09

Immunity

09-01/P

EFFECTS OF VARIOUS IMMUNOMODULATORS ON THE ANTIMICROBIAL ACTIVITY OF CLARITHROMYCIN IN COMBINATION WITH RIFAMPIN AGAINST *MYCOBACTERIUM AVIUM* COMPLEX WITHIN MACROPHAGES

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Refractory *Mycobacterium avium* complex (MAC) infections are frequently encountered in immunocompromised hosts, particularly AIDS patients. Because MAC organisms show moderate to high resistance to common antituberculosis drugs, there is an urgent need for new anti-MAC antimicrobial drugs, although the development of such antimycobacterial drugs is difficult. In this context, one promising strategy is to devise regimens to treat infected patients with ordinary antimycobacterial agents in combination with appropriate immunomodulators. Here, we examined the effects of chitin-related agents (chitin, chitosan and oligochitosan) and a number of traditional Chinese medicines on the antimicrobial activity of clarithromycin (CLR) in combination with rifampin (RIF) against MAC organisms replicating within macrophages (MΦs). As a result, (1) Chitin-related agents did not inhibit intramacrophage bacterial growth, although chitin and chitosan, but not oligochitosan, potentiated the bactericidal activity of CLR/RIF against intramacrophage MAC organisms. (2) Mao-Bushi-Saishin-To (MBST) treatment of MΦs promoted the CLR/RIF-mediated killing of intramacrophage MAC organisms, whereas forty-five other Chinese traditional medicines did not exhibit such effects. (3) The effects of chitin and chitosan on therapeutic activities of CLR/RIF against MAC infection were examined in mice. CLR/RIF displayed significant therapeutic efficacy in terms of reducing bacterial growth in the lungs and spleen of CLR/RIF-treated mice compared to that in untreated control mice. However, neither chitin nor chitosan increased the anti-MAC therapeutic activity of CLR/RIF. In separate experiments, MBST significantly increased the anti-MAC therapeutic activity of rifalazil (RLZ) when administered in combination with RLZ, although MBST alone did not exhibit such effects. These findings suggest potential benefits of chitin, chitosan and MBST as immunoadjuncts in combination with antimycobacterial drugs to treat patients with MAC infections. Further studies are desired to devise an optimal antimycobacterial drug-based regimen that includes chitin, chitosan and MBST to treat MAC infections.

09-02/P

ENDOGENOUS CYTOPLASMIC DSRNA CAN TRIGGER BOTH RNAI AND INNATE IMMUNE RESPONSES.

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RNA interference (RNAi) currently represents one of the most promising tools to decipher the human genome, and holds great potential in therapy. Yet, little is known about the possible implications of RNAi proteins in antiviral immunity in mammals. In this work, we studied the over-expression of a transcript with the potential to form a long double stranded RNA (dsRNA). Our data support previous reports that some dsRNA molecules can evade nuclear retention, and be exported to the cytoplasm. We also provide evidence for a simultaneous activation of both RNAi and innate immune responses by these endogenous dsRNAs. These findings together with the recent discovery that TRBP is involved in both RNAi and innate immunity highlight the complex interplay between these two pathways. This also further raises the question of a cytoplasmic role for naturally occurring dsRNAs in mammals.

09-03/O

AUGMENTATION OF ANTIGEN-PRESENTING AND TH1-PROMOTING FUNCTION OF DENDRITIC CELLS BY WSX-1 (IL-27R)-DEFICIENCY

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WSX-1 is a component of the IL-27 receptor. While the roles of IL-27/WSX-1 on T cell differentiation and activation has been extensively examined and it has been shown that IL-27/WSX-1 is critical for T cell regulation, either as a promoter of Th1 responses or as an attenuator of immune responses, little is know about the role of WSX-1 on dendritic cells (DCs). In the current study, we examined the function of WSX-1 on DC functions. WSX-1 is weakly expressed in immature DCs and the expression was augmented during their maturation by LPS stimulation. Expression of CD80 and 86 (B7.1 and B7.2, respectively) was higher in LPS-stimulated WSX-1-deficient DCs than in wild-type DCs. WSX-1-deficient DCs showed augmented antigen-presenting function over wild-type DCs in allo-reactive mixed lymphocyte reaction. When transferred to wild-type mice, WSX-1-deficient DCs pulsed with KLH induced more Th1-inclined immune responses than wild-type DCs. In addition, when wild-type mice were transferred with DCs pulsed with *Leishmania major* antigens at their footpad and subsequently infected with *L. major*, WSX-1-deficient DCs were more protective than wild-type DCs in terms of footpad swelling after infection. Finally, when syngeneic NK cells were cocultured with LPS-treated WSX-1-deficient DCs, these NK cells showed higher cytolytic activity with higher level of IFN-gamma and perforin expression over those cultured with wild-type DCs. These data clearly demonstrated that IL-27/WSX-1 had a suppressive effect on DC activation and that WSX-1-deficiency resulted in augmented antigen-presenting and Th1-promoting function of DCs. It is thus suggested that, while indicating a complex role of IL-27/WSX-1 during immune reaction, augmentation of DC function by regulating IL-27/WSX-1 signaling is a therapeutic target of diseases such as infection and cancer.

09-04/P

SUPPRESSOR OF CYTOKINE SIGNALING-1 SELECTIVELY INHIBITS LIPOPOLYSACCHARIDE-INDUCED IL-6 PRODUCTION BY REGULATING JANUS KINASE-SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION

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Suppressor of cytokine signaling-1 (SOCS-1) is one of the negative feedback regulators of Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling. Here, we demonstrate that SOCS-1 selectively inhibits lipopolysaccharide (LPS)-induced IL-6 production through regulation of JAK-STAT, but not LPS-induced other cytokines including Tumor Necrosis Factor-α (TNF-α). We found that LPS directly activated Jak2 and Stat5, whereas SOCS-1 inhibited LPS-induced Jak2 and Stat5 activation. Furthermore, AG490, a Jak-specific inhibitor, and dominant negative Stat5 only reduced LPS-induced IL-6 production. A recent study has demonstrated that NF-κB p50 associates with IκBζ induced by LPS and subsequently induced IL-6 production. We found that Stat5 interacted with p50, resulting in recruitment of Stat5 to the IL-6 promoter together with p50 in response to LPS stimulation. These findings suggest that the JAK-STAT pathway participates in LPS-induced IL-6 production and that SOCS-1 suppresses LPS signaling by regulating JAK-STAT.

09-05/O

CD14 FACILITATES INVASIVE RESPIRATORY TRACT INFECTION BY *STREPTOCOCCUS PNEUMONIAE*

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CD14 is a pattern recognition receptor that can interact with a variety of bacterial ligands. During Gram-negative infection CD14 plays an important role in the induction of a protective immune response by virtue of its capacity to recognize lipopolysaccharide in the bacterial cell wall. Knowledge of the contribution of CD14 to host defense against Gram-positive infections is limited. To study the role of CD14 in Gram-positive bacterial pneumonia, CD14 knockout (KO) and normal wild-type (WT) mice were intranasally infected with *Streptococcus (S.) pneumoniae,* the most frequently isolated pathogen in patients with community-acquired pneumonia. CD14 KO mice demonstrated a strongly reduced lethality, which was accompanied by a more than 10-fold lower bacterial load in lung homogenates but not in bronchoalveolar lavage fluid at 48 hours after infection. Strikingly, CD14 KO mice failed to develop positive blood cultures, whereas WT mice had positive blood cultures from 24 hours onward and eventually invariably had evidence of systemic infection. Lung inflammation was attenuated in CD14 KO mice at 48 hours after infection, as evaluated by histopathology and cytokine and chemokine levels. Intrapulmonary delivery of recombinant soluble CD14 to CD14 KO mice rendered them equally susceptible to *S. pneumoniae* as WT mice, resulting in enhanced bacterial growth in lung homogenates and bacteremia, indicating that the presence of soluble CD14 in the bronchoalveolar compartment is sufficient to cause invasive pneumococcal disease. These data suggest that *S. pneumoniae* uses (soluble) CD14 present in the bronchoalveolar space to cause invasive respiratory tract infection.

09-06/P

THE PPAR-GAMMA AGONIST CIGLITAZONE REDUCES BOTH BACTERIAL OUTGROWTH AND INFLAMMATION IN A MURINE PNEUMONIA MODEL

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Thiazolidinediones (TZDs) are a group of drugs of which some are currently in use as oral glucose lowering drugs. TZDs are synthetic agonists for the PPAR-gamma receptor though their exact mechanism of action remains unknown. Besides their insulin-sensitizing effect, TZDs were found to have immune modulating effects, both *in vitro* and *in vivo*. As type 2 diabetes patients have an increased risk for pneumonia, we evaluated the influence of ciglitazone, a TZD, on markers of inflammation and outcome during a pneumonia caused by *S. pneumoniae*. C57Bl/6 mice were inoculated with 10^5 CFU of *S. pneumoniae* intranasally. The following interventions were studied: (1) vehicle at t = 0, (2) ciglitazone 5 mg/kg intraperitoneally at t = 0, (3) ciglitazone 5 mg/kg intraperitoneally at t = 0 and 24 h. Mice were killed at either 24 or 48 h after infection (N = 8 per treatment group at each time point). Single treatment with ciglitazone reduced bacterial loads at 24 h but not at 48 h, whereas repeated ciglitazone treatment did diminish bacterial loads at 48 h. After 24 h cytokine levels in lung homogenate were lower in single dose ciglitazone treated mice; however after 48 h there was no difference in lung cytokines between all experimental groups. On both time points there was no difference in plasma cytokine levels, pathology inflammation scores and lung myeloperoxidase levels between all experimental groups. In an additional experiment, ciglitazone treatment (given once daily) tended to reduce mortality (P = 0.08). We conclude that ciglitazone reduces bacterial outgrowth and local inflammation at least during the early stage of *S. pneumoniae* pneumonia in mice.

09-07/P

HCV CORE PROTEIN INHIBITS INTERFERON-STIMULATED GENES THROUGH IRF-1 REPRESSION

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Efficiency of Hepatitis C virus (HCV) in establishing persistent infection implies that it has evolved numerous strategies in evading the host immune response. Indeed, HCV proteins have been shown to interfere at several levels with both the innate and adaptive response of the host. Key targets of HCV over the host response are found in the Interferon (IFN) signaling. While the effects of nonstructural proteins in counteracting the IFN response has been well established, controversial remains the role of structural proteins due to conflicting results. Here we investigated the effect of the HCV structural proteins on the expression of Interferon regulatory Factor-1 (IRF-1) a secondary transcription factor in the IFN system, responsible for the induction of several antiviral and immunomodulatory genes, key in the innate as well as in the adaptive immune response. We found that in cells expressing the entire HCV replicon a substantial inhibition of IRF-1 expression occurs. Suppression of IRF-1 synthesis was mainly mediated by the core structural protein and occurred at the transcriptional level by inhibition of the IRF-1 promoter activity. The core protein in turn exerted a transcriptional repression of several Interferon stimulated-genes (ISGs) target of IRF-1, including IL-15, IL-12 and LMP2. These results recapitulate in a unifying mechanism i.e. repression of IRF-1 expression, many of the so far described pathogenetic effects of HCV core protein and suggest that the HCV core-induced IRF-1 repression may play a pivotal role in establishing persistent infection by dampening an effective immune response.

09-08/P

INTERFERON REGULATORY FACTOR-1 IS REQUIRED FOR FULL ACTIVATION AND FUNCTION OF DENDRITIC CELLS

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Members of the Interferon regulatory factors (IRFs) family are transcriptional regulators that play essential roles in the homeostasis and function of the immune system. Recent studies indicate a direct involvement of some members of the family in the development of different subsets of dendritic cells (DC). Here, we report that IRF-1 is a potent modulator of the development and functional maturation of DC. IRF-1 deficient mice (IRF-$1^{-/-}$) exhibited a predominance of plasmacytoid DC and a selective reduction of conventional DC, especially the $CD8\alpha^+$ subset. IRF-$1^{-/-}$ splenic DC (s-DC) were markedly impaired in their ability to produce proinflammatory cytokines such as IL-12. By contrast, they expressed high levels of IL-10, TGF-β and the tolerogenic enzyme indoleamine 2,3 dioxygenase (IDO) indicative of a tolerogenic phenotype. As a consequence, IRF-$1^{-/-}$ s-DC were unable to undergo full maturation and retained a plasmacytoid and tolerogenic phenotype following virus infection both *ex vivo* and *in vivo*. Finally, s-DC from IRF-$1^{-/-}$ mice were less efficient in stimulating the proliferation of allogeneic T cells and instead induced an IL-10-mediated suppressive activity in allogeneic $CD4^+CD25^+$ regulatory T cells. Together, these results indicate that IRF-1 is a key regulator of DC differentiation and maturation, exerting a variety of effects on the functional activation and tolerogenic potential of these cells.

09-09/P

TOLL-LIKE RECEPTOR 2 CONTRIBUTES TO ANTIBACTERIAL DEFENSE DURING PNEUMONIA CAUSED BY PNEUMOLYSIN-DEFICIENT BUT NOT BY WILD-TYPE *STREPTOCOCCUS PNEUMONIAE*

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Streptococcus (S.) pneumoniae is a common cause of community-acquired pneumonia which becomes more difficult to treat due to emerging antibiotic resistance. Extending research about the interaction between *S. pneumoniae* and innate immunity may result in new therapeutic tools to treat pneumococcal pneumonia. Toll-like receptors (TLR) are pattern recognition receptors which recognize conserved molecular patterns expressed by pathogens. Pneumolysin, an intracellular toxin found in the pneumococcus, is an importance virulence factor of *S. pneumoniae* that is recognized by TLR4. Besides TLR4, TLR2 is of importance for the recognition of *S. pneumoniae* by immune cells. In previous research we established that TLR2 KO mice have an unremarkable antibacterial defense during pneumonia caused by serotype 3 *S. pneumoniae* (J. Immunol. 2004; 172: 3132). We here hypothesized that TLR2 KO are still able to mount an effective immune response to *S. pneumoniae* because they rely on activation of TLR4 by pneumolysin. To test this hypothesis we intranasally inoculated wild type and TLR2 KO mice with either wild-type *S. pneumoniae* D39 (serotype 2) or pneumolysin deficient *S. pneumoniae* D39. In accordance with our previous study, TLR2 KO mice displayed a normal defense against wild-type D39. In contrast, infection of TLR2 KO mice with pneumolysin deficient D39 resulted in an enhanced growth of bacteria relative to wild-type mice, indicating that in the absence of the TLR4 ligand pneumolysin TLR2 does contribute to antibacterial defense during pneumococcal pneumonia. These data suggest that pneumolysin-induced TLR4 signalling can compensate for TLR2 deficiency during the induction of an adequate innate immune response to pneumonia caused by *S. pneumoniae*.

09-10/O

CD27 DEFICIENT MICE HAVE AN IMPROVED DEFENSE AGAINST *STREPTOCOCCUS PNEUMONIAE* PNEUMONIA

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The tumor necrosis factor receptor family member CD27 has been mainly implicated in T and B cell co-stimulation. Recently, it was suggested that the interaction of CD27 and its ligand CD70 in early progenitor cells provides a negative feedback mechanism that regulates hematopoiesis during immune activation. To study the role of CD27 in pulmonary infection and inflammation, we intranasally infected wild-type (WT) and CD27 knock-out (KO) mice with $5x10^4$ CFU of *Streptococcus* (*S.*) *pneumoniae* and sacrificed the animals 24 and 48 h later. CD27 KO mice had a strongly reduced outgrowth of pneumococci in the lungs, a decreased dissemination of the infection and a better survival rate. Pulmonary levels of Interleukin (IL)-1β and KC were reduced throughout infection in the CD27 deficient animals and TNF (48h) and IL-6 (48 and 24h) concentrations were lower in the systemic compartment. Moreover, the increased resistance of CD27 KO mice was associated with reduced inflammation scores but higher neutrophil counts in bronchoalveolar lavage fluid at 48 h post infection. To investigate the role of CD27 in cellular recruitment from the bone marrow during pneumococcal pneumonia, we transferred mixtures of WT and CD27 KO bone marrow to irradiated WT recipient mice. No differences in host inflammatory responses, antibacterial defense and infiltrating cell populations were found in mice that underwent this mixed bone marrow transplantation, thus ruling out a possible role of CD27 in a negative feedback on inflammation induced hematopoiesis. In addition *in vitro* migration and phagocytosis capacity of CD27 KO neutrophils did not differ from WT neutrophils and

natural antibody levels were normal in CD27 KO mice. However, the observed induction of CD70 mRNA, the ligand of CD27 hints that the phenotype observed is a consequence of direct interaction of CD27 and CD70 and not due to an indirect effect.

09-11/P

ANTI-INTERFERON AUTOANTIBODIES IN AUTOIMMUNE POLYENDOCRINOPATHY SYNDROME TYPE 1

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The AutoImmune Regulator (*AIRE*) gene influences thymic self-tolerance induction. In Autoimmune Polyendocrinopathy Syndrome type 1 (APS1), recessive *AIRE* mutations lead to autoimmunity targetting endocrine and other epithelial tissues, although chronic candidiasis usually appears first. We tested for serum autoantibodies to type I IFNs and other immunoregulatory cytokines using specific binding and neutralisation assays. Unexpectedly, in 74/74 Nordic APS1 patients with both *AIRE* alleles mutated, we found high titre neutralising autoantibodies (NAbs) to all IFN-α subtypes and especially IFN-α (60% homologous to IFN-α) from early childhood onwards. We found lower titres against IFN-β in 23% of patients; two-thirds of these (from Finland only) also had low titres against the distantly related "type III IFN", IFN-λ1. However, autoantibodies to the unrelated type II IFN, IFN-γ, and other immunoregulatory cytokines such as interleukin-10 (IL-10) and IL-12, were much rarer and did not neutralise. The NAbs to type I IFNs preceded overt candidiasis (and several of the autoimmune disorders) in the informative patients, and persisted for decades thereafter. They were undetectable in unaffected heterozygous relatives of APS1 probands (except for low titres against IFN-λ1), in APS2, and in isolated cases of the endocrine diseases most typical of APS1, so they appear APS1-specific. These apparently spontaneous autoantibody responses to IFNs, particularly IFN-α and -ω, segregate like a recessive trait; their high "penetrance" is especially remarkable in such a variable condition. Their apparent restriction to APS1 implies practical value in the clinic, e.g., in diagnosing unusual or prodromal *AIRE*-mutant patients with only single components of APS1, and possibly in prognosis if they prove to predict its onset. They also raise numerous questions, e.g., about the rarity of other infections in APS1. Moreover, there must also be clues to autoimmunising mechanisms/ cell types in the hierarchy of preferences for IFN-ω, IFN-α8, IFN-α2, and IFN-β and IFN-λ1.

09-12/P

MIZORIBINE INHIBITS CROSS-PRESENTATION OF EXOGENOUS ANTIGEN ASSOCIATED WITH CLASS I MHC MOLECULE AND INFLAMMATION RESPONSE

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Mizoribine (MIZ) has been shown to possess an immunosuppressive action that inhibits the proliferation of lymphocytes selectively by interfering with inosine monophosphate dehydrogenase. Recent studies documented that exogenous antigens can enter the class I MHC presentation pathway, a process termed cross presentation. Since cross presentation emerges to be required for the effective generation of CTL responses, it is interesting to see whether modulation of cross presentation capability could be a way of immunosuppressants on the cross presentation capability of DC. DC2.4 cells or bone marrow derived DCs (BM-DCs) generated from C57BL/6 mouse. In this work, we tested whether MIZ inhibits the presentation of dendritic cells (DCs) when DCs were cultured in the presence MIZ with OVA-microspheres. The amount of OVA peptide-class I MHC complexes was measured by a T cell hybridoma, B3Z, which recognizes OVA (257-264)-H-2K^b complex. MIZ inhibited class I MHC-restricted presentation of exogenous antigen in both DC2.4 cells and BM- DCs cultured with GM-CSF. MIZ did not inhibit phagocytic activity of DCs, nor the total level of expression of class I MHC molecules, but decreased the expression of SIINFEKL-H-2K^b complexes in OVA-phagocytized DCs. We also examined the effects of MIZ on the class II MHC presentation pathway using CD4$^+$ T hybridoma, DOBW and showed inhibition of IL-2 production. MIZ also decreased nitric oxide (NO) production and pro-inflammatory cytokines such as TNF-α and IL-1β in a dose-dependent from murine macrophage cell line, RAW264.7. These results suggest that the immunosuppressive activity of MIZ is due to the inhibition of class I MHC antigen processing and pro-inflammatory cytokine production in antigen presenting cells (APCs).

09-13/O

IDENTIFICATION OF THE MAMMALIAN TARGET OF RAPAMYCIN (mTOR) AS A REGULATOR OF INNATE IMMUNITY

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The serine/threonine kinase mammalian target of rapamycin (mTOR) has a central role in cell-cycle progression in many cell types including malignant cell lines, endothelial cells and lymphocytes. Currently, mTOR inhibitors are employed as novel anticancer and immunosuppressive drugs. However, the role of mTOR in non-dividing innate immune cells is not defined. Here we demonstrate that Toll-like receptor (TLR) engagement activated the mTOR signaling pathway in innate immune cells in a rapamycin-sensitive manner. Pharmacological and genetic ablation of mTOR increased IL-12 but suppressed IL-10 cytokine production in TLR-stimulated monocytes and myeloid dendritic cells through a transcriptional mechanism. Furthermore, mTOR inhibition in antigen-presenting cells enhanced T cell activation and proliferation. Finally, rapamycin promoted a healing phenotype in a murine *Listeria monocytogenes* infection model accompanied by enhanced monocyte/macrophage activation and altered cytokine balance. These data identify mTOR as a novel regulator of innate immune responses with profound implications in cancer, autoimmunity, or infectious diseases.

09-14/P

ACTIVATION OF NF-κB, BUT NOT P38, IS REQUIRED FOR *MYCOBACTERIUM TUBERCULOSIS* INHIBITION OF RESPONSES TO IFNγ

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Mycobacterium tuberculosis uses multiple mechanisms to avoid destruction by the immune system. We have previously shown that *M. tuberculosis* inhibits macrophage responses to interferon gamma (IFNγ), a cytokine that activates macrophages to restrict or kill other intracellular pathogens. Two distinct components of *M. tuberculosis* are capable of mimicking the inhibitory effects of the whole bacterium. One component, a 19 kDa lipoprotein, inhibits transcriptional responses to IFNγ in a Toll-like receptor 2 (TLR2) and MyD88-dependent manner. Inhibition by the second component, peptidoglycan, is independent of TLR2 and MyD88. TLR2 signaling activates the transcription factor NF-κB and mitogen-activated protein kinase p38. To determine whether NF-κB and p38 signaling are essential for *M. tuberculosis* inhibition of responses to IFNγ, we used pharmacological inhibitors to block their activation during treatment of bone marrow derived macrophages or RAW 264.7 cells with γ-irradiated *M. tuberculosis*. We found that pharmacological blockade of NF-κB

activation restored the response to IFNγ, as assayed by induction of IFNγ-regulated gene interferon regulator factor 1 (IRF-1). However, pharmacological blockade of p38 did not restore induction of IRF-1 or class II transactivator (CIITA), a second IFNγ-regulated gene. These results indicate that activation of NF-κB, but not p38, is essential for transducing signals downstream of *M. tuberculosis* that lead to inhibition of responses to IFNγ, and suggest that *M. tuberculosis* exploits an innate immune signaling pathway to inhibit the effects of the adaptive immune system.
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09-15/P

THE ROLE OF TOLL-LIKE RECEPTORS IN MURINE ENTEROCOCCUS FAECIUM PERITONITIS

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The prevalence of nosocomial infections caused by *Enterococcus faecium* is increasing worldwide. These Gram-positive bacteria are characterized by multiple-drug resistance, which increasingly hampers antibiotic treatment. Knowledge of the pathogenesis of *E. faecium* infections is limited. Toll-like receptors (TLRs) are pattern recognition receptors that play an essential role in the initiation of the immune response. They recognize pathogen associated molecular patterns (PAMPs) expressed by microbes. TLR2 recognizes several PAMPs expressed by Gram-positive bacteria including peptidoglycan and lipoteichoic acid. TLR4 is regarded as the Gram-negative TLR, being the signaling receptor for lipopolysaccharide, but it has also been implicated in defense against certain Gram-positive bacteria. MyD88 is an adaptor molecule that mediates signaling by most TLRs. The aim of the present study was to determine the role of the TLR-family in the immune response to *E. faecium* peritonitis. For this, TLR2, TLR4, MyD88 knockout (KO) and wild type (WT) mice were intraperitoneally infected with 10^8 CFU *E. faecium*. At 2, 6 and 24 hours after infection bacterial loads were determined at the primary site of infection (peritoneal fluid) and in the liver, lungs and the circulation; in addition, the influx of granulocytes was determined in the peritoneal cavity and cytokine/chemokine concentrations in peritoneal fluid and plasma. TLR2, TLR4 and WT mice effectively cleared *E. faecium* from all body compartments. In addition, peritoneal and plasma cytokine levels and granulocyte recruitment to the peritoneal cavity were similar in these mouse strains. In contrast, MyD88 KO mice displayed higher bacterial loads in peritoneal fluid accompanied with a reduced granulocyte influx. These data show that single TLR2 or TLR4 deficiencies do not impair the host immune response to *E. faecium*, whereas deficiency of the common TLR adaptor MyD88 does.

09-16/O

THE REGULATORY ROLE OF SIGIRR IN COLON EPITHELIUM IS THROUGH INNATE RECOGNITION OF COMMENSAL MICROFLORA

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Despite constant contact with an enormous population of commensal bacteria and their products, the colonic mucosa is normally hypo-responsive to these potentially proinflammatory threats. Here we report that the single immunoglobulin IL-1 receptor related molecule (SIGIRR), a negative regulator for Toll-IL-1R signaling, plays a critical role in gut homeostasis and intestinal inflammation by maintaining the microbial tolerance of the colonic epithelium. We found that SIGIRR-deficient colon crypts are significantly elongated as compared to that in the wild-type mice. SIGIRR-deficient colonic epithelial cells displayed intrinsic homeostatic defects, including constitutive activation of signaling and increased epithelial cell proliferation and survival. The homeostatic defects in the SIGIRR-deficient colonic epithelial cells are accompanied by constitutive upregulation of inflammatory genes and increased inflammatory response to DSS challenge in the SIGIRR-deficient colon. Importantly, the homeostatic defects and increased inflammatory responses in the SIGIRR-deficient colon epithelial cells require their contact with commensal bacteria. These results suggest that the removal of the negative regulator SIGIRR causes dysregulated commensal bacteria-induced TLR-mediated-signaling in the gut epithelial cells, resulting in homeostatic defects and increased intestinal inflammatory responses in the colon epithelium. To define the role of SIGIRR in colon epithelium, we generated gut-epithelial specific SIGIRR-transgenic mice. The gut-epithelium specific expression of the SIGIRR-transgene in the SIGIRR-deficient background reduced the cell survival of the SIGIRR-deficient colon epithelium and rescued the hypersensitivity of the SIGIRR-deficient mice to DSS-induced colitis. Taken together, our results indicate that epithelium-derived SIGIRR plays a critical role in controlling the homeostasis and innate immune responses of the colon mucosal surfaces, preventing maladaptive responses to commensal bacteria and their products.

09-17/P

EXPRESSION PROFILE OF TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS (TREM)-1 IN GRAM-NEGATIVE SEPSIS

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TREM-1 is a recently discovered neutrophil surface receptor that amplifies Toll-like receptor (TLR)-initiated responses against microbial challenges by potentiating the secretion of proinflammatory cytokines. Our aim was to characterize the expression and function of TREM-1 in sepsis. Melioidosis, a severe infection caused by the Gram-negative bacterium Burkholderia pseudomallei that is endemic in SE-Asia and characterized by a high mortality, is seen as a model for Gram-negative sepsis. First we investigated 34 patients with culture proven septic melioidosis, who demonstrated strongly increased sTREM-1 plasma levels (ELISA), TREM-1 cell surface expression on monocytes and granulocytes (flowcytometry) and TREM-1 mRNA levels in whole blod leukocytes (Lightcycler) when compared to healthy controls. Secondly, wild-type mice were intranasally inoculated with a lethal dose of B. pseudomallei in order to examine the soluble and cell surface TREM-1 expression over time (0,24,48,72 hrs after inoculation), in different compartments (lung/bronchoalveolar-lavage fluid (BALF)/blood) and different cell types (monocytes, macrophages and granulocytes). sTREM was strongly upregulated in lung homogenates and plasma but not in BALF. TREM-1 surface expression was enhanced at the site of the infection as well as in blood. TREM-1 was undetectable on lymphocytes. Thirdly, to investigate the function of the observed TREM-1 upregulation, we stimulated isolated TREM-1^+ and TREM-1^- monocytes and granulocytes obtained from blood from healthy volunteers (using cell sorter) with lipopolysaccharide (LPS) and heat-killed B. pseudomallei. TREM-1^- granulocytes displayed diminished pro-inflammatory responses as measured by the release of IL-6 and IL-8 after LPS and B. pseudomallei stimulation. Interestingly, there was no difference in LPS induced release of pro-inflammatory cytokines between TREM-1^+ and TREM-1^- monocytes, suggesting the existence of additional amplifiers of the TLR-cascade. In conclusion, these results provide new information on to the regulation of TREM-1 during sepsis and underscore the potential usefulness of TREM-1 as a diagnostic and therapeutic target in sepsis.

09-18/P

INFLAMMATION PATTERNS OF DIFFERENT *BURKHOLDERIA* SPECIES FURTHER CHARACTERIZE VIRULENT AND AVIRULENT STRAINS

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Burkholderia pseudomallei, a Gram-negative bacterium, is a potential bioterror agent and the causative agent of melioidosis, a severe mainly pulmonary disease that is endemic in SE-Asia. The genus *Burkholderia* contains >30 species, ofwhich *B. pseudomallei* is considered the most pathogenic. *B. thailandensis* however does not cause overt disease and is considered avirulent. Nonetheless it is unknown whether *B. thailandensis* really is harmless. Therefore, we determined the differences in patterns of inflammation of *B. pseudomallei* 1026b (clinical virulent isolate), *B. pseudomallei* AJ1D8 (an *in vitro* invasion deficient mutant generated with Tn5-OT182-mutagenesis) and *B. thailandensis* by inoculating C57B/6 mice intranasally with 1x10e3 CFU of each *Burkholderia* strain. As expected, mice infected with *B. thailandensis* showed markedly decreased bacterial outgrowth in their lungs, spleen and blood 24 hrs after inoculation compared to the groups infected with *B. pseudomallei* and the invasion mutant AJ1D8. 48 hours after inoculation *B. thailandensis* was not detectable anymore. These findings were consistent with a marked pro-inflammatory cytokine (TNFα, IL-6, MCP-1) profile in the *B. pseudomallei* and AJ1D8 groups and absence of detectable inflammatory cytokines 48 hours, but not 24 hours, after inoculation in the *B. thailandensis* group. Interestingly however, histological examination did show a marked inflammation in the lungs of the mice infected with *B. thailandensis*, corresponding with substantial granulocyte-influx and raised MPO-levels at 24 and 48 hrs after infection (although to a lesser extent than in the two other groups). Finally, mice survival experiments showed that infection with 1x10e3 CFU *B. thailandensis* was not lethal, whereas inoculation with 1x10e6 CFU *B. thailandensis* was equally lethal as inoculation with 1x10e3 CFU *B. pseudomallei* or AJ1D8. In summary, these patterns of inflammation suggest that (1) AJ1D8 is just as lethal as *B. pseudomallei* in an *in vivo* C57B/6 mouse model and (2) *B. thailandensis* is not as avirulent as is often recognized.

09-19/P

MYCOBACETERIUM TUBERCULOSIS 30KDA ANTIGEN INDUCES NF-κB ACTIVATION AND CYTOKINE PRODUCTION IN MACROPHAGES

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Mycobacterium tuberculosis bacilli contain distinct ligands that activate cells through TLR2 or TLR4. Among several mycobacterial antigens (purified 10, 22, 30, 38kDa, recombinant 6, 16, 19, 38kDa and Ag35A antigen) we demonstrate that 30 kDa antigen induces NF-κB activation and production of IL-6 or TNF-α in THP-1 macrophages cells. We also found that the 30 kDa antigen activated NF-κB in TLR2-transfected cells, not TLR4-transfected cells. When THP-1 cells were treated with 30kDa antigen strong phosphorylation of ERK occurred. These results indicate that TLR2 may act as an important mediator with stimulation of 30 kDa antigen in macrophage activation.

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09-20/P

ACTIVATION OF THE ACTIVIN/FOLLISTATIN SYSTEM BY TOLL-LIKE RECEPTOR PATHWAYS

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Activins are members of the TGF-β superfamily and systemic activin release occurs following acute inflammatory challenge. Activin A (the prototype activin) is elevated in patients with septicemia and we have shown that its release occurs rapidly in animal models following lipopolysaccharide (LPS) challenge. This response is directly downstream of Toll-like receptor (TLR)-4, as C3H/HeJ mice do not have an activin release in response to LPS. Our working hypothesis is that activin and related proteins are responsive to the activation of other TLR pathways in addition to TLR-4. To address this hypothesis, male C57/Bl6 mice were injected with agonists to TLR-2 (Pam3Cys), TLR-3 (Poly I: C), TLR-4 (LPS) or TLR-7 (loxoribine) and blood and tissues collected at various timepoints (up to 24 hours) post-injection. Circulating concentrations of activin A and its binding protein, follistatin, were measured using specific immunoassays, and activin β_A, activin β_B and follistatin mRNAs quantified using real-time PCR. As expected, LPS induced a rapid increase in circulating activin A and a more delayed response in follistatin concentrations, with profound increases in activin β_B ($\approx$ 90-fold) and follistatin ($\approx$ 15-fold) mRNA levels; activin β_A mRNA levels were largely unaffected. While TLR-2 and TLR-3 activation induced somewhat smaller rises in circulating activin A or follistatin, there were robust increases in activin β_B and follistatin mRNA levels ($\approx$ 15-30-fold). The TLR-7 agonist had little effect on circulating activin A or follistatin, but induced 5-10-fold increases in β_B and follistatin mRNA. Finally, injection of MyD88 knockout mice with LPS did not induce significant responses in protein release or in mRNA levels of the activins or follistatin. These data infer that activins are responsive to a number of TLR pathways and that MyD88, a key adaptor protein in many of the TLR pathways, is necessary for their activation and release during acute inflammatory processes.

09-21/O

PIVOTAL ROLE OF SOCS5 IN T CELLS IN INNATE IMMUNITY DURING SEPTIC PERITONITIS

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STAT proteins are key physiological regulators of the immune system. We have recently shown in a murine model of septic peritonitis that mice deficient in Stat4 and Stat6 are resistant to the lethality by balancing local type-1 and systemic type-2 cytokine responses. Macrophage- and neutrophil-specific Stat3 is crucial in not only modulating multiple organ failure associated with systemic inflammation but also intensifying the bactericidal activity. SOCS proteins are feedback inhibitors of cytokine receptor signaling by inhibiting the JAK-STAT signal transduction pathway, but their role in innate immunity remains to be investigated. In the present study, we explored the role of SOCS5 in innate immunity during septic peritonitis induced by cecal ligation and puncture (CLP). Mice with overexpression of SOCS5 in T cells (SOCS-5Tg) were resistant to the lethality, as compared to the wild-type (WT) mice. In SOCS5-Tg mice, bacterial burden was significantly lower than WT mice, which was accompanied by increases in the peritoneal levels of IL-12, IFNg and TNFa. Bacteria killing activity was augmented in phagocytes (macrophages and neutrophils) from SOCS5-Tg mice. Adoptive transfer of CD4 T cells from SOCS5-Tg mice into the WT mice or Rag2 deficient (Rag2KO) mice resulted in increases in the peritoneal level of cytokines after CLP, as compared to the control. Under the conditions, mice harboring SOCS5-Tg CD4 T cells exhibited an augmented bacterial killing relative to those

received control CD4 T cells, an event that was associated with improved mice survival post-CLP. Altogether, these data suggest that SOCS5 expressed in CD4 T cells play pivotal roles in host defense during septic peritonitis by augmenting the innate immune response of macrophages and neutrophils. Thus, SOCS5 may be a target molecule for the treatment of life-threatening sepsis.

09-22/P

LUNG INFLAMMATION UPON BRONCHIAL INSTILLATION OF LIPOPOLYSACCHARIDE AND LIPOTEICHOIC ACID IN HUMANS.

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Toll-like receptors (TLRs) play a crucial role in the recognition of 'pathogen-associated molecular patterns' in the lung, which is considered to be important for an appropriate immune response against pathogens that enter the lower airways. Here, we studied the effects of two different TLR agonists relevant for respiratory infections in the human alveolar space: lipoteichoic acid (LTA, TLR2 agonist) and lipopolysaccharide (LPS, TLR4 agonist). A dose-finding pilot experiment was performed with bronchial LTA instillation (4-100 ng/kg body weight). In an additional experiment sixteen healthy non-smoking male subjects were given either LPS (*E. coli*, 4 ng/kg body weight) or LTA (S. *aureus,* 100 ng/kg body weight): by bronchoscope sterile saline was instilled into a lung subsegment followed by instillation of either LTA or LPS into the contralateral lung. Six hours later a broncheoalveolar lavage was performed and inflammatory reactions were determined in the fluid obtained. The pilot study showed a significant dose-dependant increase of neutrophil counts and cytokine release upon LTA instillation compared to saline. In the additional experiment both LTA (100 ng/kg) and LPS mounted an inflammatory response, as reflected by increased neutrophil counts ($P < 0.01$). Moreover there was an increased release of pro-inflammatory cytokines and chemokines upon LTA or LPS instillation compared to saline. Both LTA and LPS induced activation of macrophages and neutrophils as shown by increased surface expression of CD71 on macrophages and CD11b on neutrophils, as measured by flow cytometry. For the first time this study shows that instillation of LTA in a subsegment of the lung induces a significant inflammatory response in human volunteers. This novel human model may be used to evaluate pathogenetic mechanisms and new interventions.

09-23/P

ENHANCED CYTOKINE RELEASE IN KLEBSIELLA PNEUMONIA IN ST2 DEFICIENT MICE.

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The immune system needs to constantly strike a balance between activation and inhibition to avoid detrimental inflammatory responses. ST2, a member of the IL-1 receptor family, is thought to play an important role in negatively regulating the pro-inflammatory response and subsequently achieving immunological homeostasis. Here, we tested the hypothesis that ST2 deficiency leads to an enhanced immune response in lung inflammation *in vivo*. For this 9- week old female BALB/c wild-type and ST2 deficient mice were inoculated intranasally with either 100 µg lipopolysaccharide (LPS) derived from *Klebsiella pneumoniae* or $3x10^3$ live *Klebsiella pneumoniae*; 6, 24 and 48 hours thereafter mice were sacrificed and bronchoalveolar lavage fluid (BALF) and lungs were harvested. No differences between the two mouse strains were found with regard to release of pro-inflammatory cytokines TNF-α and IL-6 six hrs after intrapulmonary delivery of LPS. However, a significant increase was found in the release of chemokines KC ($p < 0.01$) and MIP-2 ($p < 0.05$). Relative to wild-type mice, ST2 deficient mice demonstrated an enhanced inflammatory response in their lungs 24 and 48 hrs after infection with live *Klebsiella pneumoniae*, as reflected by increased release of pro-inflammatory cytokines IL-6 and TNF-α ($p < 0.05$). No differences were found in bacterial outgrowth in the lung, however bacterial outgrowth of *Klebsiella pneumoniae* in blood tended to be decreased after 48 hrs in ST2 deficient mice compared to their wild-type controls. These data suggest that ST2 inhibits the local production of pro-inflammatory cytokines and chemokines during gram-negative lung infection induced by *Klebsiella pneumoniae*.

09-24/P

ACT1 MODULATES THE TRANSITION FROM IMMATURE TO MATURE B CELLS

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The transition from immature to mature B-cells in the periphery is a critical step for depletion of auto-reactive B cells and establishing of mature B cell repertoire. This process requires finely tuned signals through the BCR and B cell-activating factor (BAFF) receptors. We have shown previously that Act1 is an important negative regulator of BAFF-mediated signaling and Act1 deficiency results in dramatic increase of peripheral B cell number and development of autoimmunity. Here we report that Act1 plays a critical role in modulating the transitional B cell maturation through its impact on BAFF-mediated cell survival. We found that the ratio of late-immature (T2 and T3) to early-immature (T1) cells is significantly increased in the Act1-deficient spleen as compared to the control mice, implicating that the transition from T1 to T2/T3 cells is more efficient in the absence of Act1. Furthermore, *ex vivo* experiments showed that Act1-deficient T1 cells survived better in response to BAFF stimulation as compared to the wild-type T1 cells, which may provide the Act1-deficient T1 cells greater opportunity to develop to T2/T3 stage *in vivo*. In support of this, we observed an increased BrdU incorporation and higher T2/T3 to T1 ratio in the Act1-deficient mice as compared to the control, confirming increased cell survival and a higher turnover rate of the transitional cell subsets. Importantly, BAFF-mediated cell survival of T1 cells upon BCR cross-linking was markedly increased in the absence of Act1, suggesting the important regulatory role of Act1 in the process of negative selection of auto-reactive B cells. Finally our biochemical studies strongly suggest that the impact of Act1 on BAFF-mediated survival of transitional T1 cells is through a mechanism involving regulation of certain pro- and anti-apoptotic Bcl-2 members, bringing a new insight of the molecular mechanism of BAFF-signaling and its function in B cell development.

09-25/P

SELECTIVE REQUIREMENT FOR THE JANUS KINASE TYK2 IN TLR3 AND TLR4 SIGNALING DEPENDENT ON THE MACROPHAGE POPULATION

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Tyk2 is a member of the non-receptor Janus kinase (Jak) family. Gene targeting in mice revealed a partial requirement of Tyk2 in the

responses to IFNα/β, IFNγ and IL12. Previously we demonstrated an essential role of Tyk2 in the progression of LPS induced shock.
Macrophages are crucial effectors in the LPS/TLR4 biology. In this study we compared the response of wildtype, Tyk2- and IFNAR1-deficient peritoneal exudate macrophages (PMs) and bone marrow derived macrophages (BMMs) to LPS and poly(IC). As readout we chose IFNβ mRNA induction and nitric oxide (NO) production.
In general, TRL3 and TLR4 engagement of wildtype cells resulted in significantly higher IFNβ mRNA induction in PMs compared to BMMs whereas NO release was similar. Tyk2-/- PMs show impaired IFNβ expression and NO production upon stimulus with LPS and poly(IC), while responses to both stimuli are nearly normal in Tyk2-/- BMMs. A similar situation was found upon LPS induction of IFNAR1-/- PMs *versus* BMMs. In contrast, IFNAR1 was absolutely required for the response to poly(IC) in both cell types.
In summary this is the first report of Tyk2 involvement in TLR3 responses. The requirement was found to be in a cell type specific manner. The higher responsiveness of PMs *versus* BMMs per se could be attributed to the higher differentiation grade and/or the greater diversity of PMs. In BMMs poly(IC) triggers an IFNAR1-dependent and Tyk2-independent signal – we suggest the IFNαβamplification loop – which is not or to a lower degree initiated by LPS.
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09-26/P

THE NEW LPS RESISTANT MOUSE STRAIN SPRET/Ei IS DEFECTIVE IN PRODUCTION OF IFN-β.

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Bacterial lipopolysaccharides (LPS) activate inflammation and the immune system through Toll-like receptor 4 (TLR4). Although this effect is clearly essential for the elimination of Gram-negative bacteria, over-activation of the TLR4-expressing cells by large amounts of LPS initiates a systemic inflammatory reaction and shock. Here we demonstrate that SPRET/Ei, a mouse inbred strain derived from *Mus spretus*, exhibits an extreme and dominant resistance against LPS-induced lethal inflammation, a phenotype mediated, as described by transplantation studies, by bone-marrow derived cells. Macrophages from SPRET/Ei mice show a normal MyD88-dependent response, but an impairment in IFN-β production and subsequent STAT-1 phosphorylation and IRF-7 stimulation. IRF-3 phosphorylation appears normal as well as induction of ISG15 and IP-10. The signaling defect appears specific for IFN-β, although the SPRET/Ei and C57BL/6 IFN-β promoters have no sequence variations. Also *in vivo* IFN-β induction seems very low in SPRET/Ei, after LPS or after *Influenza* virus infection and IFN-β-treatment of SPRET/Ei mice restores the sensitivity to LPS. As a consequence of the defective induction of IFN-β, SPRET/Ei mice completely resist *Listeria monocytogenes* and are very sensitive to *Leishmania major* infection. In SPRET/Ei BMDM, LPS or IFN-β stimulation lead to acute downregulation of the IFNAR1 mRNA expression, which remains stable in C57BL/6 BMDM, indicating that the resistance of SPRET/Ei to LPS is due to a disturbed positive-feedback loop for amplification of IFN-β production. Interestingly, in contrast to TLR4-deficient mice, which resist LPS but as a consequence become extremely sensitive for gram-negative sepsis, SPRET/Ei mice resist LPS-toxicity but also significantly resist Gram-negative infection with *Klebsiella pneumoniae*.

09-27/P

CONTRIBUTION OF INTERFERON-β TO THE MURINE MACROPHAGE RESPONSE TO THE TLR4 AGONIST, LIPOPOLYSACCHARIDE

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Interferon (IFN)-β has been identified as the signature cytokine induced *via* the Toll-like receptor (TLR) 4, "MyD88-independent" signaling pathway in macrophages stimulated by Gram negative bacterial lipopolysaccharide (LPS). In this study, we analyzed the responses of macrophages derived from wild-type (IFN-$\beta^{+/+}$) mice or mice with a targeted mutation in IFN-β (IFN-$\beta^{-/-}$) to the prototype TLR4 agonist, *E. coli* LPS. A comparison of basal and LPS-induced gene expression (by real-time PCR and Affymetrix microarray analyses) resulted in the identification of four distinct patterns of gene expression affected by IFN-β deficiency. Analysis of a subset of each group of differentially regulated genes by computer-assisted promoter analysis revealed IFN-responsive elements in all genes examined. LPS-induced activation of intracellular signaling molecules, STAT1 Tyr701, STAT1 Ser727, and Akt, but not p38, JNK, and ERK MAPK proteins, was significantly diminished in IFN-$\beta^{-/-}$ *versus* IFN-$\beta^{+/+}$ macrophages. "Priming" of IFN-$\beta^{-/-}$ macrophages with exogenous rIFN-β significantly increased levels of LPS-induced gene expression for induction of MCP-5, iNOS, IP-10, and IL-12 p40 mRNA, while no increase or relatively small increases were observed for IL-1β, IL-6, MCP-1, and MyD88 mRNA. Finally, IFN-$\beta^{-/-}$ mice challenged *in vivo* with LPS exhibited increased survival when compared to wild-type controls, indicating that IFN-β contributes to LPS-induced lethality; however, not to the extent that one observes in mice with more complete pathway deficiencies (*e.g.*, TRAM$^{-/-}$ or TLR4$^{-/-}$ mice). Collectively, these findings reveal unanticipated regulatory roles for IFN-β in response to LPS *in vitro* and *in vivo*.

09-28/O

AN RNAi APPROACH TO STUDY JAMIP 1 FUNCTION IN CYTOTOXIC T LYMPHOCYTES

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Jamip1 (Jak and microtubule interacting protein) was identified in a two-hybrid screen as binding to the FERM domain of Tyk2, a Jak family member involved in signaling through several class 2 cytokine receptors. Jamip1 belongs to a family of three conserved genes. It is expressed uniquely in neuronal cells and in lymphocytes (T, B and NK). Our analysis of Jamip1 expression in T lymphocytes shows a remarkably higher Jamip1 mRNA and protein level in CD8 than in CD4 T cells. Since Jamip1 co-localizes with microtubules and the microtubule organizing center and affects the microtubule dynamic equilibrium, we are investigating its involvement in cytoskeletal rearrangements occurring upon T cell activation. We focused on cytotoxic T lymphocytes (CTL) as their killing activity strongly relies on the integrity of the microtubule network and the transport of lytic granules along microtubules. To address the question of Jamip1 function in CTL, we set up a lentiviral system for delivery of siRNA into CD8 T cells purified from human peripheral blood. A GFP-expressing vector was used to transduce cells. We have studied CTL effector functions in GFP sorted cells. We will present data indicating a negative regulatory role of Jamip1 in cytotoxicity.

09-30/P

TLR7-INDUCED ACTIVATION OF PLASMACYTOID DENDRITIC CELLS

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Toll-like receptor 7 (TLR7) is essential in sensing viral infections and signaling anti-viral as well as anti-tumor host responses. TLR7 is spe-

cifically expressed on plasmacytoid dendritic cells (pDC) and induces a strong type I interferon response in these cells following activation. We have isolated pDC from human blood and analyzed the overall activation pattern of different IFNA-subtypes that are encoded by 13 different genes. Furthermore we performed a microarray analysis to assess the TLR7-induced gene regulation in human pDC beyond interferons and describe here the global activation pattern and dendritic cell functions triggered by TLR7.

09-31/O

INTERFERENCE OF LIVER X RECEPTORS WITH DENDRITIC CELL PHENOTYPE AND FUNCTION

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Liver X receptors (LXR) are activated by oxidized sterols and induce the expression of genes involved in lipid and cholesterol metabolism. Recent data revealed a crosstalk between LXR and Toll-like receptor (TLR) signaling in macrophages. TLRs are expressed by professional antigen-presenting cells (APCs), the most potent of which are dendritic cells (DCs). A role for LXR in DCs has not been described yet. Here we studied expression of LXR in human DCs and its function in DC differentiation, maturation and function. LXR alpha expression was strongly induced during differentiation of myeloid DCs isolated from human blood as well as on human monocyte-derived DCs, which were used for functional studies. Treatment of monocyte-derived DCs with, LXR agonists (T0901317, GW3965) altered DC morphology, and inhibited their endocytic activity. LXR agonists also interfered with LPS-induced DC maturation, by markedly decreased production of IL-12 and increased IL-10 secretion. Moreover, treatment of DC with LXR agonists reduced their ability to stimulate T cell proliferation. although MHC II expression was not altered. As a potential cause for the reduced T cell stimulatory capacity of LXR agonist-treated DC we found a marked inhibition of immunological synapse formation. DC expression of the actin bundling protein fascin that is required for immunological synapse formation was largely abolished by LXR agonist treatment. Moreover, overexpression of fascin in LXR agonist-treated DCs not only restored their ability to form immunological synapses they also partially restored the expression of CD25 and CD69 on T cells. In conclusion, our data reveal LXR as a potent modulator of DCs differentiation and function which could in part be mediated by downregulation of fascin expression. Due to the central position of DCs at the interface between innate and adaptive immunitiy, these results emphasize LXRalpha as a potential novel target for immunosuppressive drugs.

09-32/P

INNATE ACTIVATION OF TNF-ALPHA GENE EXPRESSION BY ORIENTIA TSUTSUGAMUSHI OCCURS INDEPENDENTLY OF TLR4 BUT INVOLVES MITOGEN ACTIVATED PROTEIN KINASES, ERK1/2, JNK1/2, AND P38.

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Orientia tsutsugamushi, an obligate intracellular bacterium that freely replicates in the host cell cytoplasm, is the causative agent of scrub typhus which is histopathologically characterized by inflammatory manifestations, indicating that orientiae induce mechanisms that amplify the inflammatory response. To understand the pathogenesis of scrub typhus, we examined the induction of tumor necrosis factor alpha (TNF-a) after infection with O. tsutsugamushi in mice, peritoneal macrophage, and macrophage cell line. Peak expression of TNF-a gene was observed between 4 and 8 days after infection in mice. Gene induction was followed by the secretion of cytokine proteins. The TNF-a mRNA were induced and showed a transitory peak for 6 to 24 h after infection in macrophage cell line. Orientia inactivation by heat did not abolish induction of TNF-a production. However, inhibition of cellular invasion by treatment of host cell with cytochalasin D led to a diminished TNF-a induction, suggesting requirement of invasion by bacteria for this host cell response. Western blot analysis of cell lysates indicates that extracellular signal-regulated kinase 1/2 (ERK1/2), JNK1/2, and p38 MAPKs become phosphorylated, and hence activated in O. tsutsugamushi-stimulated macrophages. Selective inhibitors of ERK1/2 (PD98059), JNK1/2 (SP600125), p38 (SB203580) MAPKs could all completely prevent TNF-a secretion. However, these drugs did not prevent either bacterial internalization and invasion into the host cells or TNF-a processing and secretion. Host TNF-a production *via* p38 & JNK pathways by this bacterium was found to be regulated by post-transcriptional mechanism, mainly by translational control. In contrast, ERK pathway mainly control the transcription step of TNF-a gene regulation. Although O. tsutsugamushi activates adaptive immunity, the major pathogen associated molecular patterns (PAMPs) which play in the activation is not known. Since there is no LPS and very little (or no) peptidoglycan, it is likely that these bacteria do not activate TLR4. We demonstrate that TLR4 defective mice lack the ability to respond to O. tsutsugamushi as measured by secretion of cytokines by macrophages. In conclusion, our data indicate that MAPKs pathways are required to induce maximal TNF-a production in Orientia tsutsugamushi infection.

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CELL INTERACTION KNOWLEDGEBASE: A WEB-BASED PLATFORM FOR INNATE IMMUNE CELLS AND CYTOKINE/CHEMOKINE NETWORK

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The innate immune system shows enormous diversity with regard to the stimulus-specific activation of pattern recognition receptors (eg. Toll-Like Receptors, TLR), their attendant signaling molecules, pathway crosstalks, the cytokine/chemokine genes induced and the positive/negative feedbacks these mediators exert on the inflammatory network. Understanding the complexities of such a decentralized, yet highly connected biological network calls for a systems biology approach. Inspite of the overwhelming information available on the immune cells and their associated cytokine/chemokine networks, the multidisciplinary and dispersed nature these data together with the sheer volume of information pose significant barriers to their integration towards an overall understanding of innate immunity. The Cell Interaction Knowledgebase (http://cell-interaction.bii.a-star.edu.sg/) represents one of the first attempts to create a web-based, unified platform for comprehensive information and computational resource pertaining to the interaction of innate immune cells with the cytokines/chemokine network, under normal or selected pathological conditions. The knowledgebase is organized into three sections 1) The Cell Types repository which constitute characterization of innate immune cells (eg macrophages, dendritic cells), their distinct functional phenotypes, cytokine/chemokine profiles and major intracellular signaling pathways, 2) The Cytokines/Chemokines repository which contains individual descriptions of 76 cytokines/chemokines with regards to their genomic, proteomic and knockout phenotype information and 3) Visual resources that describe the immune cells in relation to the cytokine/chemokine networks as interactive, dynamic, query-driven Cell Maps. At present, there are three categories of visual maps viz. Cell Phenotype maps (for macrophages, dendritic cells phenotypes), Cell Interaction maps and animated Molecular (intracellular) Pathway maps (e.g. TLR pathway, JAK/STAT pathway). Additional resources like links to cell-line repositories, PCR primer sequences and siRNA design tools are also being provided. Here, we introduce the first version of the Knowledgebase and its potential use towards an easier understanding of immune cell repertoire.