Stellacci E[1], Ciccaglione AR[1], Marcantonio C[1], Muto V[1], Equestre M[2], Marsili G[1], Rapicetta M[1], Battistini A[1]

[1]Department of Infectious, Parasitic and Immunomediated Diseases and [2]Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, ITALY.

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

Fragale A[2], Gabriele L[1], Borghi P[1], Sestili P[1], Stellacci E[2], Lanciotti A[1], Venditti M[1], Schiavoni G[1], Belardelli F[1], Battistini A[2]

[1]Department of Cell Biology and Neurosciences and [2]Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy.

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 CD8α$^+$ 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*

Dessing MC[1,2], Florquin S[3], Akira S[4], Paton JC[5], Van der Poll T[1,2]

[1]Center of Infection and Immunity Amsterdam (CINIMA), [2]Center of Experimental and Molecular Medicine and [3]Department of Pathology, Academic Medical Center, University of Amsterdam, the Netherlands. [4]Exploratory Research for Advanced Technology, Japan Science and Technology Agency Suita, Osaka, Japan. [5]Department of Molecular Biosciences, University of Adelaide, Adelaide, Australia

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

Wieland CW[1,2], Nelte MA[1,2], Kerver ME[1,2], Dessing MC[1,2], Florquin S[4], Borst J[5], Van Oers MHJ[1,6], Van Lier R[1,2], Van der Poll T[1,2]

From the [1]Center of Infection and Immunity Amsterdam, [2]Center of Experimental and Molecular Medicine, the [3]Department of Experimental Immunology, the [4]Department of Pathology and the [6]Department of Hematology, Academic Medical Center, University of Amsterdam, The Netherlands and the [5]Division of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

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 und 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 und phagocytosis capacity of CD27 KO neutrophils did not differ from WT neutrophils and