11

Infection

11-01/P

EFFECTS OF BACTERIAL TOXINS ON THE GROWTH, DIFFERENTIATION AND TOLL-LIKE RECEPTOR GENES OF HUMAN MESENCHYMAL STEM CELLS

Mo FY[1], Yip KHK[2], Law HKW[1], Lau YL[1], Chan GCF[1]

[1]Department of Paediatrics & Adolescent Medicine, Faculty of Medicine; [2]Department of Prosthodontics, Faculty of Dentistry, the University of Hong Kong HKSAR, China

Human Mesenchymal Stem Cells (MSCs) are multipotential stem cells and its potential therapeutic value gained substantial interest in recent years. Due to the osteogenic potential of MSCs, MSCs are used to aid osseointegration in dental implants and bone grafting. They are therefore subjected to continuous exposure of microflora in oral cavity or peridontopathic bacteria during infection. Whether these bacteria have any effects on the growth and osteogenic differentiation of human MSCs remains unknown. We therefore investigated the effect of both of the gram-positive and gram-negative bacterial cell wall components: lipoteichoic acid (LTA, *Streptococcus pyogenes*) and lipopolysaccharides (LPS, *Escherichia coli*) respectively on the proliferation and osteodifferentiation of MSCs. Since Toll-like receptors (TLRs) are recently identified to be the receptors for LTA and LPS which trigger inflammatory responses, we also explored the expression kinetics of TLR2 and TLR4 genes in MSCs and its derived osteprogenitors during osteogenic differentiation. We found that both LPS and LTA did not affect the proliferation of MSCs, but prolonged LPS challenge upregulated the osteogenic differentiation of MSCs as shown by the increase in ALP activity and calcium deposition ($p < 0.05$). We also demonstrated that during MSC osteodifferentiation, the MSC-derived osteoprogenitors gradually expressed TLR2 ($p < 0.001$) and TLR4 ($p < 0.01$) gene with time even without any external stimuli. Interestingly, when under the continuous exposure of LPS, the MSC-derived osteoprogenitors showed a significant reduction in TLR2 ($p < 0.05$) and TLR4 ($p < 0.01$) gene expression during osteogenic differentiation. This adaptive response to LPS found in MSC-derived osteoprogenitors might suggest a regulatory measure to maintain homeostasis in the microflora-rich environment such as the oral cavity. Further studies are required to explore the cytokine profiles involved in altering the osteogenic activities (such as IL-6) and the TLR genes in MSCs and its osteoprogenitors.

11-02/O

ACTIVATION OF αβTCR$^+$ T-CELLS BY *S. AUREUS* CAPSULAR POLYSACCHARIDE REGULATES LOCAL NEUTROPHIL RESPONSE TO INFECTION

McLoughlin RM, Zaleski KJ, Kasper DK, Lee JC, Tzianabos AO

Channing Laboratory, Brigham & Womens Hospital and Harvard Medical School, Boston, MA, USA.

Increasing prevalence of nosocomial and community-acquired *Staphlococcus. aureus* infections necessitates a better understanding of host-pathogen interactions. *S. aureus* virulence factors contribute to its disease causing ability however the host response to *S. aureus* pathogenesis is less well understood. Neutrophils represent the first line of host-defense and are critical in determining infection outcome. Conversely, evidence suggests that survival of *S. aureus* inside neutrophils may actually contribute to pathogenesis. T-cells are also implicated in regulating *S. aureus* infection. T-cells control abscess development in animal models of *S. aureus* intra-abdominal abscess formation, and the *S. aureus* capsular polysaccharide (CP8) can activate CD4$^+$T cells *in vitro*. Here, we identify a mechanism by which *S. aureus* activates αβTCR$^+$T-cells to regulate CXC-chemokine induced neutrophil recruitment to the infection site. Using a murine model of surgical wound infection we demonstrate that αβTCR-deficient mice are less susceptible to *S. aureus*-infection than wild-type mice. Furthermore, αβTCR$^{-/-}$-mice exhibit reduced CXC chemokine (MIP-2, KC) production and neutrophil recruitment to the wound site. Local administration of MIP-2 increased bacterial burden at the wound and increased the number of bacteria internalized per neutrophil. To establish the mechanism by which *S. aureus* activates αβTCR$^+$T-cells, *in vitro* we demonstrate uptake of *S. aureus*-CP8 by antigen presenting cells, depolymerisation and presentation in the context of MHC-class II molecules. Resulting αβTCR$^+$-T-cell activation leads to cytokine (IFN-γ, IL-17) and chemokine (IL-8) secretion, which have the potential to regulate neutrophil recruitment and function. Indeed, administration of purified-CP8 in the wound-infection model results in CXC-chemokine production and neutrophil recruitment to the wound site in wild-type mice. This effect is significantly attenuated in αβTCR$^{-/-}$-mice. We conclude that *S. aureus,* by virtue of its capsular-polysaccharide, can activate αβTCR$^+$T-cells to produce cytokines and chemokines that impact upon the neutrophil arm of the host-response to infection. Appropriate neutrophil recruitment is vital for optimal clearance of the infection and return to tissue homeostasis.

11-03/P

GENE THERAPY OF CHRONIC HBV USING A HIGH-CAPACITY ADENOVIRUS EXPRESSING IL-12.

Crettaz J[1], Pañeda A[1], Ochoa L[1], Berraondo P[1], Otano I[1], Olague C[1], Vales A[1], Kochanek S[2], Prieto J[1], González-Aseguinolaza G[1]

[1]Division of Hepatology and Gene Therapy, Center for Investigation in Applied Medicine (CIMA), University of Navarra, Pamplona, Spain; [2] Division of Gene Therapy, University of Ulm, Ulm, Germany.

Three hundred fifty millions people are estimated to be infected by the hepatitis B virus (HBV). Eighty-seven millions of them are at risk of developing HBV-related liver cancer or cirrhosis. In this study we developed a high-capacity adenovirus (HC-Ad), encoding for the murine IL12 directed by a liver-specific promoter and regulable by the administration of mifepristone (HC-RU-mIL12). We assayed its antiviral effect using two animal models of chronic HBV: HBV transgenic mice and woodchucks chronically infected with the woodchuck hepatitis virus (WHV).
HBV transgenic mice received $2x10^8$ infective units (iu) of HC-RU-mIL12. Two weeks later they received five daily doses of inducer (mifepristone). Control groups received the virus but were not induced or received the inducer alone. Treated animals showed a complete abrogation of serum viral load and the concentration of viral antigens (HBsAg y HBeAg) decreased significantly, whereas the viremia in control groups remained unchanged.
Three woodchucks were included in the second study. Two of them (T1 and T2) received $9x10^9$ iu of HC-RU-mIL12. The third woodchuck (C1) received no virus. Woodchucks were treated daily during eleven days with 500 µg of mifepristone per kg of body-weight. Woodchuck T2, expressed low levels of mIL12 and its viremia remained unchanged after treatment. However, woodchuck T1expressed high levels of mIL12 and suffered a spectacular decrease of its viremia. In both treated woodchucks the expression of IL-12 induced a significant activation of cellular immune response against core antigen (WHcAg). No changes were observed in control woodchuck. These experiments demonstrate the antiviral efficacy of the intrahepatic expression of mIL12. We validate the HC-RU-mIL12 as a safe and innocuous vector since no toxicity was detected, probably due to the localized and controlled expression of the cytokine. Studies with a larger number of animals are being conducted and new data will be presented.

11-04/P

THE CANDIDA ALBICANS ORTHOLOGUES OF THE DIT1 AND DIT2 YEAST MEIOTIC GENES CONTRIBUTE TO VIRULENCE OF CANDIDA.

Bito A[1], Costantino G[2,3], Briza P[4], Müller M[2, 3], Breitenbach M[1]

[1] Department of Cell Biology, University of Salzburg, Austria;[2] Institute of Animal Breeding and Genetics, Veterinary University of Vienna, Austria; [3] Austrian Center for Biomodels and Transgenetics, Veterinary University of Vienna, Austria; [4] Department of Molecular Biology, University of Salzburg, Austria

Candida albicans is the most prevalent human fungal pathogen. It can cause mucosal and - in immunocompromized patients - severe systemic infections. Although meiosis has never been observed in this dimorphic yeast its genome contains many orthologous genes to bakers yeast meiotic genes. We have isolated two genes, *CaDIT1* and *CaDIT2*, from a *C. albicans* gene library. The products of the orthologous genes in *S. cerevisiae* are required for the synthesis of dityrosine which is the major compound of the outermost wall layer of ascospores, the products of yeast meiosis. In contrast to wild type ascospores, the meiotic products of *S. cerevisiae dit1* and *dit2* null mutants do not contain any dityrosine and are sensitive to some several conditions (enzymatic lysis of spore wall, heat shock, mechanical shearing, killing by organic solvents).
We could show that, when the native promoter of the *Candida DIT* genes has been replaced by the *S. cerevisiae DIT1* promoter, either gene could complement the dityrosine deficiency of the corresponding *S. cerevisiae dit* mutant. This strongly indicates that *C. albicans* is able to form dityrosine. Expression analysis by RT-PCR and Northern blotting revealed expression of the genes at a low level in *Candida* yeast and hyphal cells. However, we could not detect any dityrosine in *Candida* yeast or hyphal cells. *C. albicans dit1* and *dit2* null mutants did not show any obvious phenotype *in vitro* so far and formed hyphae and chlamydospores (which are formed asexually) as efficiently as the wild-type strain. In order to investigate a putative role of the *CaDIT* genes in the host, the mutants were used for virulence studies in a mouse systemic model. Both the *C. albicans dit1* and the *dit2* null mutants were less virulent compared to the wild-type strain. Therefore, both genes contribute to *C. albicans* virulence and represent a potential target for antifungal therapy.

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11-05/P

EXPLORING THE ROLE OF MURINE IRF-5 IN THE ANTI-VIRAL RESPONSE

Paun A, Reinert JT, Pitha-Rowe PM

Division of Viral Oncology, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD USA

In vivo experiments have indicated that murine IRF-5 (mIRF-5) is involved in the innate antiviral response and induction of interferon genes and cytokines. However, until very recently little was known about the role of mIRF-5 in the immune response *in vivo*. Recent data has shown mIRF-5 interacts with Traf6 and MyD88, downstream of Toll like receptors, and is involved in the expression of proinflammatory cytokines such as IL-6, TNFα and IL-12. To address the role of mIRF-5 in viral infection we are using an IRF-5 knockout mouse (IRF-$5^{-/-}$) and have found that IRF-5 is involved in both the IFN and proinflammatory cytokine response to NDV and Sendai virus. IRF-$5^{-/-}$ mice were found to have lower serum levels of TNFα at all time points examined. In vivo Il-12p70 induction was also found to be impaired, however no significant difference was found in serum levels of IL-6. Furthermore, both *in vivo* and *in vitro* data indicate impairment in type I interferon production in response to NDV infection. Serum IL-6 and TNFα levels were also found to be reduced following infection with Sendai virus. IRF-$5^{-/-}$ Flt3L-induced pDCs were found to produce lower levels of IL-6 and TNFα after 16 hours of *in vitro* stimulation with NDV or the TLR7 ligand R848. In vitro, we have found that mIRF-5 is able to activate the IFNα4 and IFNβ promoters in response to NDV infection, however no cooperation with IRF-3 or IRF-7 was detected. In contrast to IRF-3, RIG-I did not cause transcriptional activation of mIRF-5. These data suggest that as well as being important for the regulation of proinflammatory cytokines, mIRF-5 also plays a role in the innate *in vivo* response to viral infection.

11-06/P

HEPATITIS C VIRUS SERINE PROTEASE IS A POTENT INHIBITOR OF HOST CELL CYTOKINE/CHEMOKINE GENE EXPRESSION

Kaukinen P[1], Sillanpää M[1], Kotenko S[2], Lin R[3], Hiscott J[3], Melén K[1], Julkunen I[1]

[1]Department of Viral Diseases and Immunology, National Public Health Institute, Helsinki, Finland; [2]Department of Biochemistry and Molecular Biology, University of Medicine and Dentistry-New Jersey Medical School, Newark, NJ, USA; [3]Lady Davis Institute for Medical Research, Department of Microbiology & Immunology, McGill University, Montreal, Canada

Hepatitis C virus (HCV) encodes for several proteins that interfere with host cell antiviral response. Previously, serine protease NS3/4A was shown to inhibit IFN-β gene expression by blocking dsRNA-activated

retinoic acid-inducible gene I (RIG-I) and Toll-like receptor 3 (TLR3)-mediated signaling pathways. In the present work, we systematically studied the effect of all HCV proteins on IFN gene expression. Although NS3/4A was found to be the only specific inhibitor of IFN gene activation, metalloprotease NS2 inhibited and NS4B activated a control reporter gene expression as well. NS3/4A inhibited the Sendai virus-induced expression of multiple IFN (IFN-α, IFN-β and IFN-λ1/IL-29) and chemokine (CCL5, CXCL8 and CXCL10) gene promoters. Recent studies showed that RIG-I, Cardif (or IPS-1/MAVS/VISA), IKKε and TBK1 activate IRF3 and NFκB transcription factors and induce IFN (IFN-β) gene expression. Wild type NS3/4A, but not its proteolytically inactive form NS3/4A-S139A, was found to inhibit promoter activity induced by RIG-I or its adaptor protein Cardif. Both endogenous and transfected Cardif were found to be proteolytically cleaved by NS3/4A. Cardif was also strongly colocalized with NS3/4A at the mitochondrial membrane suggesting mitochondria to be the site for proteolytic cleavage. In many experimental systems IFN priming can dramatically enhance RNA virus-induced IFN gene expression. Pretreatment of HEK293 cells with IFN-α strongly enhanced RIG-I expression but failed to protect Cardif from NS3/4A-mediated cleavage and failed to restore Sendai virus-induced IFN-β gene expression. Our data indicate that HCV is a strong and a general inhibitor of host cell cytokine gene expression.

11-07/O

ABSENCE OF AN EARLY CYTOKINE RESPONSE TO MALARIA INFECTION IS ASSOCIATED WITH PROGRESSION TO CEREBRAL MALARIA IN MICE

Hunt NH, Mitchell A, Hansen A, Hee L, Potter S, Ball H

Molecular Immunopathology Unit, Bosch Institute, University of Sydney, Sydney, NSW Australia

Cerebral malaria (CM) is a major cause of death in malaria infection. The pathogenesis of this condition is poorly understood. C57BL/6 mice infected with *Plasmodium berghei* ANKA (PbA) (2 X 10^6 parasitised red blood cells, PRBC) succumb to CM on days 6 – 8 post-inoculation (p.i.). In contrast, mice infected with the same size inoculum of *P. berghei* K173 (PbK) do not develop CM but, rather, die much later (around days 15 – 21 p.i.) of other complications, most likely anaemia. There was a large peak of interferon-γ (IFNγ) in the plasma 24 hours after PbK inoculation, which had returned to normal by day 2 p.i. This peak of plasma IFNγ did not occur in mice inoculated with PbA. In the spleen, mRNAs for IFNγ, IL-12, IDO, IL-10 and NOS2 all were strongly induced at 24 hours p.i. in PbK, but not PbA, infection. Thus induction of expression of these immunomodulators was not seen in an infection (PbA) that progressed to an immunopathological reaction, namely CM, but was present in an infection (PbK) that did not lead to CM. It was shown that a low inoculum size (2 X 10^4) of PbK did not induce immunomodulator gene expression in the spleen, or IFNγ levels in the plasma, at 24 hours p.i. and these mice did go on to develop CM. When PbA and PbK were co-infected, there was an early increase in immunomodulator expression and mice did not develop CM. $CD8^+$ T lymphocytes appeared to be the major source of the IFNγ in PbK infection. Thus, effective engagement of the innate immune system and immunomodulator expression, as in a high PbK inoculum, led to an active immune response but not to immunopathology. Failure to induce an immunomodulator response in PbA infection led to an ineffective immune response but an immunopathological reaction, CM.

11-08/P

HEPATITIS C VIRUS NS3/4A PREFERENTIALLY TARGETS RIG-I/IRF3 PATHWAY AND DOWNREGULATES CHEMOKINE GENE EXPRESSION

Sillanpää M, Kaukinen P, Melén K, Julkunen I

National Public Health Institute, Helsinki, Finland

Hepatitis C virus (HCV) is a single-strand RNA virus that belongs to Flaviviridae family. HCV genome encodes a large polyprotein that is cleaved into eleven structural and non-structural (NS) proteins by virus-encoded or cellular proteases. NS3/4A protein complex has previously been shown to inhibit dsRNA activated Toll-like receptor (TLR)3 and retinoic acid inducible gene I (RIG-I) pathways by proteolytically cleaving the crucial adaptor proteins of these pathways, TIR-domain containing adaptor inducing IFN-β (TRIF) and CARD containing adaptor protein Cardif. Here we have used osteosarcoma cell lines, that inducibly (kindly provided by Dr. D. Moradpour) express NS3/4A, NS4B, core-E1-E2-p7 and entire HCV polyprotein, to study the effect HCV proteins on Sendai virus-induced IFN-β, CCL5, CXCL8 and CXCL10 gene expression. The expression of NS3/4A alone or in the context of the whole HCV genome led to clear down-regulation of IFN-β, CCL5, CXCL8 and CXCL10 mRNA expression and protein production. In further studies, the expression of NS3/4A and the entire HCV polyprotein resulted in a significant decrease of IRF3 and to a lesser extent NF-κB (p50/p65) binding to their respective binding elements on CXCL10 promoter. However, the expression of NS3/4A had only a minor effect on binding of NF-κB and AP-1 (c-Jun/c-Fos) to their response elements on CXCL8 promoter. The expression of core-E1-E2-p7 proteins increased production of CXCL8, and also the binding of NF-kB to CXCL8 promoter was prolonged. In the cell lines expressing NS3/4A or the whole HCV genome a clear sequestration of mitochondria was observed which was kinetically associated with the degradation of endogenous Cardif. Our results indicate that NS3/4A targets Cardif in the RIG-I pathway specifically leading to reduced IRF3 activation and expression of IRF3 regulated genes such as IFN-β, CCL5 and CXCL10. MAP kinase and NF-κB pathways, instead are not at all or only weakly inhibited by HCV NS3/4A.

11-09/O

THE ROLE OF TRIF AND MYD88 DEPENDENT SIGNALING IN THE RESISTANCE TO *E. coli* PERITONITIS.

van 't Veer C[1,2], van den Pangaart P[1,2], Hoebe K[3], Beutler B[3], Takeda K[4], Akira S[4], van der Poll T[1,2]

[1]Center for Infection and Immunity Amsterdam (CINIMA), [2]Center of Experimental and Molecular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, [3]Department of Immunology, The Scripps Research Institute, La Jolla, CA, USA, [4]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Japan

The innate immune system may sense the presence of bacteria by recognition of pathogen associated molecular patterns (PAMP) through Toll-like receptors (TLR). Lipopolysaccharide (LPS) is the most potent PAMP on gram-negative bacteria activating the innate immune sytem *via* both MyD88 and Trif. TLR4, MyD88, and Trif deficient mice are protected against a lethal dose of LPS. We determined the contribution of TLR4, MyD88 and Trif in the resistance to *E. coli* peritonitis. Wild type (WT), TLR4-KO, Trif-mutant and MyD88-KO mice were injected intraperitoneally with 10^4 CFU of the virulent strain *E. coli* 018:K1, and sacrificed after 6 or 20 hours. Twenty hours after the i.p. challenge with *E. coli* the bacterial load ($\approx 10^9$ CFU/ml peritoneal lavage fluid) was comparable in the peritoneal cavity of the different mice. The main difference at this initial site of infection was the lack of leukocyte recruitment in the MyD88-KO mice, while recruitment was normal in TLR4-KO and Trif-mutant mice. Dissemination of *E. coli* bacteria to blood and liver was ≈ 50-fold increased in TLR4-KO and MyD88-KO mice and associated with elevated plasma TNFα levels compared to WT, while Trif-mutant mice tended to have lower bacterial counts than WT at this point and lower plasma TNFα. Trif-mutant mice displayed a moderate tendency to live longer in survival studies ($p = 0.1$). Interestingly, in the early phase of the infection, at t = 6 hours after i.p. inoculation, the Trif-mutant mice had significantly elevated bacterial counts in blood and liver compared to WT, which was associated with lower TNF-α levels in peritoneal lavage fluid. Our data indicate that TLR4 and MyD88 signaling are key events in the resistance to *E. coli* peritonitis. Furthermore, signaling through Trif enhances the early resistance to *E. coli* dissemination during peritonitis, but appears to be detrimental at later stages of peritonitis caused by pathogenic *E. coli*.

11-10/O

ROLE OF THE PROTEIN KINASE IKKε AND OF THE RIG-I/CARDIF PATHWAY IN THE CONTROL OF HCV EXPRESSION

Vitour D[1], Vilasco M[1], Dabo S[1], Larrea E[2], Vidalain PO[3], Tangy F[3], Jacob Y[3], Regnault B[4], Eid P[5], Prieto J[2], Meurs EF[1*]

[1, 3, 4] Institut Pasteur : [1]Unit Hepacivirus, [3]Department of Virology, [4]« DNA chips», Paris, France; [2]Center for Applied Medical Research (CIMA), University of Navarra and Clinica Universitaria, Pamplona, Spain; [5] CNRS, UPR 9045, Laboratory of Viral Oncology, Villejuif, France

During a viral infection, binding of viral dsRNAs to the cytosolic RNA helicase RIG-1 leads to recruitment of the mitochondria-associated Cardif protein, involved in activation of the IRF3-phosphorylating IKKε/TBK1 kinases, IFN induction and development of innate immune response. The Hepatitis C Virus (HCV) NS3/4A protease cleaves Cardif and abrogates both IKKε/TBK1 activation and IFN induction. By using an HCV replicon model, we showed that ectopic overexpression of IKKε can inhibit HCV expression. Analysis of the IKKε transcriptome profile in these conditions revealed induction of several genes associated with the antiviral action of IFN. Interestingly, IKKε still inhibits HCV expression in presence of neutralizing antibodies to IFN receptors, suggesting that IKKε is able to rapidly activate the cellular antiviral response in HCV-infected cells, in addition to provoke IFN induction. To determine the physiological importance of IKKε in HCV infection, we then analysed its expression levels in liver biopsies from HCV-infected patients. This analysis also included genes of the IFN induction pathway (RIG-I, MDA5, LGP2, Cardif, TBK1), and three IKKε-induced genes (IFNβ, CCL3 and ISG15). The results show significant inhibition of expression of IKKε and of the RNA helicases RIG-I/MDA5/LGP2 in the HCV-infected patients, while expression of TBK1 and Cardif was not significantly altered. Given the antiviral potential of IKKε and of the RNA helicases, these *in vivo* data strongly support an important role for these genes in the control of HCV infection. Recent data showed close colocalization of IKKε with Cardif at the mitochondrial membrane (*Lin, R. et al, 2006*). Recently, we have determined the regions of Cardif involved in IKKε recognition and identified common partners to Cardif and IKKε by yeast two-hybrid screening. Using a cell culture system of infection with HCVcc of genotype 2a, we are currently determining the role of these new partners in the control of HCV infection.

11-11/P

ROLE OF TLR2, TLR4 and MyD88 IN RESPONSES TO INFECTION WITH *STREPTOCOCCUS PYOGENES*

Gratz N[1], Gattermeier I[1], Schaljo B[1], Vojtek I[1], Akira S[2], Charpentier E[1], Kovarik P[1]

[1]Max F. Perutz Laboratories, University of Vienna, Vienna, Austria; [2]Research Institute for Microbial Diseases, Osaka University, Japan

Streptococcus pyogenes (also called Group A Streptococcus (GAS)) is an important Gram-positive human pathogen which causes a variety of infections that can be mild, such as pharyngitis and impetigo, to extremely severe, such as necrotizing fasciitis, septicaemia, pneumonia and streptococcal toxic shock syndrome.
The primary line of innate defence against most bacterial pathogens consists of macrophages, which reside in tissues and polymorphonuclear neutrophils (PMNs) which migrate from the blood to the site of infection. The molecules responsible for the recognition of *S. pyogenes* by these cells and the downstream signalling intermediates, which are critical for the host to activate inflammatory responses, remain largely unknown. We have addressed this issue by analyzing the activation of ERK and p38 MAP kinases, and the transcription factors NFkB and Stat1 in primary mouse TLR2, TLR4 or MyD88 knockout and wild type control macrophages infected with *S. pyogenes*. Surprisingly, our data show that TLR2, the receptor for Gram-positive bacteria, is not required for macrophage activation. Macrophage responses were also TLR4-independent. However, the activation of ERK, p38 MAPK and NFkB in the early phase of infection was dependent on the adaptor protein MyD88, an important member of the TLR signaling pathway. This finding indicates that TLRs other than TLR2 or TLR4 (or a combination of them) are involved in the recognition of *S. pyogenes*. Furthermore, we have observed that the activation of the interferon-activated transcription factor Stat1 was independent of TLR2, TLR4, or the adaptor MyD88 in infected macrophages. The activation of interferon signaling by so called extracellular Gram-positive bacteria such as *S. pyogenes* in primary mouse macrophages was so far unknown and is currently under investigation in our laboratory.

11-12/P

INDUCTION OF THE ANTIVIRAL PROTEIN VIPERIN BY INTERFERON AND VIRUS DEPENDS ON INTERFERON REGULATORY FACTOR-1

Wiese A, Klages K, Hauser H, Kröger A

Department of Gene Regulation and Differentiation, Helmholtz Centre for Infection Research, Braunschweig, Germany

Enhanced expression of Interferon regulatory factor-1 (IRF-1) induces antiviral effects independent of interferon (IFN) by transcriptional activation of target genes. Viperin (Vig1) is an evolutionary highly conserved interferon stimulated gene which exhibits antiviral activity and is supposed to play an important role in limiting hepatitis C virus replication. Vig1 induction occurs not exclusively after IFN treatment but also after infection with various viruses. Our data reveal Vig1 as a new IRF-1 target gene that contributes to the antiviral effects of IRF-1. We show that enhanced expression of IRF-1 leads to induction of endogenous Vig1. The Vig1 promoter contains three IRF-1 response elements (IRF-E) that are critical for IRF-1 and IFN induced gene transcription. Mutation of these promoter elements inhibits IRF-1 mediated Vig1 expression. IRF-1 KO fibroblasts fail to activate Vig1 in response to IFN or vesicular stomatitis virus (VSV) infection, whereas IRF-3 KO cells respond to IFN-β by increasing Vig1 expression. These data elucidate a pathway how IRF-1 enforces its antiviral activities.

11-13/P

NITRIC OXIDE PLAYS A CRITICAL ROLE IN PLATELET-ACTIVATING FACTOR-INDUCED ENHANCEMENT OF RESISTANCE AGAINST SYSTEMIC CANDIDIASIS

Park SJ[1], Kim SH[1], Kim KJ[1], Kim HA[1], Im SY[1], Lee HK[2]

[1]Department of Biological Sciences, Chonnam National University, Kwangju 500-757, [2]Department of Immunology, Chonbuk National University Medical School, Chonju 561-180, Republic of Korea.

In studying the mechanism of *C. albicans* resistance, we have recently reported that endogenously produced platelet-activating factor (PAF) in response to *C. albicans* renders the animals resistant to the fungus by promoting the activation of NF-κB. In this study, we investigated the role of nitric oxide in the PAF-induced enhancement of the resistance to *C. albicans* infection. *C. albicans* infection itself expressed minute levels of mRNA expression and protein synthesis of iNOS in the organs examined, whereas pretreatment of the mice with PAF resulted in the strong expression of both mRNA and protein levels. These effects of PAF were significantly inhibited by pretreatment with the NF-κB inhibitor. iNOS inhibitor, aminoguanidine reduced the protective activity of PAF in a dose dependent manner. In addition, the significant protective activity of PAF was not observed in $iNOS^{-/-}$ mice. The nitrite-producing capacity of cultured splenocytes by PAF was completely abrogated in iNOS. These data indicate that NO plays an important role in PAF-induced protection against *C. albicans*.