order to modulate uncontrolled inflammation.

ABSTRACT 186

TLR-independent Type I interferon induction in response to an extracellular bacteria is based on an intracellular receptor.

Charrel-Dennis M , Latz E., Halmen K., Trieu-Cuot P, Fitzgerald KA, Kasper DL and Golenbock DG

University of Massachusetts, Worcester MA USA

We have identified and characterized the pathway leading to type I interferon (IFN) production by macrophages infected with group B streptococcus (GBS). IFNβ was produced by macrophages upon stimulation with both heat-killed and live GBS. Exposure of macrophages to heat-killed GBS activated a Toll-like receptor (TLR)-dependent pathway, whereas exposure to live GBS activated a TLR/NOD/Rig-like receptor (RLR)-independent pathway. This latter pathway requires bacterial phagocytosis, proteolytic degradation and destruction of the phagolysosomal membrane by GBS pore-forming toxins, leading to the release of bacterial DNA into the cytosol. GBS DNA then triggers IFNb by interacting with a cytosolic receptor, with consequent activation of the serine-threonine kinase TRAF-associated NFkB activator (TANK)-binding kinase 1 (TBK1) and phosphorylation of IFN regulatory factor 3 (IRF3). Thus, activation of IFN̰ab production during infection with GBS, commonly thought of as an extracellular pathogen, appears to result from the intracellular interaction of GBS DNA with a DNA-binding receptor.

Cancer

ABSTRACT 187

TLR3 activation induces various fates of Multiple Myeloma cells

David Chiron1, Catherine Pellat-Deceunynck1, Martine Amiot1, Régis Bataille1, and Gaëtan Jego1

1INSERM U892, Nantes, France

Multiple Myeloma (MM) is a fatal plasma cell malignancy localized in the bone marrow. MM patients are strongly vulnerable to bacterial, fungal and viral infections. Furthemore, next to the general organ failure, recurrent infections remain a major cause of death in MM patients. MM cells express Toll-like receptors (TLR). It has been shown that TLR ligands induce proliferation, survival and immune surveillance escape of MM cells through MyD88-TLR pathways. Thus, deciphering TLR function in MM cells would help to understand the mechanism of tumor cell growth. We have examined the response of MM cells to the TRIF-dependent/MyD88-independent TLR3. We show that most of MM cell express TLR3 but response to TLR3 synthetic ligand (Poly(IC)) is heterogeneous. Indeed, 20% of MM cells release IFNa and IL-6 after TLR3 triggering, and then die by apoptosis. The apoptosis is mediated by IFNa through a p38 Mapk- and Erk1/2-dependent mechanism. The secretion of IFNa is controlled by p38 Mapk. Poly(IC)- and IFNa -induced apoptosis involves Mcl-1 cleavage and the BH3-only molecule Noxa up-regulation. On the contrary, 40% of MM cells show an increase of proliferation upon TLR3 triggering. This proliferation is mediated by either NFkB or Notch pathways activation. In conclusion, our results suggest that some MM cells retain the capacity to secrete IFNa and are induced in apoptosis in response to TLR3 ligands. This effect has also been observed in melanoma and breast cancer cells. On the contrary, a majority of MM cells could take advantage of TLR3 ligand to proliferate through mechanisms that are distinct from those triggered by MyD88-dependent TLR

ABSTRACT 188

Investigation of the effects of treatment with Toll like receptor ligands on plasmacytoid dendritic cell leukemia cells

Zoltán Magyarics1, Attila Bácsi1, Kitti Pázmándi1, Katalin Pálóczi2, Éva Rajnavölgyi1
1Institute of Immunology, Medical and Health Sciences Center, University of Debrecen, Debrecen, Hungary

2Department of Immunology, National Medical Center, Budapest, Hungary

Plasmacytoid dendritic cells (pDC) are able to recognize nucleic acids of host and pathogens by their special set of pattern recognition Toll-like receptors (TLR) represented by TLR7 and TLR9. They can prime and polarize naive T-cells, and have an important effector function in anti-viral immunity through the production of IFN-alpha. pDC-leukemia is a rarely diagnosed malignancy, characterized by high number of malignant cells in the peripheral blood as well as in the bone marrow. Based on the scarcity of pDC in the peripheral blood of healthy subjects, leukemic pDC may represent an especial opportunity for collecting information about phenotypic and functional properties of these cells. The aim of this study was to characterize the changes in the expression of cell surface molecules and in the cytokine production of leukemic pDC after treatment with different TLR ligands. To achieve this, pDC leukemia cells were isolated by cell sorting from cryopreserved peripheral blood and bone marrow samples of a 71-year old male patient (Gopcsa et al. Eur J Haematol. 75:346-51, 2005). The expression of co-stimulatory molecules (ICOS-L, CD80, CD86) and antigen presenting molecules (HLA-DQ, HLA-DR) on the surface of pDC leukemia cells was monitored by flow cytometry at 24 and 48 hours after treatment with TLR-9 ligand type A (CpG2216) and type B (CpG2006) CpG oligonucleotides, and the TLR-7 ligand imiquimod, respectively. The levels of secreted IFN-alpha, TNF-alpha and IL-6 in the culture supernatants were determined by ELISA. Treatment with type B CpG oligonucleotide or imiquimod alone induced expression of co-stimulatory molecules as well as production of TNF-alpha and IL-6, while the production of IFN-alpha was not detectable. We was unable to detect IFN-alpha even after type A CpG oligonucleotide stimulation, most likely because the effect of cryopreservation, as we observed higher IFNA1 levels using Q-PCR. Simultaneous stimulation with type B CpG oligonucleotide and imiquimod proved to be the most potent inducer of surface markers of activation; however it was less effective in TNF-alpha and IL-6 induction than the ligands alone. On the basis of our data, it seems that leukemic pDC share several functional and phenotypic features with their normal counterpart. Based on the functional properties the studied leukemic pDC are useful models for pDC research, as they can be isolated in higher amount than normal cells.

ABSTRACT 189

THE EXPRESSION AND FUNCTIONALITY OF TLR3 IN DIFFERENT HUMAN TUMOR CELL LINES

Tanja Matijevic1, Jelena Knezevic1, and Jasminka Pavelic1

1Division of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Croatia

Toll-like receptor 3 (TLR3) is a member of Toll-like receptors who recognize structurally conserved molecules derived from pathogens and trigger the immune response. TLR3 is activated by dsRNA and is usually connected with viral infections. However, TLR3 and its synthetic ligand poly I:C have recently been connected with the induction of apoptosis in tumor cells. In order to clarify if TLR3 is expressed in certain tumor cell lines and whether it is functional and what is the response of these cell lines to different concentrations of poly I:C treatment, we have screened SW480, SW620 and Detroit 562 cell lines by Real Time PCR, Flow Cytometry and ELISA. We have shown that all these cell lines express TLR3 on mRNA and protein level but it is only functional in Detroit 562 cell line since only in these cells poly I:C treatment triggered the IL-6 secretion. By using annexin-V apoptosis detection kit, we have found that poly I:C triggers apoptosis in Detroit 562 cell line.

ABSTRACT 190

Toll like receptors in chronic lymphocytic leukemia cells

Marta Muzio, Cristina Scielzo, Maria TS Bertilaccio, Michela Frenquelli, Benedetta Apollonio, Giorgia Simonetti, Paola Portararo, Paolo Ghia, Federico Caligaris-Cappio
Department of Oncology, Unit and Laboratory of Lymphoid Malignancies, Istituto Scientifico San Raffaele and Università Vita-Salute San Raffaele, Milano, Italy

Mature B-lymphocytes can recognize microbial antigens via B cell receptor (BCR) in a specific way and via Toll like receptors (TLR) in a costimulatory manner. A wealth of information is gathering on the possible role of antigenic stimulation in the natural history of chronic lymphocytic leukemia (CLL), a disease characterized by the accumulation of CD5+ monoclonal B lymphocytes in primary and secondary lymphoid tissues. However little is known regarding the expression and function of TLR in CLL cells. We studied fresh leukemic B-cells purified from the peripheral blood of CLL patients for the expression pattern and function of TLR 1 to 10, NOD1, NOD2 and TIR8. We found that CLL cells express several functional pattern-recognition molecules; leukemic cells upon stimulation with distinct TLR ligands upregulate surface levels of CD86 and CD25 activation molecules, and are protected from spontaneous apoptosis. These findings suggest a potential role of costimulatory signals in modulating CLL cell response in the context of specific antigen recognition, and further support the hypothesis that CLL cells resemble antigen-activated B-cells.

ABSTRACT 191

5´-triphosphate-siRNA: turning gene silencing and RIG-I activation against melanoma

Hendrik Poeck1, 2, 3, Cornelius Maihoefer1, Robert Besch4, Damia Tormo5, Svetlana Shulga Morskaya6, Susanne Kirschnek7, Johannes Hellmuth1, Andreas Schmidt1, David Anz1, Michael Bscheider1, Ulrich Kalinke8, Hiroki Kato9, Shizuo Akira9, Rachel Meyers6, Georg Häcker7, Simon Rothenfusser1, Veit Hornung1, Stefan Endres1, Thomas Tüting5 and Gunther Hartmann2.

1Division of Clinical Pharmacology, Department of Internal Medicine, University of Munich, 80336 Munich, Germany;

2Institute of Clinical Chemistry and Pharmacology, University of Bonn, 53127 Bonn, Germany
3Medizinische Klinik III, Klinikum rechts der Isar, Technische Universität München, 81675 Munich, Germany

4Department of Dermatology and Allergology, Ludwig-Maximilian University, 80337 Munich, Germany;

5Laboratory for Experimental Dermatology, Department of Dermatology and Allergology, University of Bonn, 53105 Bonn, Germany;

6Alnylam Pharmaceuticals, Cambridge, MA 02142, USA;

7Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, 81675 Munich, Germany;

8Division of Immunology, Paul-Ehrlich-Institut, 63225 Langen, Germany;
9Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Japan

Two hallmarks of tumor development are increased tumor cell survival and immune escape. Genetic and epigenetic plasticity allows tumors to evade single-targeted treatments. Here we direct short interfering RNA (siRNA) containing triphosphate groups at the 5´ends (3p-siRNA) against melanoma. The 3p-siRNA used comprises two distinct and independent functional activities in one molecule: silencing of anti-apoptotic bcl-2 and activation of the cytosolic helicase RIG-I. Systemic treatment with bcl-2-specific 3p-siRNA elicited strong anti-tumor activity in a metastatic melanoma model. Like TLR agonists, RIG-I ligation by 3p-siRNA activated innate immune cells such as dendritic cells; unlike TLR agonists, activation of RIG-I directly induced a type I IFN response and apoptosis in murine and human tumor cells; RIG-I-induced apoptosis of tumor cells synergized with apoptosis induced by siRNA-mediated silencing of bcl-2 in tumor cells. In vivo, these mechanisms acted in concert to provoke massive apoptosis of tumor cells in lung metastases. The overall therapeutic activity of 3p-siRNA in vivo required NK cells, type I IFN and silencing of bcl-2 as evidenced by rescue with a mutated bcl-2 target, by site-specific cleavage of bcl-2 mRNA in lung metastases and downregulation of bcl-2 protein in tumor cells in vivo. Together, 3p-siRNA represents a novel single molecule-based combinatorial approach in which RIG-I activation on both the immune- and the tumor cell level corrects immune ignorance and in which gene silencing is used to correct key molecular events that govern tumor cell survival.

ABSTRACT 192

Evaluation of expression of Toll-like receptros 2, 3, and 4 in cancer of the larynx

Jan Żeromski, Mirosław Szczepański, Husam Samara, Wojciech Golusiński

Depts Clinical Immunology and Otolaryngology, University of Medical Sciences, Poznań, Poland

Among head and neck cancers laryngeal carcinoma remains one of the most common and unpredictable tumors, as far as survival is concerned. Tumor cells are often heavily infiltrated by inflammatory ones, including significant proportion of T lymphocytes. In spite of it, both local and systemic anti-tumor response is usually markedly suppressed, presumably due to inhibitory substances secreted by tumor. Toll-like receptors (TLRs) have been shown to be of crucial importance for the induction of innate and subsequently acquired immunity. They are expressed not only on cells of the immune system, but also on several epithelia.

The aim of study: was to search the expression of some TLRs in the microenvironment of larynx carcinoma in order to seek their effects on cancer biology.
Materials and methods: Frozen sections of tumor specimens (n=10) of male patients aged 53-75 years (mean 62 yrs) obtained after total laryngectomy, were subjected to ABC immunohistochemistry and/or immunofluorescence, using polyclonal antibodies vs. TLR2, TLR3, TLR4 and monoclonal ones vs. HLA-DR and lymphocyte subsets.
Results: TLRs could be demonstrated both, on tumor cells and inflammatory ones. Expression on tumor cells was focal, usually membrane-bound. TLRs and HLA-DR were shown to be co-expressed, what may suggest the role of the former in antigen presentation.

ABSTRACT 193

Toll-like receptor 5 engagement with flagellin mediating the innate immunity elicits anti-tumor activity in mouse xenograft model of human colon cancer.

Sang Hoon Rhee, Eunok Im, and Charalabos Pothoulakis

Division of Digestive Diseases, David Geffen School of Medicine, UCLA, Los Angeles, CA, 90095, USA

Toll-like receptors (TLRs)-dependent signaling pathways have been proposed as immunotherapeutic targets against invading pathogens and tumorigenesis. Moreover, TLRs-dependent host-commensal interactions in the human gut play an essential role in inflammation, allergy, and host immunity. Here we investigated whether TLR5 engagement with bacterial flagellin mediates innate immunity and elicits anti-tumor activity in a mouse xenograft model of human colon cancer. Lack of MyD88 or TLR5 expression dramatically enhanced tumor growth and inhibited tumor necrosis. Moreover. TLR5 activation by peritumoral flagellin treatment substantially suppressed tumor growth and increased tumor necrosis. The underlying mechanism likely involves reduced production of chemokines (ENA-78, MIP3a, and IL-8) responsible for recruiting neutrophils and consequently diminished neutrophil infiltration in MyD88- or TLR5- deficient tumors. In contrast, tumor-associated macrophage infiltration or tumor angiogenesis was not changed in MyD88 or TLR5 deficient tumors. These data demonstrate that TLR5 engagement by flagellin elicits potent anti-tumor activity, suggesting a potential immunotherapeutic application against tumorigenesis and tumor growth. This study also implies an essential role of host-commensal interactions by TLR5 in tumor immunology.

New advances, Techniques and Models to study Innate Immunity

ABSTRACT 194

Functional Proteomics Analysis of E3-Ubiquitin Ligases and Deubiquitinating Enzymes involved in Innate Immunity

Evren Karayel, Tilmann Bürckstümmer, Melanie Planyavsky, André Müller, Keiryn Bennett, Sebastian Nijman, Giulio Superti-Furga

CeMM- Research Center for Molecular Medicine by the Austrian Academy of Sciences

Ubiquitination is a regulatory event that either triggers proteasomal degradation or mediates interactions with other proteins. It is a reversible process that requires ubiquitin ligases for addition and deubiquitinating enzymes (DUBs) for removal of ubiquitin. Innate immunity signalling is also regulated by uniquitination. For instance, several ubiquitin ligases seem to target RIG-I with different outcomes: While RIG-I ubiquitination at its N-terminal CARD domain. by TRIM25 leads to its activation, the ubiquitination of the same domain by RNF125 results in its degradation. From the opposite point of view, little is known about deubiquitination in innate immunity. Recently, DUBA, an ovarian tumour domain-containing deubiquitinating enzyme, was discovered as a negative regulator of type I IFN production.

Advances in protein purification and protein mass spectrometry have enabled the systematic analysis of signal transduction pathways by pathway proteomics. The pathway proteomics approach allows for the unbiased and comprehensive investigation of protein-protein-interactions in a given pathway, thereby enabling the construction of a coherent map of the pathway. It represents an ideal framework for integration of additional data from the literature and sets the ground for a more "systems biology" understanding of the process.

In order to provide a more comprehensive view of ubiquitination and deubiquitination events in innate immunity, we want to identify interactors of E3 ubiquitin ligases and deubiquitinating enzymes which are known to be involved in innate immunity by tandem affinity purification coupled to mass spectrometry analysis. Tandem affinity purification (TAP) is a generic two-step affinity purification protocol that enables the isolation of protein complexes under close-to-physiological conditions for the subsequent characterization by mass spectrometry. Interacting proteins will be verified by coimmunoprecipitation. Interesting candidates from these analyses will be validated by loss-of-function and gain-of function experiments using functional read-outs.

ABSTRACT 195

Binding of a tick saliva protein to DC-SIGN inhibits cytokine expression by impairing both nucleosome remodeling and mRNA stabilization

JW Hovius 1, MA de Jong 2, J den Dunnen 2, M Litjens 2, E Fikrig 3, T van der Poll 1, SI Gringhuis 2, TB Geijtenbeek 2

1 Center for Experimental and Molecular Medicine, Academic Medical Center and 2 Department of Molecular Cell Biology & Immunology, VU Medical Center, Amsterdam, The Netherlands, 3 Section of Infectious Diseases, Department of Internal Medicine, Yale University, School of Medicine, New Haven, Connecticut, USA

Ixodes ticks are major vectors for human pathogens, such as Borrelia burgdorferi, the causative agent of Lyme disease. Tick saliva contains immunosuppressive molecules that facilitate tick feeding and Borrelia infection. We here demonstrate, for the first time, that the Ixodes scapularis salivary protein Salp15 inhibits adaptive immune responses by suppressing human dendritic cell (DC) functions. Salp15 inhibits both Toll-like receptor ligand- and B. burgdorferi-induced production of the pro-inflammatory cytokines IL-12p70, IL-6 and TNF-alpha by DCs, and DC-induced T cell activation. Salp15 interacts with DC-SIGN on DCs, which results in activation of the serine/threonine kinase Raf-1. Strikingly, Raf-1 activation by Salp15 leads to mitogen-activated protein kinase kinase (MEK)-dependent decrease of IL-6 and TNF-alpha mRNA stability and impaired nucleosome remodeling at the IL-12p35 promoter. These data demonstrate that Salp15 binding to DC-SIGN triggers a novel Raf-1/MEK-dependent signaling pathway acting at both cytokine transcriptional and post-transcriptional level to modulate Toll-like receptor-induced DC activation, which might be instrumental to tick feeding and Borrelia infection, and an important factor in the pathogenesis of Lyme disease. Insight into the molecular mechanism of immunosuppression by tick salivary proteins might provide innovative strategies to combat Lyme disease and could lead to the development of novel anti-inflammatory or immunosuppressive agents.

ABSTRACT 196

A novel functional screening approach identifies CD47 as a target of dectin-1-dependent stimulation of macrophages

Gerben Ferwerda, Sigrid Heinsbroek, Siamon Gordon

Sir William Dunn School of Pathology, Oxford University, United Kingdom

Dectin-1 is a C-type lectin expressed on myeloid cells and is a pattern recognition receptor for β-glucans. The role of dectin-1 for the modulation of membrane bound proteins involved in immune responses is not known. We have employed a functional approach to screen for the effect of dectin-1 engagement on the expression of membrane proteins by stimulating murine peritoneal macrophages overnight with zymosan. Changes in expression of 60 'at-random' selected proteins were analyzed by FACS. To determine the role of dectin-1 in this process we compared WT and dectin-1 -/- macrophages. The most pronounced differences in expression between the two mouse strains was the up-regulation of co-stimulatory molecules CD80 and CD86, and the down regulation of integrin-associated protein CD47. Because the relation between CD47 and dectin-1 was not known, we further focused on this protein. Low expression of CD47 on apoptotic cells induces phagocytosis of the apoptotic bodies by phagocytes. However, there was no difference in apoptosis between dectin-1 -/- and WT macrophages measured by rhodamine 123 and 7-AAD. By blocking phagocytosis with cytochalasin D or by enhancing phagocytosis by opsonization of zymosan, the difference in expression of CD47 between dectin-1 -/- and WT macrophages persisted, excluding a secondary effect to phagocytosis of zymosan. Incubation with β-glucan alone or heat killed C. albicans lowered the expression of CD47, whereas incubation with Pam3Cys had no effect on the expression of CD47. These experiments demonstrate that screening membrane bound proteins by FACS after overnight stimulation with zymosan in dectin-1 -/- and WT macrophages can reveal novel interactions. Dectin-1 stimulation with β-glucan leads to a lower expression of CD47 on murine macrophages, which might play a role in the immune response to fungal antigens.

ABSTRACT 197

Synthetic Pathogens for the Integrated Biophysical and Genetic Dissection of Antigen Presentation

Freitas1, R.P., Moita1, C.F.,Irvine2, D.J., Moita1, L.F.

1Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Lisboa 1649-028, Portugal 2Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

Antigen uptake and processing is a key step in the induction of adaptive immunity. Classical studies focused on soluble extracellular antigens, but much evidence suggests that particulate antigens, such as bacteria, fungi and microparticles, are processed much more efficiently (10^4-fold) to stimulate CD8+ T cells.

To understand how the physical nature of particulate antigens influences their uptake and fate in antigen presenting cells, we are studying the internalization, traffic and processing of 'synthetic pathogens' - model particles with distinct, well-defined physical and biochemical properties. We have begun to engineer synthetic pathogen particles to deliver a protein antigen and model phagocytic ligand(s) to antigen presenting cells.
Our preliminary results suggest that particle size influences antigen presentation efficiency and that this effect is not the result of different amounts of antigen delivered to the cell. We are now testing how the presence of modulation ligands (mainly TLR agonists) influences antigen presentation. We have found dramatic effects in the context of cross-presentation and are currently exploring the molecular mechanisms that might explain these observations using a combination of cell biology and biochemistry approaches.

In parallel we have used shRNA to generate loss-of-function phenotypes in antigen presenting cells and to systematically probe the role of both known genes and genes newly identified by the Ferreira-Moita Lab, in internalization, membrane recruitment, cellular trafficking and antigen presentation to effector cells.

By combining these strategies we hope to gain a deeper understanding of how pathogen structure and chemistry dictates signaling, intracellular traffic, antigen processing, immune responses and pathogen survival or elimination.

ABSTRACT 198

Introduction of zwitterionic motifs into bacterial polysaccharides generates TLR2 agonists : Can we use them to improve vaccines?

Simona Gallorini, Francesco Berti, Gianfranco Volpini, Pierino Parente, Chiara

ammicheli, John L. Telford, Domenico Maione, and Andreas Wack

Department of Immunology, Novartis Vaccines Research Center, Siena, Italy

Bacterial capsular polysaccharides (PS) which naturally contain zwitterionic charge motifs (ZPS) possess specific immunostimulatory activity, leading to direct activation of antigen-presenting cells (APCs) through Toll-like receptor 2 (TLR2) and of T cells in co-culture systems. To generate vaccine candidates with antigen and adjuvant properties in one molecule we have chemically introduced a positive charge into naturally anionic PS from group B streptococcus (GBS). The resulting zwitterionic PS (ZPS) has the ability to activate human and mouse APCs, and in mixed co-cultures of monocytes and T cells, ZPS induce MHC II-dependent T-cell proliferation and up-regulation of activation markers. TLR2 transfectants show reporter gene transcription upon incubation with ZPS and these stimulatory qualities can be blocked by anti-TLR2 mAbs or by the destruction of the zwitterionic motif.

When ZPS are used in vivo as adjuvant in combination with the prototype antigen tetanus toxoid (TT), they are able to increase the TT-specific antibody titre, thus acting as classical adjuvants. Glycoconjugates made with ZPS induce a higher T cell response to the protein carrier and accordingly, a higher antibody response than the control glycoconjugates made with the native form of the same polysaccharide. Moreover, neonate offspring from mothers immunized with ZPS-glycoconjugates are better protected from lethal challenge with GBS than the counterparts immunized with the control glycoconjugates. We conclude that glycoconjugate vaccines containing ZPS are highly efficacious vaccines, presumably through targeting the Ag to TLR2-expressing APCs and activation of these APCs, leading to better T cell priming and ultimately to higher specific Ab titers.

ABSTRACT 199

Human-like responses to LPS in a mouse model

Lynn Hajjar, Lindsay Newlon, Ed Fortuno, Alicia Brasfield, Shawn Skerrett*, Bob

Ernst*, Chris Wilson

Departments of Immunology and *Medicine, University of Washington, Seattle, WA

Lipopolysaccharide (LPS), composing the outer leaflet of the outer membrane of Gram-negative bacteria, is a ligand for TLR4/MD-2. It is now clear that the structure of lipid A, the component of LPS that directly interacts with TLR4/MD-2, varies not only between bacterial species but also within the same bacterium depending on growth conditions. For example, Yersinia pestis produces a hexa-acylated lipid A at room temperature (the flea temperature) whereas it switches to a tetra-acylated structure at 37°C (mammalian temperature). The hexa-acylated structure is an agonist to mouse and human TLR4/MD-2, whereas the tetra-acylated structure is an antagonist to the human receptor complex while it remains an agonist to the mouse receptor. To further study the role of this potential immune evasion strategy, we have developed a mouse model that displays human-like responses to various LPS structures. We generated transgenic mice expressing human TLR4 or human MD-2 from human genomic bacterial artificial chromosomes (BAC) and bred these transgenes, present in 2-4 copies, onto the mouse TLR4 and MD-2 knockouts. We thus compared responses in 1° splenocytes from mice of 4 genotypes: wild-type B6 mice, humanized TLR4/MD-2 mice (both transgenes on a double-knockout background), humanized TLR4 mice (human TLR4 on a mouse TLR4 KO background), and humanized MD-2 mice (human MD-2 on a mouse MD-2 KO background). We found that while the response to E. coli LPS was equivalent in B6 mice and in humanized TLR4/MD-2 mice, the humanized mice lost the response to LipidIVA as well as to LPS from Y. pestis grown at 37°C. Furthermore, the response to penta-acylated Pseudomonas aeruginosa LPS was lost in cells from humanized TLR4/MD-2 mice. Data from the mixed receptor complexes (i.e. the humanized TLR4 or humanized MD-2 mice) showed that the response to penta-acylated PA LPS appears to be mainly dependent on the species source of TLR4 whereas the response to LipidIVA and LPS from Y. pestis grown at 37°C is dependent on both TLR4 and MD-2. We are currently testing whether the humanized TLR4/MD-2 mice are more susceptible than WT mice to Y. pestis in vivo.

ABSTRACT 200

FUNCTIONAL CHARACTERIZATION OF A NOVEL TLR9 GENE VARIANTS

Jelena Knezević1, Dinko Pavlinić2, Jasminka Pavelić1, Zlatko Dembić3

1 Ruđer Bosković Institute, Division for Molecular Medicine, Zagreb, Croatia

2 Medical School, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

3 Faculty of Dentistry, University of Oslo, Oslo, Norway

Polymorphisms in innate immunity genes may influence their function and can be clinically relevant, contributing to development of different diseases. Toll-like receptors (TLR) are pattern recognition receptors that bind distinct pathogen-associated molecular patterns. TLR9 is mainly expressed by plasmacytoid dendritic cells (pDC) and B cells, which function as central mediator in recognizing unmethylated CpG motifs present in bacterial and viral DNA. The aim of this study was to investigate the function of TLR9 gene variants at the cellular level. It is very likely that there are more genetic variations of TLR9 (alleles) in general population than it is known for now. For this purpose we analyzed the sequence of coding region of TLR9 gene in two populations: 100 healthy blood donors and 100 patients with diagnosed breast cancer. We found 11 different gene sequence variants, four of which have a change in the amino acid sequence (111X, F667L, R863Q and R892W). We investigated all TLR9 non-synonymous variants functionally, by dual-glo luciferase assay of transiently transfected HEK293 cells. Genetic variant 111X completely abrogates signaling pathway by TLR9, while others are hyporeactive. In the same system we demonstrated the dominant-negative effect of those variants.

ABSTRACT 201

Structural modeling of Toll-like receptors: science or fiction?

Andriy Kubarenko, Martin Frank, Alexander Weber

German Cancer Research Center, Im Neuenheimer Feld 242, 69120 Heidelberg, Germany

Microorganisms that invade a vertebrate host are detected by the innate immune system through pattern-recognition receptors, for example Toll-like receptors (TLRs), based on distinct microbial components, known as pathogen-associated molecular patterns (PAMPs). New insights into the structures and functions of PRRs challenge and shape our understanding of pathogenesis and treatment of infectious diseases, allergy, autoimmunity and cancer.

An essential requirement for the study of TLR signalling is the availability of molecular structures. X ray crystallography, NMR and Cryo-EM are valuable experimental methods to obtain structural information, but are not feasible for all proteins of interest. In silico "modelling" has therefore become a straightforward alternative way to obtain structural information in the absence of experimental structural data, and, could meaningfully contribute to structure-function investigations and therapeutics design. Although comparisons exist on how "close" modelling "gets" to experimental structures (e.g. CASP), benchmarks do not exist for the TLR family. Therefore initially we have carried out comprehensive comparison of recently published TLR1, 2, and 4 ectodomains x-ray structures with 3D models generated independently in our lab using a combination of homology modelling and molecular dynamics simulation, which showed good agreement between crystal structures and models. Subsequently, we have extended our modelling approach and generated models of other members of the human and murine TLR family, illustrating both structural patterns common to all TLRs and distinct features of individual TLRs.

ABSTRACT 202

A Potent therapeutic anti-human monoclonal Ab defines a role for FcgRIIA in TLR4-mediated inhibition.

Olivier Leger1, Irene Dunn-Siegrist2, Fabien Depis1, Bruno Daubeuf1, Anne-Catherine Raby1, Marie Kosco-Vilbois1, Yann Dean1, Jerome Pugin2 and Greg Elson1
1NovImmune SA, Geneva, CH. 2University Medical Center (CMU), Geneva, Switzerland.

Activation of TLR4 induces a signalling cascade that, when exaggerated or uncontrolled, has been associated with acute inflammatory disorders such as septic shock. We have generated an anti-human TLR4 MAb, 15C1 (mouse IgG1), which potently blocks the effects of TLR4 ligands on a panel of primary cells and cell lines in vitro and in a whole blood assay ex vivo. The binding of 15C1 was mapped to an epitope in the second portion of the extracellular region of TLR4, which has previously been shown to be functionally important in the recognition of LPS. Furthermore, we demonstrate that the mechanism of inhibition of 15C1 is partially dependent on the mouse IgG1 Fc portion of the MAb, and involves FcgRIIA. A humanized (human IgG1) version of 15C1 (hz15C1) generated by CDR grafting retained affinity for TLR4 but demonstrated a significant loss in its ability to inhibit TLR4. Potency could be completely regained by restoring FcgRIIA interaction via the replacement of 3 amino acids within the CH2 domain of human IgG1 with the corresponding residues from mouse IgG1. We propose this modified version of hz15C1 as a potent therapeutic agent in diseases involving stimulation of TLR4. Optimisation of CDR sequences for preclinical development will be discussed.

ABSTRACT 203

MOLECULAR MODELLING OF NOD-LIKE RECEPTORS - TOWARDS AN UNDERSTANDING OF PROTEIN FUNCTION

Tom P. Monie*, Clare E. Bryant**, and Nicholas J. Gay*

*Department of Biochemistry, University of Cambridge, Cambridge, UK, **Department of Veterinary Medicine, University of Cambridge, Cambridge, UK

The NOD-like receptors (NLRs) are cytoplasmic multipartite proteins with critical roles in; the detection of pathogens; the recognition of cellular danger signals; and the regulation of inflammation and apoptosis. In general they constitute an N-terminal effector signalling domain, a central NACHT (domain present in NAIP, CIITA, HET-E, TP-1) domain and a C-terminal leucine rich repeat region (LRR). The study of the NLRs is a rapidly expanding area of research fuelled by their multifunctional cellular roles. Functionally it is believed that the LRR domain is involved in ligand recognition, the NACHT domain in receptor oligomerisation and the effector domain in signal transduction following homotypic interactions. Following activation some members of this family (such as NALP1, NALP3, Ipaf) form large multiprotein complexes, called inflammasomes. To date limitations on the expression of soluble NLR protein has delayed determination of NLR molecular structure. We have used fold recognition and homology modelling software to generate molecular models of the domains from the NLR proteins NOD1 and NOD2. These models are discussed in light of both sequence variation and conservation observed in species orthologues and in single-nucleotide polymorphisms. In particular we find that electrostatic forces may play an important role in the recognition of ligand by both NOD1 and NOD2.

ABSTRACT 204

SPRET/Ei, an important mouse model on the study of new anti-inflammatory molecules.

Iris Pinheiro, Lien Dejager, Filip Van Hauwermeiren and Claude Libert

Molecular Mouse Genetics Unit, DMBR/VIB - Ghent University, Belgium.

SPRET/Ei, a mouse strain derived from Mus spretus, is extremely resistant to the lethal effects of several inflammatory triggers, as LPS or TNF, when compared to the common laboratory mouse strain, C57BL/6 (BL6). The resistance of SPRET/Ei (S) to LPS is dominant, and two major protective loci, namely on chromosomes 2 and X and two minor protective loci on chromosomes 10 and 13 were identified by linkage analysis. Moreover, females resulting from the cross between BL6 females and S males display higher resistance than males, a fact confirming the contribution of the X-chromosome to this trait. Also against TNF, S mice resist up to 1000 microgram of TNF whereas BL6 mice succumb from 25 microgram. This trait was found to be closely linked to the gene encoding the major TNF receptor Tnfrsf1a on chromosome 6. In the light of these findings, we believe that different signaling pathways, namely the TLR4 pathway, are altered in S macrophages. Upon LPS challenge, several genes (e.g. type I-IFNs or MMPs) seem to be only mildly expressed in S macrophages, and are currently under investigation. Additionally, a genome-wide expression profile on BL6 and S macrophages stimulated with LPS was performed by using Affymetrix GeneChip arrays. With this, we hope to bring forward new anti-inflammatory molecules and/or genes important for therapeutic targeting. SPRET/Ei mice are not only hyporesponsive to the toxic effects of LPS, but can also still resist gram-negative infections, which is in contrast with the mouse strains C3H/HeJ and C57BL10/ScCr, defective in TLR4. Therefore, we believe that this mouse model is useful on the study of new therapies for sepsis.

ABSTRACT 205

TLR3 AS THE MOLECULAR RULER FOR SELECTIVE ACTIVATION BY dsRNA OF DIFFERENT LENGTH: RATIONALISATION OF CONFORMATIONAL HYPOTHESIS OF INTERFERON ACTIVATION

Nina Pirher1, Karolina Ivičak1, Jelka Pohar1, Mojca Benčina1 and Roman Jerala1,2
1Department of biotechnology, National institute of chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia;

2Faculty of chemistry and chemical technology, University of Ljubljana, 1000 Ljubljana, Slovenia.

Toll-like receptor 3 is activated by dsRNA above the threshold of 21 base pairs. We show that this recognition is accomplished by the existence of two binding sites at two ends of the TLR3 ectodomain separated by 50 Å. In our study we establish the existence of an N-terminal dsRNA binding site in addition to the previously identified C-terminal site, located on the nonglycosylated surface of the TLR3 solenoid, comprising residues His39, His60, Gln62 and Arg64. This is in complete agreement with the recently determined crystal structure of the TLR3-RNA complex. Distance between the two sites corresponds to two turns of an RNA duplex in A-type conformation. Fixed distance between binding sites provides support for the "conformational hypothesis of interferon activation" postulated several decades ago based on chemically modified 2'-site RNA derivatives. TLR3 can differentiate between nucleic acids in A or B conformation as the B-type duplex has a significantly larger helical pitch and its periodicity does not fit to the distance between the two binding sites. 21 bp long dsRNA could interact with all crucial residues of both sites and explains the ability of siRNA to activate TLR3. We propose that different arrangements of TLR3 ectodomain pairs along the opposite sides of dsRNA can occur, which can modulate strength of the interferon response.

ABSTRACT 206

Development of a Practical Assay to Evaluate Toll-Like Receptor Function in Different Immune Cells

Chin-An Yang, Nadine Unterwalder, Holger Pöhlmann, Manuel Guerreiro, Hans-Dieter Volk, Carmen Scheibenbogen

Institute of Medical Immunology, Charité Campus Mitte, Berlin, Germany

Toll-like receptors (TLRs) are important in antiviral immunity. Patients with recurrent viral infections may appear normal in quantitative cellular parameters, but may have an impairment of TLR recognition or signalling. In addition, responses to TLRs vary among different cell populations. Our goal was to characterize TLR-signalling in each immune cell subpopulation by using unseparated peripheral blood mononuclear cells. In this assay, secretion of tumor necrosis factor (TNF) or interferon-γ (IFN-γ) in monocytes, myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC), B cells, natural killer cells (NK) and T cells from 10 healthy donors was studied by intracellular cytokine staining cytometry after stimulation with ligands for TLRs 1/2, 3, 4, 7/8, 9. Monocytes, mDCs and B cells showed a consistent pattern of TLR signaling as previously reported. Unexpectedly, TNF production was detected in Pam3CSK4 (TLR2 ligand) and LPS (TLR4 ligand) stimulated pDCs. Furthermore, in the presence of a suboptimal dose of IL-12, NK cells responded to agonists of TLRs 1/2, 3, 4, 7/8, and T cells responded to agonists of TLRs 3, 4, 7/8. When brefeldin A was added together with TLR stimulators, the responses of NK cells to TLR ligands (except for TLR 1/2) were much lower and the responses in T cells were completely abrogated, indicating that accessory cell stimulation plays a role. In conclusion, our assay provides information of TLR function in each cell population, and can be applied to evaluate TLR signalling in patients with recurrent viral infections.

ABSTRACT 207

TLR ACTIVATION PROFILE OF GENETICALLY MODIFIED LACTOBACILLI: THERAPEUTIC IMPLICATIONS

Gregg A. Dean1, Shila K. Nordone1, Sara Bumgardner1, Laura Stoeker1, Alora LaVoy1, Richard Tallon, Jun Goh, Todd Klaenhammer2

1Center for Comparative Medicine and Translational Research, 2Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC, USA.

The safety of Lactobacillus (Lb), its historical use as a probiotic, and more recent data showing the relationship of Lb with the immune system, particularly dendritic cells, has raised interest in exploiting Lb as an oral vaccine vector. In the present study we investigated the Toll-like receptor activation profile of L. acidophilus and L. gasseri and whether genetic modification of cell surface proteins would alter the TLR profile. We found the greatest activity is mediated through TLR2/6 with moderate activity through TLR2 alone and the least with TLR2/1. Mutation of Lb surface layer proteins or mucus binding proteins enhanced TLR2/6 activation, most likely due to increased contact between the bacterial ligands and TLR2/6. In some cases, lactobacilli engineered to express HIV-1 antigens enhanced TLR2/6 activation. Transfection of the fliC gene, encoding Salmonella flagellin, into Lb imparted strong TLR5 activation. Human myeloid dendritic cells isolated directly from human blood that were co-cultured with wild-type or flagellin-expressing Lb showed increased expression of CD80 but not CD86 or MHCII. DC cocultured with Lb expressed high levels of inflammatory cytokines, several chemokines, and a mix of type 1 and type 2 cytokines and induced proliferation in approximately 25% of naïve CD4+CD25- T cells. Because exposure to Lb in the gastrointestinal tract involves the intestinal epithelium, and it is the epithelium that expresses TLR5 and not DC, we are investigating the activity of Lb with and without flagellin in a coculture system with intestinal epithelial cells, DC, and T cells. We hypothesize that in this coculture system, wild-type Lb will favor T cell anergy while flagellin-expressing Lb will favor T cell activation.

ABSTRACT 208

UV-irradiation affects TLR Expression in Human Endothelial Cells

Fitzner, N. and Kolb-Bachofen, V.

Research Group Immunobiology at the Institute of Molecular Medicine, Heinrich-Heine-University of Duesseldorf, Germany

Endothelial cells (EC) fulfill important functions in the regulation of inflammatory reactions and act as "gate keepers" for infiltration of immune cells at the interface between blood and tissue. As a result of their localization EC are directly exposed to pathogens or pathogenic structures as well as to endogenous molecules circulating within the bloodstream. We have recently described that human skin endothelial cells can express all 10 currently known TLRs and also demonstrated their functionality. Since UV-irradiation may also reach into the vascular bed and is known to exert an immunosuppressive effect, we determined the impact of UV-irradiation on skin-derived primary endothelial cells and screened for modulation of TLR expression.

Independent of the donor (n = 3), primary human dermal microvascular EC (HDMEC) express 7 out of 10 TLRs as verified by quantitative 2-step real-time PCR as well as functional testing and following proinflammatory conditions will express all known TLRs. Irradiation of HDMEC with different subtoxic doses of UVA- or UVB-light leads to a differential modulation of TLR expression and this response appears to be donor-specific. Notably, highest differences were seen with TLR7 and TLR10, where responses differ between UVA or UVB treatment and donors.

In conclusion, our results demonstrate that UV-light modulates TLR expression in human skin-derived EC in a differential and donor-dependent way. This UV-induced modified expression might contribute to immune responses and immunosuppression.

ABSTRACT 209

CIGARETTE SMOKE INDUCES TOLL-LIKE RECEPTOR 4 AND INTERLEUKIN 8 RELEASE VIA REACTIVE OXYGEN SPECIES IN HUMAN MONOCYTES.

Gert Folkerts1, Hadi Sarir1, Esmaeil Mortaz1, Irfan Rahman2, and Frans P. Nijkamp1
1 Division of Pharmacology and Pathophysiology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands. 2 Department of Environmental Medicine, Lung Biology and Disease Program, University of Rochester Medical Center, Rochester, NY, USA.

Toll-like receptors (TLRs) are present on monocytes and alveolar macrophages that form the first line of defense against inhaled particles. The importance of those cells in the pathophysiology of lung emphysema has well been documented. Cigarette smoke can stimulate immune-inflammatory cells to produce reactive oxygen species (ROS), cytokines and chemokines. In this study, we evaluated the effects of cigarette smoke medium (CSM) on TLR4 expression and interleukin (IL)-8 production using different inflammatory cells and investigated the involvement of ROS. Due to CSM stimulation, TLR4 surface expression was downregulated on short term exposure (3 h) and upregulated on long term exposure (7 h) in response to CSM treatment. The downregulation could be explained by internalization/shedding of the TLR4 and the upregulation by an increase in TLR4 mRNA. IL-8 mRNA and protein were also increased by CSM, probably via activation of IRAK) and hence the degradation of Iκ-B. Glutathione levels were decreased and intracellular ROS-production was increased after CSM stimulation. The modulation of TLR4 mRNA and surface receptors, IRAK activation, I-KB degradation, IL-8 mRNA and protein levels, GSH depletion and ROS production were all prevented by the thiol antioxidant N-acetyl-L-cysteine. In conclusion, TLR4 is likely to be involved in the pathogenesis of COPD and oxidative stress seems to be a crucial contributor.

ABSTRACT 210

Physical and Functional characterization of the kinases involved in Innate Immunity

Adriana Goncalves1, Tilmann Bürckstümmer1, Hannah Jahn1, Melanie Planyavsky1, André Müller1, Keiryn L Bennett1, Jacques Colinge1 and Giulio Superti-Furga1
1CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences

The innate immune response against pathogens is a dynamic process that involves the coordinated action of many different types of proteins and, hence, it must be tightly regulated. Posttranslational modification of proteins by phosphorylation is a key regulatory event used in signal transduction. Kinases, the mediators of protein phosphorylation, play an essential role in all fundamental cellular processes including innate immune pathways. The important role in signaling allied with the enzymatic activity of kinases makes them attractive drug targets and a valuable object of study. Consequently, we propose to characterize the kinases involved in innate immunity and map their protein complexes by tandem affinity purification and subsequent mass spectrometry analysis. In order to provide a more comprehensive picture of the kinases involved in innate immunity, we are developing a kinome-wide siRNA screen that will allow us to identify new players in innate immunity. By combining loss of function of each individual kinase with overexpression of proteins that activate the pathway at different levels, we will perform an epistasis analysis which will allow us to localize the identified complexes into a global functional network.

ABSTRACT 211

Live imaging of transgenic zebrafish reveals a conserved capacity for Toll-like receptor-mediated signalling within embryonic immune cell compartments

Chris Hall, Maria Vega Flores, Annie Chien, Kathy Crosier and Phil Crosier
Department of Molecular Medicine and Pathology, School of Medical Sciences, The University of Auckland, Auckland, New Zealand

Real-time imaging of immune responses is beginning to reveal new insights into the cellular dynamics of the immune compartment. Transgenic zebrafish provide an exceptional system to investigate immune cell responses within a whole living animal at single cell resolution. We have generated a unique set of transgenic zebrafish lines that will enable chemical and genetic approaches to further understand aspects of innate immune cell function and Toll-like receptor signalling.

We have performed live real-time imaging of fluorescently tagged innate immune cells during wound healing and bacterial infection that has helped resolve how these cells participate during embryonic inflammatory responses. In addition we have helped clarify that embryonic myeloid leukocytes express the Tlr adaptors, myd88, mal/tirap, trif and sarm suggesting a conserved capacity for Tlr-mediated signalling in these earliest innate immune cells. Furthermore, this potential continues within adult zebrafish myelomonocytes, lymphocytes and haematopoietic precursor cells. Live in vivo phagocytosis, infection and wound healing assays within Tg(myd88:EGFP/DsRED2) embryos and larvae have validated the immunocompetence of these myd88-expressing myelomonocytic cells in contributing to the inflammatory response. Using these lines in chemical and forward genetic screens will provide an unbiased means to identify novel regulatory pathways involved in Toll-like receptor signalling.

ABSTRACT 212

THE INFLUENCE OF PROLONGED CYCLING AND BLOOD TEMPERATURE ON MONOCYTE TOLL-LIKE RECEPTOR 1-4 EXPRESSION IN HEALTHY MEN

Oliveira, M. (1), Gleeson, M. (1)

(1) Exercise Immunology Laboratory, Loughborough University, Loughborough, Leicestershire, UK

The purpose of this study was to examine the effects of 2.5 h cycling at 60% of maximal oxygen uptake (VO2max) on human monocyte TLR1, 2, 3 and 4 expression. Six healthy males (20±1 years; body mass 71.0±8.3 kg; VO2max 57.3±9.0 ml.kg-1.min-1, means ± s) had REST, POST and 1H POST exercise blood samples collected and analysed using four colour flow cytometry. At 1H POST CD14+ monocyte TLR expression (GMFI) was significantly lower than REST for all TLR and for TLR4 was also lower at POST (all $P<0.05$). Participants with low TLR expression at REST had a smaller decrease in expression than participants with high expression of TLR at REST. Also, in order to assess the possibility that blood temperature influences TLR expression, blood samples from 6 other male participants (27±11 years; body mass 71.9±5.2 kg; VO2max 54.0±8.3 ml.kg-1.min-1) were incubated for 1.5 h at different temperatures (22ºC, 37ºC and 40ºC) and analysed. No significant differences in TLR expression were found between temperatures for any TLR ($P>0.05$). In conclusion, this study has shown that an acute bout of prolonged exercise reduces TLR1, 2, 3 and 4 expression on the cell surface of CD14+ human monocytes and that TLR expression down-regulation is not caused by an elevation of blood temperature. It is possible that a lowering of TLR expression may contribute to the anti-inflammatory effect of exercise.
Key-words: Toll-Like Receptor, Exercise, Blood temperature, Flow Cytometry.

ABSTRACT 213

Somatic cell genetics for the study of signalling in innate immunity

Felix Randow

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK

To gain further insight into IRF and NF-kB signalling we have isolated mutants from mammalian somatic cell lines. For the IRF pathway we used homologous recombination in human cells to knock out candidate genes. For the NF-kB pathway we isolated mutants after random genome-wide mutagenesis based on their unresponsiveness to TLR agonists. From these mutants we have identified one novel gene, gp96, which is specifically required for the maturation of TLRs in the endoplasmic reticulum. We have also isolated clones deficient in Tlr9, Unc93b1, Myd88, Irak1, IKKbeta, Nemo and RelA.

I will describe insights we have obtained, using our collection of mutant cells, into how the NF-kB and IRF pathways are organized. Emphasis will be given a) to the TBK1 complex, where at least three adaptors (TANK, NAP1 and SINTBAD) compete for binding, and b) to Nemo, for which we have isolated a gain of function allele. This Nemo mutant, even when disabled to bind ubiquitin chains, constitutively activated the IKK complex. This finding suggests that Nemo is not a mere ubiquitin binding adaptor but rather harbours latent activation potential and signal processing ability. The implication of this finding for our understanding of NF-kB activation and potential parallels in IRF signalling will be discussed.

ABSTRACT 214

Histone deacetylase inhibitors (HDIs) impair innate immune responses

Thierry Roger, Didier Le Roy, Jérôme Lugrin, Isabelle Miconnet*, Patrice François1, Jacques Schrenzel1 and Thierry Calandra

Infectious Diseases Service and *Division of Immunology and Allergy, CHUV & University of Lausanne, Lausanne and 1 Infectious Diseases Service, HUG, Geneva, Switzerland

Background: Originally developed as anticancer drugs, HDIs have emerged to be potent anti-inflammatory agents. Here we investigated the effects of 2 prototypical HDIs, trichostatin A (TSA) and valproic acid (VPA), on innate immune responses in vitro and in experimental models of sepsis.

Methods: Mouse BMDMs or BMDCs and human PBMCs or moDCs were preincubated with HDIs and then stimulated with microbial products. The expression of cytokines and of co-stimulatory molecules and activation of intracellular signalling pathways were analyzed using DNA arrays, RT-PCR, ELISA, Luminex assay, FACS, EMSA and Western blotting. The effect of VPA (200 mg/kg i.p.) was examined in a model of toxic shock (1.6 mg/kg of Pam3CSK4 ip) in D-gal-sensitized mice and in a K. pneumonia (10 CFU i.n.) pneumonia model.

Results: HDIs modulated the expression of 40% to 60% of the genes either up- or down-regulated by LPS or Pam3CSK4 in BMDMs. Moreover, HDIs strongly inhibited the release of cytokines (TNF, IL-6, IL-12, IL-10) and chemokines (CXCL10, CCL2) and the up-regulation of CD40 and CD86 induced by LPS or Pam3CSK4 in BMDMs, BMDCs, PBMCs and moDCs. Surprisingly however, HDIs did not interfere with stimulus-induced activation of MAPKs, NF-kB, IRF3 and STAT-1 in BMDMs, suggesting that HDIs does not inhibit signal transduction pathways. Consistent with its anti-inflammatory effects, VPA protected mice from lethal Pam3CSK4-induced toxic shock and induced death in a sub-lethal K. pneumonia pneumonia model.
Conclusions: HDIs exert profound inhibitory effects on the host innate immune antimicrobial defense response. While these results suggest a possible role for HDIs as anti-sepsis agents, they also indicate that cancer patients treated with HDI may be at increased risk of developing severe infections.

ABSTRACT 215

Identification of Listeria monocytogenes Mutants that Affect Cytosolic Detection and Inflammasome Activation

John-Demian Sauer, Chelsea Witte, Jason Zemansky and Daniel Portnoy
University of California, Berkeley

Listeria monocytogenes is a gram positive, facultative intracellular, foodborne pathogen which has been used for decades as a model system for studying many aspects of bacterial pathogenesis and host biology. L. monocytogenes that access the host cell cytosol stimulate a specific host transcriptional response but the host receptor(s) and bacterial ligand(s) involved are unknown. Additionally, it is now appreciated that L. monocytogenes stimulates one or more types of inflammasomes upon access to the host cytosol, however many of the receptor(s) and ligand(s) involved in this process are also unknown. To understand the complex detection of L. monocytogenes by the innate immune system we performed a forward genetic screen to identify bacterial mutants that affect these processes.

We developed a new mariner based transposon delivery system and screened mutants for their ability to induce secretion of IFNβ, a hallmark of the cytosolic transcriptional activation pathway, or to induce LDH release, a correlate of inflammasome mediated cell death. Three independent mutations, affecting either one or both phenotypes, were located in a single operon, the lmo2477-lmo2471 locus. Mutants in lmo2477 (galE) resulted in a modest ~2-3 fold induction of IFNβ above wild type whereas mutants in lmo2473 (yvcK) resulted in ~10 fold increase in induction of IFNβ. In both instances the induction of IFNβ was independent of MyD88 and Trif indicating that TLRs are not involved. In addition to affecting host IFNβ induction, mutants in lmo2473 also caused increases in both host cell death and subsequent IL-1β secretion, ~5 fold above wild type levels. Mutants in lmo2474 (yvcJ) also cause increases in host cell death and IL-1β secretion, although only ~2 fold more than wild type. We found that caspase-1, NLRP3, and ASC are all involved, to varying degrees, in both mutant and wild type induced cell death and IL-1β secretion. We are continuing analysis of these mutants and the mechanisms by which they induce host responses in an attempt to understand the bacterial ligands being recognized.

ABSTRACT 216

Oxidized phospholipids are CD14 receptor antagonists

Elena Schlieffen, Olga Oskolkova, Gernot Schabbauer, Bernd R. Binder, Valery Bochkov
Department of Vascular Biology and Thrombosis Research, Center for Biomolecular Medicine and Pharmacology, Medical University of Vienna, Austria

Oxidized phospholipids (OxPLs) are increasingly recognized as inducers of chronic inflammation in atherosclerosis. More recent data indicate that in addition to their proinflammatory role, OxPLs demonstrate antiinflammatory activity as well. In particular, OxPLs antagonize effects of bacterial lipopolysaccharide (LPS, endotoxin) in vitro and protect animals from LPS-induced sepsis in vivo. The goal of this study was to investigate the role of CD14 as a target of the anti-endotoxin action of OxPLs. CD14 is a lipotransferase presenting LPS to its signalling receptor, Toll-like receptor 4 (TLR4). We found that preincubation of recombinant soluble CD14 with oxidized phosphatidylserine enhanced electrophoretic mobility of CD14 in non-denaturing gels indicating that a long-living CD14-OxPL complex was formed. Phospholipid oxidation was a prerequisite for CD14 mobility shift. OxPLs changed the mobility of endogenous soluble CD14 present in mouse and human plasma, suggesting that the interaction was highly selective and occurred in the presence of high excess of blood proteins and lipids. In addition, we showed that binding of OxPLs to CD14 prevented further binding of LPS. In summary, our data suggest that OxPLs act as competing ligands preventing LPS-CD14 interaction necessary for activation of TLR4. We conclude that since OxPLs do not activate TLR4, they can be characterized as LPS receptor antagonists.

ABSTRACT 217

Modulation of microRNA by Toll-like receptors

Frederick J. Sheedy, Elizabeth Hennessy, Sinead Corr, Luke AJ O'Neill,
School of Biochemistry & Immunology, Trinity College Dublin

MicroRNAs (miRNA) have recently emerged as a novel class of negative regulators of gene expression. They have been shown to have roles in cellular development, growth and differentiation. More recently microRNAs have emerged as key regulators of the immune response. More specifically, activation of the key pathogen recognition receptors, Toll-like receptors (TLRs) has been shown to induce the expression of two miRNA, miR-146 and miR-155. miR-146 functions to negatively regulate the expression of two key TLR-signalling proteins, IRAK-1 and TRAF-6. miR-155 expression is required for appropriate B-cell and Th1 responses and may also stabilise TNF-alpha expression and negatively target the key transcription factor c-Maf.
We have examined the expression of 150 microRNA in murine dendritic cells following treatment with ligands for Toll-like receptors (TLRs) by TaqMan PCR. As well as confirming the induction of miR-146 and miR-155 we have demonstrated that miR-21 is strongly up-regulated in dendritic cells following TLR activation. The induction of miR-21 is later than miR-146 or miR-155 and is NF-kappaB dependant. The induction of miR-21 by TLR ligands also occurs in murine macrophages as well as primary human cells and a human glioblastoma cell line.

miR-21 has been shown to be up-regulated in many human cancers and targets the tumour suppressor genes tropomyosin, PDCD4 and PTEN. We have examined the expression of both the cytoskeletal protein tropomyosin and the tumour suppressor protein PDCD4 in dendritic cells following TLR activation and found both are induced. However at later time points levels of both tropomyosin and PDCD4 protein decrease. This decrease is miR-21 dependant. miR-21 has been implicated in the control of cell migration in cancer through its control of the above proteins. We demonstrate here that TLR activation induces migration in dendritic cells and this process can be affected by miR-21. Profiling of the actin cytoskeleton by phalloidin staining reveals the occurrence of many lamellapodia and microspikes following TLR stimulation which are not as obvious at later time points and the influence of miR-21 on this process is being investigated.
We propose that following TLR activation the actin-binding protein tropomyosin and PDCD4 are induced to promote cellular migration and rearrangement of the actin cytoskeleton as seen in other cell types. miR-21 is also induced at later timepoints and this functions to negatively regulate expression of tropomyosin and PDCD4. The resulting decrease in both tropomyosin and PDCD4 protein levels leads to a decrease in cell migration and a stability of the actin cytoskeleton.
We have not only identified new components of TLR signalling but also novel processes which regulate key events downstream of TLR activation.

ABSTRACT 218

IDENTIFICATION OF THE PORCINE C-TYPE LECTIN DECTIN-1

Eva Sonck1, Edith Stuyven1, Bruno Goddeeris1,2, Eric Cox1

1 Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, 2 Department of Biosystems, Faculty of Bioscience Engineering, K.U.Leuven, Heverlee, Belgium.

Beta-glucans are conserved glucose polymers found in the cell walls of plants, fungi, yeasts and bacteria. They have a backbone of beta-(1-3)-linked glucose units with beta-(1-6)-glucan-linkages. Although a number of receptors are thought to play a role in mediating the biological response to beta-glucans, Dectin-1, a C-type lectin, was described as the most important receptor. Dectin-1 belongs to the large family of pattern recognition receptors (PRRs) which recognize conserved pathogen-associated molecular patterns (PAMPs). It has a carbohydrate recognition domain with a stalk or neck region, a transmembrane region and an intracellular ITAM motif within a cytoplasmic region. Here, we report the identification and characterization of Dectin-1 in the pig. We identified at least two major isoforms, which differ by the presence of a stalk region separating the carbohydrate recognition domain from the transmembrane region. At the nucleotide level, the full length Dectin-1 comprises 744bp and is 88% identical to the bovine Dectin-1 and 82% identical to the human Dectin-1. Messenger RNA transcripts for porcine Dectin-1 were detected in the stomach, the small intestine (duodenum, jejunum and ileum), colon and rectum of the large intestine, the spleen, the mesenterial lymph nodes and the lungs. The transcript was not expressed in the liver, kidneys, the bladder, the heart, the brains and the skin.

ABSTRACT 219

Gestational age-dependent maturation of innate immune responses to S. epidermidis

Tobias Strunk1, Peter Richmond1, Karen Simmer1, 2, Andrew Currie1, Michael Otto3, Ofer Levy4 and David Burgner1

1Schools of Paediatrics & Child Health and 2Women's and Infants Health, University of Western Australia, Perth, Australia, 3Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT and 4Division Infectious Diseases Children's Hospital Boston and 3Harvard Medical School, Boston, MA.

Coagulase-negative staphylocci, with S. epidermidis being the predominant strain, have emerged as the most commonly isolated pathogens in neonatal intensive care units around the world. This study aimed to investigate the innate immune responses to S. epidermidis in preterm and term neonates and healthy adults. S. epidermidis activates a TLR transcriptome in all age groups, however, the magnitude of responses is dependent on gestational age (GA). These findings were confirmed at protein level on a selection of cytokines, including IL6, IL10, TNF, MCP1 and MIP1a. Stimulation with TLR1/2 and TLR2/6 agonists demonstrated a similar pattern of GA-dependent cytokine induction.

The basal expression of TLRs on mononuclear cells were similar between the groups. However, S. epidermidis significantly altered the expression levels of CD14, TLR2, TLR4 and TLR6. Experiments employing neutralizing anti-TLR antibodies revealed a role of TLR2 and TLR6 for recognition of S. epidermidis.

ABSTRACT 220

EFFECT OF BETA-GLUCANS IN HORSES

E. Stuyven1, E. Sonck1, B.M. Goddeeris1,2, E. Cox1

1Laboratory of Immunology, Faculty of Veterinary Medicine, UGent, Merelbeke, Belgium
2Faculty of Bioscience Engineering, Department of Biosystems, K.U.Leuven, Heverlee, Belgium

There is increasing demand for new, safe and efficient nonspecific immunomodulators in the horseracing industry. Beta-glucans are a heterogeneous group of glucose polymers found in fungi, yeast, plants and some bacteria. Biologically efficient beta-glucans consist of linear backbones of beta (1,3) bound glycopyranose to which some beta (1,6) glycosidic linkages are connected. In the present study, seven beta-1,3/1,6-glucans who differed in origin (fungi, yeast, seaweed, bacteria or algae) and structure (linear or branched; soluble, gel or particulate) were analysed in vitro for their effect on proliferation of lymphocytes and induction of reactive oxygen species by neutrophils (chemiluminescence) of horses. Beta-glucan preparations from baker's yeast (Macrogard, Saccharomyces, Zymosan) stimulated O2-radical production best, while glucans from bacteria (Curdlan), seaweed (Laminarin) and fungi (Scleroglucan) had no effect. Paramylon, a beta-glucan from algae, stimulated ROS production moderately. Highest proliferation of T-lymphocytes occurred with Curdlan and Zymosan. However, beta-glucans derived from baker's yeast rather seemed to suppress proliferation. The other beta-glucans Scleroglucan, Paramylon and Laminarin induced moderate stimulation indexes. From these data, beta-1,6-branches don't seem to be necessary to stimulate neutrophils and lymphocytes, but solubility seems to play a significant role. The beta-glucan receptors involved in these biological activities, still have to be identified.

ABSTRACT 221

MAPPIT (Mammalian Protein-Protein Interaction Trap) analysis of early steps in TLR signalling

Peter Ulrichts, Frank Peelman and Jan Tavernier

Ghent University – VIB

The mammalian protein-protein interaction trap (MAPPIT) is a two-hybrid technique founded on type I cytokine signal transduction. Thereby, bait and prey proteins are linked to signalling deficient cytokine receptor chimeras. Interaction of bait and prey and ligand stimulation restores functional JAK-STAT signalling, which ultimately leads to the transcription of a reporter or marker gene under the control of the STAT3-responsive rPAP1 promoter.

We used the MAPPIT technique to study the molecular interactions downstream of Toll-Like receptor activation. We demonstrate pathway walking from TLR4 to IRAK-1 and identified Mal as a bridging adaptor, linking MyD88 to the activated TLR4.

ABSTRACT 222

Identification of novel viral inhibitors of innate immune signalling

Leonie Unterholzner and Andrew G Bowie

School of Biochemistry and Immunology, Trinity College Dublin, Ireland

The innate immune system recognises viral infection by making use of pattern recognition receptors (PRRs), such as the Toll-like receptors, the RNA helicases RIG-I and Mda5 and cytoplasmic DNA sensors. These PRRs initiate signalling cascades that ultimately converge at the level of the I-kappaB kinase (IKK) family of proteins, which activate the transcription factors IRF3, IRF7 and NF-kappaB. Many viruses, having co-evolved with their host, encode proteins that block or subvert these anti-viral signalling cascades. Large DNA viruses, such as vaccinia virus, encode a particularly ample repertoire of immunomodulatory proteins.

We identified several novel viral inhibitors of innate immune signalling by screening 49 largely uncharacterised vaccinia virus open reading frames for inhibition of interferon-beta induction. Two of the most potent inhibitors that emerged from this screen are the vaccinia proteins C6 and A49. C6 is a previously uncharacterised member of a poxvirus protein family that includes the immunomodulators A52, B14 and K7. Like B14, C6 targets members of the IKK family and inhibits the induction of the interferon-beta promoter by many different PRRs. However, while B14 inhibits the activation of NF-kappaB, C6 prevents the activation of IRF3 and IRF7. A49, a vaccinia protein unrelated to C6 and B14, inhibits PRR signalling by preventing the activation of NF-kappaB as well as IRF3 and IRF7. The identification of viral inhibitors and their host targets provides further insights into the regulation of innate immune signalling.
This project is supported by a Marie Curie Intra-European Fellowship and by Science Foundation Ireland.

ABSTRACT 223

Inhibition of Toll-like receptor mediated NF-kB activation by Acidic Oligosaccharides

B vt Land1,2, F Dijk2, B vd Heijning2, J Garssen1,2 A Vriesema2 and K v Norren2
1/ Utrecht Institute for Pharmaceutical Sciences, Utrecht, 2/ Danone Research, Centre for Specialised Nutrition, Wageningen, The Netherlands.

Rationale: Circulating microbial products (eg lipopolysaccharides (LPS) and peptidoglycans (PG)) derived from the gastrointestinal tract, are a suggested cause of HIV-related chronic immune activation. It was hypothesized that chronic Toll-like receptor (TLR) activation and subsequent NF-kB activation contributes to disease progression. Dietary Acidic Oligosaccharides from pectin hydrolysate (AOS) inhibit the adhesion of bacterial pathogens in the gastrointestinal tract. It was investigated whether AOS also inhibit TLR activation, resulting in decreased NF-kB activation.
Methods: HEK293 cells were stably transfected with either TLR-4 or TLR-2 and a reporter gene (luciferase) construct containing a promoter sequence with NF-kB binding sites. LPS, PG or TNF-alpha (positive control for NF-kB activation) were added 18h after addition of AOS. At 26h luciferase production was measured.
Results: AOS inhibited both LPS-stimulated TLR-4 and PG-stimulated TLR-2 NF-kB activation in a concentration-dependent way. Incubation with 1-3 mg/mL AOS resulted in a 30-80% reversal of the activation. AOS also inhibited TNF-alpha induced NF-kB activation, suggesting an additional TLR-independent NF-kB inhibition route.
Conclusion: This study shows for the first time that specific dietary oligosaccharides (AOS) are able to block NF-kB activation. The ability of AOS to modulate different pathways leading to NF-kB activation especially in the gut designates a potential beneficial role for dietary AOS in for instance the management of HIV infection.

ABSTRACT 224

Budesonide enhances TLR2 expression in activated bronchial epithelial cells

Ida von Schéele, Kjell Larsson, Lena Palmberg

Lung and Allergy Research, The Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Introduction: Endotoxin, organic dust and TNF are pro-inflammatory stimuli in vivo and in vitro. The major receptor for endotoxin (LPS) is toll-like receptor 4 (TLR4) while peptidoglycan (PGN) binds to TLR2. In dust from a swine barn both LPS and PGN are present. The aim of the study was to elucidate if TLR2 and TLR4, expressed on primary bronchial epithelial cells are influenced by exogenous (dust and LPS) and endogenous (TNF) stimuli and whether a glucocorticosteroid (budesonide) influence this interaction.

Materials and Methods: Primary bronchial epithelial cells were exposed to LPS (10µg/ml), TNF (10ng/ml) or dust (100µg/ml) 1.5hrs, 6hrs and 24hrs, in the presence or absence of budesonide 10-6M in vitro. mRNA expression of IL-6, IL-8, TLR2 and TLR4 was measured with real-time RT-PCR. IL-6 and IL-8 release was assessed with ELISA. To elucidate the importance of TLR-signaling for IL-6 and IL-8 secretion the effect of TLR-blockers was studied.

Results: LPS, TNF and dust stimulated the release of IL-6 and IL-8 in a time dependent manner. Budesonide significantly attenuated the release of IL-6 and IL-8, induced by LPS, TNF or dust, and did also attenuate IL-6 and IL-8 mRNA expression after 1.5hrs exposure, (n=6). Simultaneous exposure of pro-inflammatory stimuli and budesonide synergistically increased the TLR2 expression. Blocking of TLR2 and TLR4 reduced IL-6 and IL-8 secretion by 60% in dust stimulated cells.

Conclusions: In the presence of pro-inflammatory stimuli, budesonide reduced IL-6 and IL-8 production and enhanced expression of TLR2 in bronchial epithelial cells. These findings may contribute to the explanation of the beneficial effects of glucocorticosteroid treatment in chronic inflammatory disorders.

ABSTRACT 225

ON THE ROLE OF THE TOLL-LIKE RECEPTOR SYSTEM IN MELIOIDOSIS

WIERSINGA WJ, de Vos AF, Wieland CW, Leendertse M, Roelofs JJTH, van der Poll T
Academic Medical Center, Center for Infection and Immunity Amsterdam, the Netherlands

Background
Melioidosis, a severe infection caused by the gram-negative bacterium Burkholderia pseudomallei, is endemic in SE-Asia. We have recently shown that TLR2 - and not TLR4 - detects the LPS of B. pseudomallei and only TLR2 impacts on the immune-response of the intact host in vivo (PLoS Medicine, 2007). We now aimed to characterize function of CD14 and the key TLR-signaling proteins myeloid-differentiation-primary-response-gene-88 (MyD88) and TIR-domain-containing-adaptor-protein-inducing-IFNb (TRIF) in melioidosis.

Methods & Results

CD14 and MyD88, but not TRIF, deficient leukocytes released less TNFa upon in vitro stimulation with B. pseudomallei compared to wild-type (WT) cells. During experimentally induced melioidosis in vivo, MyD88 knock-out (KO), but not TRIF mutant mice demonstrated a strongly accelerated lethality, which was accompanied by significantly increased bacterial loads in lungs, liver and blood, and grossly enhanced liver damage compared to WT mice. The decreased bacterial clearance capacity of MyD88 KO mice was accompanied by a markedly reduced early pulmonary neutrophil recruitment and activation after infection with B. pseudomallei. In contrast, CD14 KO mice demonstrated a strongly reduced lethality, which was accompanied by significantly decreased bacterial loads compared to WT mice. Administration of recombinant soluble CD14 to CD14 KO mice partially reversed their phenotype into that of a WT-mouse, increasing bacterial loads in their brochoalveolar space and liver. Deficiency in CD14 or MyD88 did not alter the capacity of neutrophils to phagocytose or kill B. pseudomallei.

Conclusions
MyD88 dependent signaling, but not TRIF dependent signaling, contributes to a protective host response against B. pseudomallei at least in part by causing early neutrophil recruitment towards the primary site of infection. In contrast, CD14 plays a remarkable detrimental role during experimental melioidosis. Inhibition of CD14 may be a novel treatment strategy in melioidosis.

ABSTRACT 226

Impaired TLR and NLR pathway responses in the preterm infant

Andrew Currie[1], Tobias Strunk[1], Peter Richmond[1], Karen Simmer[1,2], Ofer Levy[3,4] and David Burgner[1]

[1]Schools of Paediatrics & Child Health and [2]Women's and Infants Health, University of Western Australia, Perth, Australia. [3]Division Infectious Diseases Children's Hospital Boston and [4]Harvard Medical School, Boston, MA

Preterm infants (<37 wks gestational age; GA) are extremely susceptible to life-threatening bacterial infections, not only in the neonatal period, but also in early childhood. The lack of protection against infection in preterm infants with either exogenous pathogen-specific or polyclonal antibodies is in keeping with the central role of the innate immune system in the newborn, where adaptive immune responses are largely ineffective. We therefore examined the responsiveness of cord blood mononuclear cells from very preterm (<30 wk GA) and moderately preterm (31-33 wk GA) infants to a range of TLR and NLR agonists and compared cytokine (IL-6 and TNF) responses to those of term infants and adult PBMC. There was a clear and significant inverse relationship between GA and responsiveness to all TLR agonists tested. Cytokine production by cells from very preterm infants was <30% of that by adult cells in response to TLR1/2, 2/6, 4, and 7 agonists and <50% in response to TLR7/8 stimulation. Responses by preterms to high doses (50 mcg/ml) of the NOD2 agonist, MDP, were > 1 log-fold lower than those by term and adult cells, as were responses to the complex NOD2 agonist, peptidoglycan. In contrast, responses to similar doses of NOD1 agonist, TriDAP were equivalent among all groups tested. Preterm infants have profound deficiencies in innate immune responses, but with differential effects for TLR and NOD agonists. We are currently investigating the function of TLR and NLR signalling pathways in preterm monocytes and its relevance to neonatal infection.

ABSTRACT 227

TLR RESPONSIVENESS IS DEFICIENT IN LATE PRETERM NEONATES.

SJ Macpherson1, J Xie1, I Ostopowich1, G Srinivasan2 C Ellison3 and Kent T HayGlass1,2 CIHR National Training Program in Allergy and Asthma Research 1Dept. Immunology1, Pediatrics and Child Health2 and Pathology3 Univ. of Manitoba, CANADA

Late preterm human neonates (34-36 weeks) are historically grouped immunologically and clinically with full term babies. Emerging evidence reveals that premature infants are at increased risk of infection, respiratory distress and subsequent development of asthma. We sought to assess how gestational age affects the pattern and intensity of human innate immune capacity at birth. Cord blood was collected following vaginal delivery from full-term (n=77), early (<33 wk, n=7) and late preterm (34-37 wk, n=40) infants. Mononuclear cells (CBMC) were cultured with a panel of TLR ligands for 22 h and cytokine/chemokine production quantified. The frequency of CBMC producing IL-6 and IL-10 was measured via ELISPOT. Flow cytometry was used to identify cytokine producing cell populations and to quantify absolute numbers of CD14+ monocytes. mRNA levels of TLR and related signaling molecules were assessed by Q-PCR.
Results: Over 99% of cytokine producing cells were CD14+ monocytes. Proinflammatory (IL-1 β , CCL2), anti-inflammatory (IL-10) and global (IL-6) markers of activation were all markedly reduced in the late preterm group relative to full term controls. The frequencies of IL-6 and IL-10 producing cells and the absolute number of CD14+ monocytes were not different in the preterm and full term populations. However, expression of TLR2,4,6,7,8 MyD88 and TRIF mRNA was significantly reduced in late preterm compared to full term infants.

Conclusions: Late preterm neonates demonstrate substantively impaired innate immune responses to both bacterial and viral stimuli. Our data suggest that CBMC from late preterm neonates possess intrinsic deficiencies in TLR signaling pathways which could contribute to the hyporesponsiveness observed in this population.

Funded by AllerGen NCE, CIHR and Canada Research Chair programs

ABSTRACT 228

Beta-Arrestins Differentially Regulate TLR4-Induced Gene Expression

Jane E. Lattin1, Kathryn P. Greenwood2, Norelle L. Daly2, David J. Craik2, Stuart Kellie1,3, David A. Hume4 and Matthew J. Sweet1,3

1Cooperative Research Centre for Chronic Inflammatory Diseases, Institute for Molecular Bioscience, University of Queensland, Australia, 2Australian Research Council Special Research Centre for Functional and Applied Genomics, Institute for Molecular Bioscience, University of Queensland, Australia, 3School of Molecular and Microbial Sciences, University of Queensland, Australia, 4The Roslin Institute, University of Edinburgh, Scotland, UK.

Beta-arrestins, ARRB1 and ARRB2, are highly homologous scaffolding proteins that mediate G-Protein Coupled Receptor (GPCR) internalization and downstream signalling. However, recent studies have shown ARRBs also impact on TLR signalling; they negatively regulated TLR signalling by interacting with TRAF6, but positively regulated ERK activation. We report here that primary murine macrophages and murine cell lines constitutively express elevated levels of ARRB1 and 2 mRNA and protein in comparison to other non-macrophage cell types. By expression profiling we identified a selective deficiency in expression of negative regulators of LPS signalling, including histone deactylase 1 (Hdac1) and complement component 1q (C1q) in ARRB2-/- bone marrow-derived macrophages. Basal and LPS-inducible expression of Hdac1 mRNA was repressed in ARRB2-/- BMM and consequently TLR4-inducible IFN-beta expression was amplified in these cells. Similarly, C1qa, C1qb, and C1qc mRNA and protein expression was significantly impaired in ARRB2-/- BMM. This effect was apparent in ARRB2-/-, but not ARRB1-/- BMM. ARRB2, but not ARRB1, contains a JNK docking domain and a cell permeable peptide encompassing this domain also reduced C1q expression. Thus, ARRB2 is required for the inducible expression of negative feedback regulators of TLR4 signalling in macrophages. These effects may underlie the hypersensitivity of ARRB2-/- mice to LPS-induced shock.

ABSTRACT 229

5'-Triphosphate RNA and non-CpG DNA induce a common immune response program in glomerular mesangial cells.

Ramanjaneyulu Allam and Hans-Joachim Anders

Medical Policlinic, University of Munich, Germany.

Viral infections are often associated with immune complex disease and glomerulonephritis. Mesangial cells (MC) localize between glomerular capillaries and contribute to the structure and function of the glomerular filter. It is known that MC handle and internalize immune complexes via Fc receptors and that poly I:C RNA activates MC most likely via TLR3 while they do not express TLR7 and TLR9. We have recently shown that systemic exposure to 5'-triphosphate RNA (3P-RNA) or non-CpG DNA causes an aggravation of SLE and lupus nephritis in MRLlpr mice via different effects on systemic autoimmunity of these mice. We hypothesized that the aggravation of lupus nephritis may also involve local effects of 3P-RNA and non-CpG DNA on intrinsic renal cells, like MC. We hypothesized that, in contrast to the systemic effects of 3P-RNA and non-CpG-DNA, these nucleic acids would elicit a common antiviral response program in MCs.

Comparing the transcriptome of 3P-RNA- and non-CpG-DNA-stimulated MC revealed largely overlapping expression profiles. We identified three common groups of genes being induced by 3P-RNA and non-CpG-DNA. These were 1) Proinflammatory cytokines and chemokines (Il-6, Ccl2, Ccl6, and Cxcl10), 2) Interferon-related genes (Ifit1, Mx1, Oasl2, Ifnb1, Ifih1, and Zc3hav1), 3) cell-cycle and apoptosis-related genes. The induction of the listed genes was confirmed by RT-PCR, the induction of apoptosis was confirmed by FACS. Additional siRNA studies confirmed that 3P-RNA recognition in MC depends on RIG-I but that the recognition of non-CpG-DNA is independent of DAI. Our studies show that 3P-RNA and non-CpG DNA aggravate lupus nephritis by activating different adaptive immune responses. However, in glomerular MC, 3P-RNA and non-CpG-DNA induce similar local antiviral mechanisms. Interestingly, in MC another DNA sensor, besides DAI, must exist

ABSTRACT 230

Essential components for transferring lysine-type peptidoglycan and beta-1,3-glucan recognition signals to Toll receptor in Tenebrio molitor larvae

Kyung-Baeg Roh, Hyun-Mi Kwon, Ji-Won Park, Hanna Lee, Nam-Chul Ha and Bok Luel Lee

National Research Laboratory of Defense Proteins, College of Pharmacy, Pusan National University, Busan 609-735, Korea

The Drosophila Toll signaling pathway is responsible for defending against Gram-positive bacteria and fungi by inducing the expression of antimicrobial peptides (AMPs) via NF-KB-like transcription factors. The elegant genetic studies in Drosophila have been and remain very powerful for characterizing and arranging the components of the Drosophila Toll pathway. However, there would be a limit with only genetic studies if it turns out that there is redundancy in the components involved in regulating and controlling this proteolytic cascade. Recently, we found that three serine proteases (SPs) were involved in the activation of the lysine (Lys)-type peptidoglycan (PG)-dependent Toll pathway in Tenebrio molitor, and we indicated the sequence in which they are activated in vitro [Kim et al., J. Biol. Chem. 283: 7599-7607 (2008)]. This three-step proteolytic cascade linking the Lys-type PG recognition complex and subsequent Spätzle processing is essential for the PG-dependent Toll signaling pathway. However, fungal recognition signal pathway during Toll pathway activation, which links between beta-1,3-glucan recognition complex and Spätzle, is not clearly determined yet. Here, we purified essential components involved in beta-1,3-glucan recognition signal pathway to homogeneity from the hemolymph of T. molitor and investigated how the initial beta-1,3-glucan recognition signal is transferred to Spätzle resulting in Toll pathway activation.

ABSTRACT 231

Mouse ficolin-B is expressed in neutrophils, dendritic cells, and macrophages and is down-regulated upon cell maturation

Valeria L. Runza, Johanna Kürchner, Dorothea Weber-Steffens and Daniela N. Männel

Institute for Immunology, University of Regensburg, Germany

Ficolins are plasma proteins able to activate the complement system upon pathogen encounter. Recently, we described the detection of mouse ficolin-B protein in peritoneal macrophages only after cell permeabilization, suggesting a novel function of ficolin-B as an intracellular pattern recognition molecule. However, little is still known about the kinetics of the expression of ficolin-B mRNA.

In this study, we screened different immune competent cells for ficolin-B expression and investigated its regulation upon differentiation, maturation, and stimulation by real time-PCR. We found ficolin-B to be highly expressed in bone marrow-derived Gr-1+CD11b+ ER-Hoxb8 neutrophils. The kinetic experiments showed that the amount of ficolin-B mRNA peaked around day (d) 3 and still remained high after complete differentiation (d5) in comparison to precursor cells. Stimulation on d5 with IFN-alpha and LPS, but not with TNF or IFN-gamma, resulted in a 2.5-fold increase of ficolin-B mRNA. Furthermore, ficolin-B expression was also detected in both bone marrow-derived macrophages (BMM) and dendritic cells (BMDC) but not in bone marrow-derived mast cells. In addition, expression of ficolin-B in BMM and BMDC was studied during 7-9 days of differentiation and after LPS induction, and showed to decrease during terminal differentiation and upon maturation.

In conclusion, these results indicate that ficolin-B may play a role as a novel pathogen receptor in myeloid immature cells during early stages of infection. The described characterization of ficolin-B expression provides new approaches to study the biological function of this lectin on which we currently focus.

ABSTRACT 232

Antidepressants inhibitors of TLR signalling and collagen induced arthritis.

Sandra Sacre, Alexandra Lo, Bernard Gregory, Mino Medghalchi, Marc Feldmann,

ichard Williams, Fionula Brennan, and Brian M. Foxwell.

The Kennedy Institute of Rheumatology, Imperial College London, 65 Aspenlea Road, Hammersmith, London W6 8LH, UK.

Toll-like receptors are increasingly being suggested as candidates for therapy in rheumatoid arthritis (RA), a chronic autoimmune inflammatory disease that is characterized by a destructive inflammation of the joints. We and others have been able to show expression of TLRs in RA tissue and we have also previously demonstrated a role for MyD88 and Mal in human RA synovial tissue cultures. In an attempt to address which TLRs are important in the disease process, we discovered that some antidepressants are able to inhibit endosomal TLRs and were also able to reduce established disease progression in the murine collagen induced (CIA) arthritis model. Most importantly in human RA synovial tissue cultures they were able to inhibit spontaneous cytokine release. These molecules may help us gain further insight into the mechanism of endosomal TLR signalling and the role of these receptors in RA.

Sponsored by The Kennedy Trustees and The Arthritis Research Campaign.

ABSTRACT 233

An African Swine Fever Virus gene manipulating Toll Like Receptor (TLR) signalling

Soares H.R., Oliveira V., Almeida S., Parkhouse R.M.

Instituto Gulbenkian de Ciência, Apartado 14, 2781-901 Oeiras, Portugal

African swine fever virus (ASFV) is a large double stranded DNA virus able to infect both a vertebrate (swine) as well as an invertebrate (Tick) host. As ASFV also replicates in pig macrophages, a key cellular component of the innate immune system, this virus may have evolved immune evasion genes to manipulate innate immunity; specifically, a strategy to interfere with the evolutionary conserved Toll-like receptor (TLR) signaling system. We therefore used a Bioinformatic approach to search for homologies to TLRs in the ASFV genome. This approach indicated a predicted transmembrane protein of ASFV with no known function, but containing putative Leucine-rich-repeats. To test for the interference of this gene in TLR signaling, we measured its impact on the activation of NFκB and IRF3 in luciferase reporter assays, and demonstrated inhibition of signaling induced by externally added poly I:C. Consistent with this the virus gene was a glycoprotein with cell surface expression These results indicate that the ASFV candidate gene may be inhibiting the TLR3 signaling pathway by interfering with TLR3 mediated recognition of dsRNA, either at the level of the dimerization of the TLR3 receptors or downstream in the intracellular signaling pathway.

Genetic insights and polymorphisms of innate immune receptors

ABSTRACT 234

Galectin-3 down-regulates macrophage TLR2 expression and IL-10 production stimulated by fungal antigen.

Luciana P. Ruas, Emerson S. Bernardes, Marise L. Fermino, Maria Cristina Roque-Barreira
Department of Cellular and Molecular Biology, Medical School of Ribeirão Preto - University of São Paulo, Ribeirão Preto, SP, Brazil.

Paracoccidioides brasiliensis is the causative agent of the most important systemic fungal disease in Latin America. Galectin-3 is a lactose-binding protein constitutively expressed by myeloid cells and implicated in the regulation of innate immunity. We have showed that galectin-3 tunes immunity against several pathogens, including *P. brasiliensis*, by altering cytokine production by host cells (Bernardes et al, 2006; Ferraz et al, 2008; Ruas et al, submitted). Because TLR2 is implicated in fungal recognition by phagocytes and its expression was verified to be regulated by galectin-3 in a bacterial infection (Ferraz et al, 2008), the TLR2 expression was compared among peritoneal macrophages from gal3-/- and gal3+/+ mice, in response to stimulus with P. brasiliensis antigens (PbAg). The TLR2 detection was higher on the surface of macrophages from gal3-/- than from gal3+/+ mice. In the absence of stimulus, gal3-/- macrophages presented a TLR2 expression almost 6 times higher than the gal3+/+ cells. When gal3-/- macrophages were cultured with PbAg, the expression of TLR2 was 10% higher than the observed in gal3+/+ macrophages. The measurement of IL-10 levels in the cultures supernatants showed a higher production of this regulatory cytokine by gal3-/- macrophages after PbAg stimulation. On the other hand, the stimulus with LPS - which is not a ligand for TLR2 and stimulates similar receptor expression on gal3-/- or gal3+/+ macrophages - induced a high IL-10 production that was not affected by galectin-3. Our results suggest that galectin-3 down-regulates the expression of TLR2 on macrophages and that, under selective stimulation with fungal antigens, gal3-/- macrophages up-regulates IL-10 production, a cytokine that deactivates macrophages and collaborates to increase the susceptibility to the fungal infection.

ABSTRACT 235

Frequency of Toll-like receptors 2 and 4 gene polymorphisms in Mexican patients and their association with Type 2 diabetes

Carmen Maldonado-Bernal 1, Alejandra Trejo-de la O 1, Ma. Elena Sánchez-Contreras 2, Nils Wacher-Rodarte 3, Javier Torres 1, and Miguel Cruz 2

1Infectious Diseases Research Unit, Hospital de Pediatría, 2Biochemistry Research Unit, Hospital de Especialidades, 3Clinical Epidemiology Research Unit, Hospital de Especialidades. Centro Médico Nacional SXXI, IMSS, Mexico City. MEXICO.

Important single nucleotide polymorphisms (SNPs) have been described in TLR4 and TLR2 genes, which have been associated with impaired inflammatory response. Type 2 diabetes (T2D) is characterized by a chronic low-grade inflammatory state, which can cause tissue injury. The aim of this study was to ascertain whether SNPs in TLR4 and TLR2 have any association with T2D in Mexican patients. The study included 851 Mexican individuals, 321 patients with clinical and laboratory diagnosis of T2D, and 538 healthy subjects. All individuals were genotyped for TLR4/D299G, TLR4/T399I and TLR2/R753Q SNPs by allelic exclusion PCR. Laboratory and clinical variables were also analyzed. The allelic frequencies in all the 851 individuals were: 94.1% for allele 1 (Wt) of TLR4/D299G, and 5.9% for allele 2; 93.7% for allele 1 of TLR4/T399I, and 6.3% for allele 2; 99.4% for allele 1 of TLR2/R753Q, and 0.6% for allele 2. All polymorphisms were in Hardy-Weinberg equilibrium. The OR for T2D of the SNPs was 1.12 (CI, 0.62-2.0; p= 0.690) for TLR4/D299G; 1.36 (CI, 0.78-2.4; p= 0.267) for TLR4/T399I; and 0.412 (CI, 0.04-3.70; p= 0.414) for TLR2/R753Q. No significant association was found with any of the SNPs studied and T2D, even after adjusting for age, gender and BMI. Only HDL-cholesterol values showed a significant difference between individuals with allele 1 and 2 of TLR2/R753Q (OR: 3.899, p= 0.049). In conclusion, a low frequency of SNPs in TLR4 and TLR2 was found; none of these polymorphisms were associated with T2D. These results suggest that TLRs polymorphisms play no major role in the pathogenesis of T2D in our population. Of note, TLR2/R753Q SNP was significantly associated with lower levels of HDL cholesterol.

ABSTRACT 236

Human dectin-1 polymorphisms are associated with mucocutaneous fungal infections

Theo S Plantinga1, Bart Ferwerda1, Gerben Ferwerda1, Janet A. Willment2, Annemiek B. van Spriel1, Alessandra Cambi1, Cristal Huysamen2, David L. Williams3, Leo A.B. Joosten1, Jos W.M. van der Meer1, Gosse J. Adema1, Bart-Jan Kulberg1, Gordon D. Brown2, Mihai G. Netea1

1Radboud University Nijmegen, The Netherlands; 2University of Cape Town, South Africa; 3East Tennessee State University, Johnson City, USA

Dectin-1, the C-type lectin recognizing the fungal cell wall component β-glucan, has previously been shown to be involved in the innate immune response towards fungi such as Candida albicans. We have sequenced dectin-1 gene in patients with mucocutaneous candidiasis, disseminated candidiasis and in healthy volunteers. We have identified in several patients with mucocutaneous candidiasis an early stop codon polymorphism (Tyr238Stop) in dectin-1 that results in a truncated and nonfunctional protein. Individuals homozygous for this polymorphism have defective cytokine production capacity after stimulation with β-glucan or C. albicans. As a consequence, these individuals cannot mount an effective immune response in the mucosal layers and therefore mucocutaneous and/or recurrent mucosal infections with C. albicans occur. In contrast, phagocytosis and killing of C. albicans by primary macrophages and neutrophils was normal, explaining why the dectin-1 deficiency is not associated with invasive fungal infections. In addition, a missense mutation in the CRD domain of dectin-1 (Ile223Ser) was associated with oropharyngeal candidiasis in HIV-positive patients. These results highlight the specific role of dectin-1 in human mucosal antifungal defense, and underline the role of cytokine dysregulation in the pathogenesis of mucocutaneous fungal infections.

ABSTRACT 237

Genetic Variability of TLR4 in Two Populations from Venezuela

Karen Sánchez 1, Mercedes Fernández-Mestre1, María Alejandra Duarte 1 and Zulay Layrisse1
1 Laboratorio de Fisiopatología, Centro de Medicina Experimental "Miguel Layrisse", Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela.

The toll-like receptor family (TLRs) is a major class of eukaryotic receptors for microbial pathogens associ¬ated molecular patterns (PAMPs) and endogenous lig¬ands. TLRs recognize PAMPs induce signals re¬sponsible for the activation of genes relevant to the host defense including inflammatory and adaptative immune-related cytokines. The allelic variants of TLR4 Asp299Gly and Thr399Ile have been associated with receptor dysfunction, impaired LPS signaling, and a subnormal inflammatory response. The aim of the present study was to estimate the distribution of two single nucleotide polymorphisms (SNPs) of TLR4 (Asp299Gly and Thr399Ile) in two Venezuelan communities: one Amerindian tribe (Yucpa) and the mestizo urban population living in Caracas. Whole blood was collected from 159 individuals: sixty Yucpa and ninety-nine mestizo individuals. A PCR-based restriction fragment length polymorphism (PCR-RFLP) was applied to establish TLR4 polymorphisms Asp299Gly and Thr399Ile. Allele and genotype frequencies were obtained by direct counting. A X2 test was used to verify whether the observed genotype frequencies agreed with those expected under the hypothesis of Hardy-Weinberg (H-W). Differences in allele frequencies between groups were analyzed using a contingency chi-square test. The TLR4 genotype frequencies in the mestizo population were not in H-W equilibrium and H-W test was not done in the Yucpa because the TLR4 variants were found to be monomorphic. Two common alleles for each variant (Asp and Gly of Asp299Gly, Thr and Ile of Thr399Ile) are present in mestizo population, in agreement with those reported in the population of Europe and mestizo American populations. Six TLR genotype combinations were identified in the Venezuelan mestizos: AspAsp/ThrThr (88%), AspAsp/ThrIle (1.01%), AspGly/ThrThr (4.04%) AspGly/ThrIle (4.04%), GlyGly/ThrThr(1.01%) and GlyGly/ThrIle (1.01%). In the Yucpa Amerindian, Gly and Ile alleles are completely absent, in concordance with results reported in Asian populations. The results support the notion that the distribution of the TLR4 alleles shows ethnic variability and that the variability of TLR4 locus is much reduced in Amerindians compared to other populations studied.

ABSTRACT 238

A novel splice variant of TRAM, TRAM-s, acts as an inhibitor of TLR4 signalling

Dr Anne McGettrick[1], Dr Eva Pålsson-McDermott[1], Dr Harald Husebye[2], Prof Terje Espevik[2] and Prof Luke AJ O'Neill[1]

[1]School of Immunology and Biochemistry, Trinity College Dublin, Ireland

[2]Institute of Cancer research and Molecular Medicine, NTNU, N-7489 Trondheim, Norway

We have found an alternatively spliced variant of the human TLR4 adapter TRAM (Trif-related adaptor molecule), TRAM-s, defined as 'TRAM-splice variant' (*TIRAP3b,* GenBank accession number AY304584,). TRAM-s was identified by northern blot analysis which resulted in two TRAM mRNA transcripts of 2.0 and 3.6 kb. TRAM shares its entire sequence with TRAM-s except for the 20 amino acids at its N-terminus. Interestingly therefore, TRAM-s lacks the N-terminal myristoylation consensus sequence of TRAM and also the phosphorylation site for Protein Kinase C-epsilon, but instead carries a predicted GOLD domain (amino acid 41-190), a domain implicated in protein shuttling from the endoplasmic reticulum to the Golgi. Overexpression of TRAM, but not TRAM-s, in HEK293-TLR4 cells activates an NFkappaB-linked reporter gene. Overexpression of TRAM-s inhibits the activation of NFkappaB by LPS and TRAM itself, but not MyD88 or the TLR2 ligand Pam3Cys, suggesting that TRAM-s when over-expressed is a negative regulator of the LPS signalling pathway. Preliminary results using siRNA targeting TRAM and TRAM-s supports these results, where cells transfected with siRNA to TRAM exhibit a reduction in the ability of LPS to activate an ISRE-linked reporter gene, whereas siRNA targeting the transcript for TRAM-s enhances LPS activation of ISRE. Furthermore siRNA targeting TRAM-s potentiates RANTES production and the phosphorylation of p38 MAPK by LPS. We also found that the mRNA levels of TRAM-s in THP-1 cells decrease after the cells were treated with LPS. Reconstituting TRAM knock out cells with TRAM, but not with TRAM-s, restores the ability of LPS to activate p38 MAPK. Preliminary results using confocal microscopy suggests a differential intracellular expression pattern of TRAM versus TRAM-s, where TRAM resides in the plasma membrane and in the early endosomes, whereas TRAM-s is expressed in a reticular pattern and colocalizes with markers of the late endosome. Our current hypothesis is therefore that TRAM-s is involved in the trafficking of TLR4 and possibly TRAM to the late endosome where it limits TLR4 signaling.

ABSTRACT 239

D96N, a TIR domain polymorphism of MAL/TIRAP functions as a hypomorph in TLR signaling

Kamalpreet Nagpal, Katherine A. Fitzgerald, Douglas T. Golenbock

Dept. of Medicine, University of Massachusetts Med. School, Worcester, MA, USA

Recently, many studies have described the contribution of host polymorphisms to disease susceptibility. With 8 known non-synonymous coding SNPs, MAL/TIRAP appears to be the most polymorphic of the adapter proteins involved in TLR signaling. We cloned the known polymorphisms of MAL and screened them for activity using NF-kB and IRF5-dependent ISRE luciferase reporter assays. The D96N polymorphism, which lies in the TIR domain of MAL, failed to drive either of the reporters. To further characterize these SNPs in a MAL deficient background, immortalized macrophage-like cell lines were generated from both wild type as well as MAL knockout mice. These cell lines mimic the primary bone marrow derived macrophages in morphology and function. The MAL knockout cell line was then transduced with retrovirus carrying either wild-type (WT) MAL or one of the polymorphic forms. The MAL knockout cell line transduced with WT MAL fully restored function. In addition, *all the polymorphisms except D96N were functional and produced pro-inflammatory cytokines upon LPS and PAM2 stimulation. D96N however acted as a hypomorphic mutation, with impaired cytokine production*. NF-kB activation (via IkB degradation) upon LPS and PAM2 stimulation was also analyzed in these cell lines. A similar difference in activity was observed: the D96N variant failed to degrade IkB upon PAM2 activation and had a delayed degradation profile in the case of LPS activation. Co-immunoprecipitation studies in HEK 293T cells showed that like wild type MAL, D96N interacted physically with both TLR2 and TLR4. However, the polymorphic form lost its ability to interact with MyD88, which appears to be a necessary event for downstream signaling to occur. We hypothesize that this disrupted interaction with Myd88 is the reason behind the significantly reduced activity of D96N.

ABSTRACT 240

Genetic Variations of Innate Immunity receptors and their influence on the course of infectious diseases

Djin-Ye Oh, Oliver Kumpf, and Ralf R. Schumann

Institute for Microbiology, Charité-Universitätsmedizin Berlin, Germany

Key elements of the innate immune system have been identified and functionally analyzed over the last ten years, with the Toll-like receptors (TLRs), nod-like receptors (NLRs) and rig-like receptors (RLRs) being the most prominent families. The function of these molecules includes the recognition of microbial cell-wall compounds and nucleic acids but also the recognition of intrinsic 'danger signals'. Ligand binding results in a signal transduction cascade initiating the host´s inflammatory response. Single nucleotide polymorphisms (SNPs) have been identified within the genes coding for these receptor and signal transduction proteins and gene-association studies have assessed the role of these genetic variations for susceptibility and course of infectious, inflammatory and other diseases. Several large studies are presented here investigating the influence of SNPs within TLRs 7 and 8 on susceptibility and course of viral diseases, and a combination of SNPs within the TLR system and TIRAP/Mal, a central signal transducer, on sepsis and pneumonia. Furthermore, a large group of healthy controls was genotyped, and cells of these individuals were analyzed for their response to a microbial stimulus according to their individual genotype. Genetic variations in TLRs 7 and 8 were found to influence the course of hepatitis and HIV disease, and this was reflected by a significant difference in cytokine release of cells stimulated ex vivo. Sepsis and pneumonia caused by gram-negative bacteria were strongly affected by the presence of a combination of SNPs in TLR4 and TIRAP/Mal. Individuals carrying this genotype furthermore were unable to release cytokines during the acute phase. Identifying high risk groups of patients might lead to better preventive strategies and might ultimately reduce mortality of these diseases.

ABSTRACT 241

Ancient infectious evolutionary pressure on functional variants of the Mal/TIRAP gene.

Bart Ferwerda1, Santos Alonso2, Kathy Banahan3, Evangelos J. Giamarellos-Bourboulis4, Peter Picckers1, Concepcion de la Rúa2, Bart-Jan Kullberg1, Jos W.M. van der Meer1, Luke A.J. O'Neill3, Mihai G. Netea1.

1Radboud University Nijmegen Medical Center, The Netherlands. 2University of the Basque Country, Bizkaia, Spain. 3Trinity College, Dublin 2, Ireland. 4ATTIKON University Hospital, Athens, Greece

Mal is an important adaptor protein downstream of the toll-like receptor (TLR) 2 and TLR4 pathways. The polymorphism Ser180Leu (TIRAP, rs8177374) of Mal has been described to be protective against a broad range of infectious pathogens. We assessed the worldwide distribution of Ser180Leu polymorphisms. Based on genetic data in 17 populations around the globe, we propose that this mutation might have been selected in West Eurasia during the early settlement of this region after the out-of Africa migration of modern Homo sapiens. Beside this we also investigated the functional consequences, in order to understand the changes in the genetic make-up of the innate immune system as a result of infectious pressures. The in-vivo model of experimental endotoxemia in human volunteers we used showed that heterozygous individuals for TIRAP 180L display an increased inflammatory phenotype that has been previously related to an increased resistance to infection. Finally the combined genetic and functional data provide new insight in our understanding of the pathogenesis of sepsis and how evolution molded our innate immune system.

Linking innate and acquired immunity

ABSTRACT 242

Protein Kinase C alpha is critically involved in MyD88-mediated activation of dendritic cells.

Christelle Langlet, Cécile Springael, Jolyn Johnson, Séverine Thomas, Véronique Flamand, Michel Goldman, Ezra Aksoy, and Fabienne Willems.

Institute for Medical Immunology, Université Libre de Bruxelles, Charleroi, Belgium.

Protein kinase C (PKC) isoforms are important mediators in the regulation of Toll like receptor (TLR) signalling pathways. Previously, we showed that conventional PKCα plays a critical role in IRF3 activation and IFN-β synthesis downstream of TLR3. Here, we investigated the implication of conventional PKCα isoform in the MyD88-dependent signalling pathway. Inhibition of conventional PKCs (cPKCs) activity in monocyte-derived dendritic cells by a cPKCs-specific inhibitor, Gö6976, downregulates the inflammatory cytokine synthesis induced by a TLR2 ligand (MyD88-dependent) but not by a TLR3 ligand (MyD88-independent). We showed that the drug inhibitory effect on cytokine synthesis occured at the transcriptional level. Furthermore, reporter gene assays indicated that overexpression of dominant negative form PKCα downregulates MyD88-dependent NF-κB and AP-1 transcriptional activites in parental HEK293 cell lines or stably expressing TLR2. Finally, bone marrow-derived DCs (BMDCs) from PKCα deficient mouse produced lower amounts of TNF-α and IL-12p40, following TLR-2 stimulation compared to wt BMDCs. In TLR2-stimulated PKCα-/- DCs, phosphorylation of p38, JNK MAPK, and IKKα/β kinases as well as IκBα degradation were severely impaired.

Collectively, this study identifies PKCα isoform as a key component of MyD88-mediated signalling that regulates the expression of inflammatory cytokine genes by targeting NF-κB and AP-1 activation pathways in DCs.

ABSTRACT 243

Both plasmacytoid and myeloid dendritic cells differentially produce cytokines after selective TLR7 or TLR8 stimulation.

Charlaftis N.1,2, Morley P.1, Fearon D.2, Schoenemeyer A.1

1 GlaxoSmithKline, Biopharm CEDD, Gunnels Wood Road, SG1 2NY

2 University of Cambridge, Hills Road, Cambridge CB2 2QH

The synthetic compounds resiquimod (R848) and imiquimod are potent inducers of antiviral and antitumour immune responses, mediated in part via dendritic cell activation. R848 can activate both TLR7 and 8 signalling whereas imiquimod mediates its activity via TLR7 only. In the accepted model, plasmacytoid dendrtitic cells (pDCs) selectively expressing TLR7 respond by making type I interferons, whereas myeloid dendritic cells (mDCs) selectively expressing TLR8 respond by making proinflammatory cytokines.

We have demonstrated that, at the RNA level, TLR7 and TLR8 expression is not absolutely restricted to pDC and mDC respectively. Until recently, there was no synthetic compound capable of specifically activating the TLR8 pathway. We have now used a small-molecule agonist which selectively acts via TLR8 to investigate differences between the TLR7 and TLR8 cytokine response in these dendritic cell subsets. For this purpose, pDCs and mDCs were stimulated with R848 or with compounds which are shown to be potent and selective agonists of TLR7 or of TLR8.

Our data support a model whereby, in general terms, mDC express higher levels of TLR8 and respond by making pro-inflammatory cytokines and IL-10, however, these cells could also induce low levels of IL-6 via TLR7 stimulation. Conversely, pDC express higher levels of TLR7 and respond to TLR7 stimulation by making type I interferons. However, in accordance with their RNA expression profile, we now show that pDC can also respond via TLR8 stimulation to secrete significant levels of IL-1β, IL-6 and IL-12p70.

ABSTRACT 244

Role of Dendritic Cells in the Innate and Adaptive Immune Responses to TLR Ligands

Baidong Hou1, Boris Reizis2 and Anthony L. DeFranco1

1Dept. of Microbiology and Immunology, UCSF, San Francisco, CA USA 94143 and 2Dept. of Microbiology, Columbia University Medical Center, New York City, NY, USA 10032

We have generated mice in which the gene encoding MyD88 is selectively deleted in conventional (97%) and plasmacytoid (80%) dendritic cells (DCs). We have used these mice to evaluate the role of DCs in the rapid innate cytokine production that occurs following TLR stimulation and also in evaluating the role of DCs in stimulating adaptive immune responses using TLR ligands as adjuvants. These experiments reveal a predominant role of DCs in most circumstances tested, but the responses to aggregated TLR ligands, such as LPS or CpG oligonucleotides complexed with the cationic lipid DOTAP, were less attenuated in these mice than were responses to uncomplexed CpG oligonucletides, to lipopeptides, or to a synthetic TLR7 agonist. Monocytes appeared to be the additional cell type in the spleen that responded to aggregated TLR ligands administered i.v. Interestingly, CpG oligonucleotides complexed with DOTAP induced strong production of type I interferons, primarily by plasmacytoid DCs. This type I interferon was only partially decreased in the DC-specific MyD88-knockout mice due to incomplete deletion of myd88 in plasmacytoid DCs. The residual type I interferon promoted interferon-gamma production by NK cells, and likely was responsible for the CD4 T cell expansion, survival, and Th1 differentiation seen in these mice, which was comparable to control mice. In contrast, use of non-complexed CpG oligonucleotides as adjuvant resulted in good NK and CD4 T cell responses in wild type mice but very poor responses in the mutant mice.

ABSTRACT 245

SRC KINASES ARE REQUIRED FOR SYNERGISTIC INDUCTION OF SELECTED NFLAMMATORY CYTOKINES BY MULTIPLE TLR IN HUMAN DENDRITIC CELLS

Mirela Kuka, Roberta Baronio, Elisabetta Monaci, Ennio De Gregorio and Ugo D'Oro
Novartis Vaccines, Siena, Italy, I-53100

Antigen presentation by mature Dendritic Cells (DC) is a key event in the initiation of adaptive immunity. A broad range of microbial products can trigger DC maturation, acting as stimuli for Toll-like receptors (TLRs) whose activation leads, through different signalling pathways, to cytokine production and increased expression of costimulatory molecules. Dendritic cells express different TLRs and thus they can be simultaneously activated by different pathogen-associated molecular patterns (PAMPs). It was previously shown that TLR4 triggered production of inflammatory cytokines by DC is dependent on src family tyrosine kinases. Since TLR4 signaling operates trough both MyD88 and TRIF adaptors we investigated if src kinases are required for cytokine production triggered by TLR3-TRIF mediated signalling or by TLRs that signal only via MyD88. We indeed found that in all cases active src kinases are critical for TLR mediated cytokine production. To better characterize the molecular mechanism of this requirement we also performed a whole human genome microarray analysis on DC stimulated with a TLR3 agonist in the presence of PP2, a src kinase inhibitor. In the case of TLR3 stimulation we identified 41 genes which up-regulation was strongly inhibited by PP2, while only partial inhibition could be observed for 38 more genes. It was reported that a concurrent engagement of TLR8 with either TLR4 or TLR3 resulted in a synergistic effect on cytokines production. We investigated the role of src kinases in this biological phenomenon in human MoDC stimulated with a TLR8 agonist and LPS, a TLR4 agonists. We found that production of inflammatory cytokines was markedly enhanced when stimuli were provided simultaneously, and that this synergistic effect was impaired when DCs were pre-treated with PP2. These findings consolidate the idea that DCs can respond specifically to different and defined sets of microbial products, and that their maturation can be modulated by specific pharmacological inhibitors, thus modifying the type of immune response.

This work was supported by a grant from the European Commission 6th Framework Program (Contract LSHB-CT-2004-512074 DC-THERA NETWORK OF EXCELLENCE)

ABSTRACT 246

Role of TLR in the development of IgM+IgD+CD27+ B cells in humans

Sandra Weller1, Héloïse Delagreverie1, Mélanie Bonnet1, Anne Puel2, Capucine Picard2,3, Jean-Laurent Casanova2, Claude-Agnès Reynaud1 and Jean-Claude Weill1
1U783 INSERM and 2U550, Université Paris Descartes, Faculté de Médecine Necker-Enfants malades Paris, France, 3Centre des déficits immunitaires, Hôpital Necker, Enfants-Malades, Paris, France

In humans, peripheral blood B cells that display the CD27 marker and express mutated Ig receptors can be divided in 2 major populations: IgM+IgD+CD27+ cells and isotype-switched IgM-IgD-CD27+ cells carrying surface IgG or IgA. While switched CD27+ cells are bona fide memory cells that are generated in germinal center during the course of T-dependent responses, we showed that blood IgM+IgD+CD27+ cells are not memory cells, but are instead circulating splenic marginal zone B cells involved in responses against T-independent (TI) antigens such as encapsulated bacteria. Moreover, our recent data support the view that these cells may develop and diversify their Ig receptor along a developmental program and we wanted to find out what could be the role of TLRs during this process, TLR9 in particular, because of its high expression on CD27+ B cells and its role in proliferation and antibody production in response to CpG DNA in vitro. The UNC93B molecule is crucial for proper TLR3, 7, 8 and 9 signaling and both IRAK-4 and Myd88 appear to be essential in the signaling pathways of all TLRs, with the exception of TLR3 and TLR4 which is partially Myd88/IRAK-4 independent (not known for TLR10). For this reason, we started to study the peripheral blood B cell subsets of patient deficient for IRAK-4 (n=7), Myd88- (n=3), or UNC93B (n=2). We observed that in Myd88- and IRAK-4-deficient patients, but not in UNC93B-deficient patients, the proportion of IgM+IgD+CD27+ cells was markedly reduced in comparison to switched cells (a 2-4 fold difference), but the mutation level of their Ig was comparable to age-matched healthy controls. Our results suggest that TLR3, 7, 8 and 9 are dispensable for the development and/or maintenance of IgM+IgD+CD27+ cells and point to a possible role for TLR10. Alternatively, an UNC98B-independent signaling pathway for TLR9 may exist in human B cells. This possibility is currently under investigation.

ABSTRACT 247

Functional role of murine TLR8 in autoantibody production

Shauna R. Hutchinson, Jin-Hwan Han and Thereza Imanishi-Kari

Program in 1Genetics and 2Immunology, Tufts University Sackler School of Graduate Biomedical Sciences, Boston, MA 02111

Equally contributed

Systemic Lupus erythematosus (SLE) is an autoimmune disease characterized by the high production of nuclear antigen specific autoantibodies. In order to study the pathogenesis of SLE, we developed 564Igi, a mouse model of SLE. This model has targeted heavy and light chain genes in the immunoglobulin heavy (Igh) and light (Igk) chain locus. 564Igi idiotype-positive (Id+) B cells in the periphery of these mice were anergic, however 564 Id+ antibodies were found in the sera (Berland et.al. Immunity, 2006). The production of these antibodies was totally MyD88 but partially dependent on TLR7 expression, suggesting the potential involvement of a different MyD88-dependent TLR. Since human TLR7, 8, and 9 are known to sense nuclear antigens in a MyD88-dependent manner, we determined a possible role of murine TLR8 and TLR9 in 564 autoantibody production. We analyzed Id+ antibodies in the sera of 564Igi in TLR9- and TLR7/TLR9 double-deficient background. 564Igi-Tlr9-/- mice showed markedly increased levels of Id+ antibodies in their sera in comparison to the 564Igi mice in the wild-type and MyD88-deficient background. These results suggest a protective role of TLR9 to the RNA-specific autoantibody production of 564Igi mice. 564Igi B cells in TLR7/ TLR9 double-deficient background still possessed Id+ antibodies when compared to 564Igi on MyD88-deficient background. In addition, in vitro culture of TLR7/TLR9 double-deficient mouse B cells with human TLR8 ligand resulted in their differentiation to antibody-secreting cells. These results suggest that murine TLR8 is functional and has a role in autoimmune disease like SLE.

ABSTRACT 248

Direct involvement of TLR2 in the maintenance of memory CD8 cells

Anne Cottalorda, Blandine C. Mercier, F. Martial Mbitikon-Kobo, Christophe Arpin, Jacqueline Marvel and Nathalie Bonnefoy-Bérard

INSERM, U851, Lyon, F-69007 France, IFR128, Biosciences Lyon-Gerland, Lyon, F-69007 France; Université Lyon 1, Villeurbanne, F-69622 France.

Previous works have shown that persistence of memory CD8 T cells is controlled by cytokines using the common gamma chain receptor like IL-2, IL-7 and IL-15. However, other molecules may be involved in this phenomenon. In particular, we had already demonstrated that TLR2 is functionally expressed on CD8 T cells. As we observed that TLR2-/- mice had a partial defect in memory CD8 T cells when compared with WT mice, we investigated the potential role of TLR2 in the homeostasis of memory CD8 T cells. We demonstrate here that TLR2 engagement on memory CD8 T cells increases their proliferation and survival induced by IL-2 or IL-7 both in vitro and in vivo. This effect is associated with augmented expression of CD25 and of anti-apoptotic proteins Bcl-xL and Bcl-2.We also reporte that TLR2 ligands synergize with IL-2, but not IL-7, to promote IFN-γ production. Collectively, our results describe a new mechanism by which TLR2 engagement on memory CD8 T cells participates to their proliferation and to their effector functions in the absence of specific antigen. These results suggest that continuous TLR signaling in response to microbial stimuli might directly promote the maintenance of memory CD8 T cells in the organism.

ABSTRACT 249

Nod1 and Nod2-dependent Th2 polarization of antigen-specific immunity is dependent of radio-resistant cells.

Joao G.Magalhaes1, Lionel LeBourhis1, Leonardo H.Travassos1, Thirumahal Selvanantham1, Jörg H.Fritz1, Stephen E.Girardin2, and Dana J.Philpott1

1 Department of Immunology, 2Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.

Nod1 and Nod2 are cytoplasmic PRMs implicated in the recognition of bacterial pepdidoglycan (PGN), a major component of bacterial cell wall. It has been previously shown that Nod1 and Nod2 agonists are able to stimulate a Th2 adaptive immunity in vivo. However, the mechanisms and the signaling pathways underlying their action have remained elusive. Here, we examined the unfolding of the adaptive immune response upon Nod1 and Nod2 activation. In contrast with the current model that DCs are the key players integrating microbial and antigen signals to instruct adaptive immune responses, our studies showed that bone marrow cell transfer from wild-type to Nod1- or Nod2-KO mice could not compensate for Nod1 and Nod2 deficiency leading to the idea that immune priming by Nod1 and Nod2 is carried by cells other than those in the myeloid compartment. Very interestingly, we have demonstrated that adaptive immunity through Nod1 and Nod2 can be induced in wild-type mice receiving Nod1 or Nod2-deficient bone marrow. This activation, however, was not optimal and required radio-sensitive cells to induce a full blow response to Nod1 and Nod2 agonists. In terms of mechanism, we have shown that IL-7RKO mice immunized with Ova plus Nod1 or Nod2 agonists have a reduced proliferation of Ova specific T cells. We are now identifying the specific cells types implicated in these processes as well as the mediators involved. Results from these studies will contribute to a better understanding of the role of Nod proteins in mucosal immunity. In particular, this study reveals an unexpected role of radio-resistant cell in driving dendritic cells to induce an adaptive immunity upon Nod1 and Nod2 activation.

ABSTRACT 250

TLR2 ENGAGEMENT ON CD8 T CELLS ENABLES GENERATION OF FUNCTIONAL MEMORY CELLS IN RESPONSE TO SUBOPTIMAL ACTIVATION

Blandine C. Mercier, Anne Cottalorda, Charles-Antoine Coupet, Jacqueline Marvel and Nathalie Bonnefoy-Bérard

INSERM, U851, Lyon, F-69007 France; IFR128, Biosciences Lyon-Gerland, Lyon, F-69007 France; Université Lyon 1, Villeurbanne, F-69622 France.

Toll-like receptors (TLR) are involved in the detection of microbial infection as well as endogenous ligands that signal tissue and cell damage in mammals. This recognition plays an essential role in innate immune response and in the initiation of adaptive immune response. We have previously shown that murine CD8 T cells express TLR2 and that costimulation of Ag-activated CD8 T cells with TLR2 ligands enhances their proliferation, survival and effector functions. We show here that TLR2 engagement on CD8 T cells lowers their threshold for the antigen concentration normally required for optimal activation, and converts a suboptimal activation into a productive process leading to significant cell expansion. Using altered peptide ligands, we demonstrate that TLR2 engagement increases CD8 T cell activation in vitro and in vivo, and enables the generation of functional memory cells in response to poorly immunogenic antigens. This increased activation is associated with an augmented activation of the PI3-K signaling pathway. Taken together, our results demonstrate that TLR2 engagement on CD8 T cells lowers their activation threshold for TCR signal strength and enables efficient memory cell generation in response to suboptimal activation signals.

ABSTRACT 251

T-cell co-stimulation by TLR7/8 ligands is dependent on the cellular environment

Denise Pargmann1, Miriam Wechsler1, Arthur M. Krieg2, Jörg Vollmer1 and Marion Jurk1 Biotherapeutics and Bioinnovation Center, Pfizer Inc., 1 Coley Pharmaceutical GmbH, Düsseldorf, Germany, 2 Research Technology Center, Cambridge, MA, USA

Toll-like receptors (TLRs) are mediators of the innate immune system detecting conserved pathogen-associated molecules. TLR 1, 2, 4, 5, 6, 10, and 11 are expressed on the cell surface, whereas TLR 3, 7, 8 and 9 are predominantly localized in endosomal compartments. The TLRs are widely expressed in different cell types. Recent studies have reported that the majority of TLRs are also expressed by T lymphocytes, resulting in direct co-stimulation of isolated CD4+ T-cells by Pam3CSK4 (TLR2 ligand), Flagellin (TLR5 ligand) or R-848 (TLR7/8 ligand). We here describe enhanced IFN-gamma production and T-cell proliferation by CD3/T-cell receptor- or antigen-stimulated purified CD4+ T-cells upon co-culture with TLR7/8 specific single-stranded oligoribonucleotides or small molecule ligands. Surprisingly, TLR7/8 stimulation of CD4+ T-cells within a whole peripheral mononuclear cell (PBMC) environment did not result in enhanced T-cell proliferation. The observed lack of proliferation was cell-cell contact dependent, and cell depletion assays point towards a monocyte-mediated effect. In these experiments, different TLR ligands influenced T-cell proliferation differently indicating that the effect is unique only for certain TLRs. Our results strongly suggest that activation of T-cell proliferation is dependent on the specific cellular context. These findings are of interest for the development of TLR therapeutics for vaccine and other disease applications.

ABSTRACT 252

The allergy protective Lactococcus lactis strain G121 requires intracellular signaling for dendritic cell activation

Karina Stein1, Jennifer Debarry1, Anna Hanuszkiewicz2, Otto Holst2, Azita Mahiny3, Michael Lohoff3, Ruth Ferstl4, Carsten Kirschning4, Holger Heine1

1Dept. of Immunology and Cell Biology, and 2Dept. of Immunochemistry and Biochemical Microbiology, Research Center Borstel, Borstel, Germany

3Institute of Microbiology, University of Marburg, Marburg, Germany

4 Department of Medical Microbiology and Hygiene, Technical University of Munich, Munich, Germany

The incidence of allergic diseases, particularly in industrialized regions, is worldwide increasing. Farming environment in early childhood reduces the occurrence of allergic reactions later in life. Recently, we showed that the cowshed isolate Lactococcus lactis G121 prevents allergic immune responses in a mouse asthma model. However, the molecular mechanisms involved in cell activation by L. lactis G121 are mainly unknown. L.lactis G121-stimulated human DCs showed an induction of IL-12p70, a main TH1-polarizing cytokine. Inhibition of uptake and endosomal acidification strongly decreased activation by L. lactis G121, indicating intracellular receptors. However, stimulation of murine bone-marrow derived macrophages from TLR-KO animals with L. lactis G121 showed no implication of the intracellular TLRs 3, 7 or 9 despite the fact that MyD88-KO, in contrast to Trif-KO, macrophages could not be activated after stimulation with L. lactis G121. Investigation of different transcription factors indicated a possible involvement of the interferon signal transduction pathway, since L. lactis-stimulated IRF-1 KO macrophages show less activation as wild-type cells. In addition, western blotting analysis of human DC lysates demonstrated an activation of Stat1 and Stat3. Overall, identifying the molecular mechanism of the activation of immune cells by L. lactis G121 might lead to a better understanding of the molecular mechanism by which farming environment prevents allergic immune responses (supported by DFG, SFB/TR22, project A2).

ABSTRACT 253

Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5

Satoshi Uematsu and Shizuo Akira

While intestine maintains homeostasis by immunological tolerance, it properly induces immune responses by recognizing the invasive pathogens. However, it remains unknown what kinds of cells in the intestine initiate immune responses and how they activate host immunity. Here we identified $CD11c^{high}CD11b^{high}$ lamina propria (LP) dendritic cells (DCs) that express Toll-like receptor (TLR) 5 in the small intestine. When stimulated by the TLR5 ligand flagellin, $TLR5^{+}$ LPDCs induced differentiation of naïve B cells into immunoglobulin A (IgA)-producing plasma cells; this plasma cell generation took place in a gut-associated lymphoid tissue (GALT)-independent fashion. In addition, in a manner dependent on TLR5 stimulation, these LPDCs promoted differentiation of antigen-specific T_H-17 and T_H1 cells. Unlike spleen DCs, LPDCs specifically produced retinoic acid, which, in a dose-dependent manner, supported the generation and retention of IgA-producing cells in the LP and positively regulated T_H-17 cell differentiation. These findings reveal unique properties of LPDCs and the importance of TLR5 for adaptive immunity in the intestine.

ABSTRACT 254

Role of TLR7 and IRAK1 for pristane-induced autoantibody production and development of lupus nephritis

Jakob Loschko*, Emina Savarese*, Christian Steinberg, Rahul D. Pawar, Wolfgang Reindl, Hans-Joachim Anders, Anne Krug (*contributed equally)

II. Medizinische Klinik, Klinikum Rechts der Isar, Technical University Munich, Germany

High titers of antibodies against small nuclear ribonucleoproteins (snRNP) are observed in the serum of patients with systemic lupus erythematosus (SLE). Endogenous RNA molecules within snRNP trigger toll-like receptor 7 (TLR7) activation in B cells and dendritic cells leading to anti-snRNP antibody production, which is associated with the development of immune complex nephritis in SLE. In this study we investigated the role of TLR7 as well as the downstream signaling molecule IRAK1 for anti-snRNP antibody production and renal disease in SLE induced by an exogenous factor in the absence of genetic predisposition using the pristane-induced murine lupus model. Anti-snRNP/Sm antibody production induced by pristane was dependent on the expression of TLR7 while anti-dsDNA antibody production was not affected by lack of TLR7. Similarly anti-snRNP/Sm antibody production was reduced in IRAK1-deficient mice, whereas anti-dsDNA antibody production was comparable between IRAK1-/- and WT mice suggesting that the IRAK1-dependent type I IFN response downstream of TLR7 is specifically required for the production of RNA-reactive autoantibodies. TLR7-deficient mice demonstrated lower glomerular IgG and complement deposition as well as less severe glomerulonephritis. In contrast, no difference in nephritis activity was observed between IRAK1-deficient and WT mice suggesting that IRAK1-independent signalling downstream of TLR7 activation is required for development of lupus nephritis. Thus, TLR7 is specifically required for production of RNA-reactive autoantibodies and development of glomerulonephritis in pristane-induced murine lupus as a model for environmentally triggered SLE. Specific interference with TLR7 activation by endogenous TLR7 ligands may therefore be a promising novel strategy for the treatment of SLE.