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Including STAT5, BTK/ITK, H2Ax, ZAP70/SYK, ERK1/2 and NF- κ B p65



In This Issue

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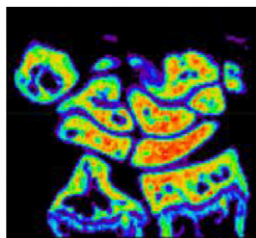
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Arthritis Assault Ag

Specific HLA alleles may contribute to autoimmune disease susceptibility through presentation of self Ags or other, as yet elusive, mechanisms. The 5-aa shared epitope (SE) motif in HLA-DRB1 increases both the risk of rheumatoid arthritis development and the severity of disease. The SE has been found to act as a signal transduction ligand that activates pro-oxidative signaling and Th17 polarization. In this issue, to better understand the SE's role in arthritis development, Holoshitz et al. (p. 48) addressed its ability to affect osteoclastogenesis. A peptide containing the SE directly stimulated NO and reactive oxygen species signaling and osteoclast differentiation in an osteoclast precursor cell line and promoted osteoclastogenesis in murine bone marrow and human PBMCs. Transgenic HLA-DRB1 expression also augmented osteoclast differentiation from bone marrow precursors and subsequent bone-degrading activity. Compared with control peptide, a peptide containing the SE upregulated production of IL-6 and TNF- α , which were important for the SE's osteoclastogenic activity. The SE could also induce upregulation of receptor activator for NF- κ B (RANK) on osteoclast precursors and, together with IL-17, synergistically promote osteoclastogenesis. Treatment of collagen-induced arthritis with the SE-containing peptide resulted in the selective expansion of collagen II-specific Th17 cells, increased numbers of osteoclasts, and significantly increased disease, relative to control treatments. Taken together, these data reveal a pathogenic mechanism that may be responsible for the strong association of the SE with rheumatoid arthritis and may advance understanding of HLA linkage to autoimmune disease in general.



Links in Lung Inflammation

The dimorphic fungus *Stachybotrys chartarum* causes conditions such as "black mold disease" and has been suggested to promote hypersensitivity pneumonitis (HP). TLR9 was previously found to be required for disease development in an *S. chartarum*-induced mouse model of HP. Because IL-17 was implicated in another model of experimental HP, Bhan et al. (p. 349) investigated whether TLR9 and IL-17 had related functions in *S. chartarum*-induced granulomatous inflammation. Sensitization and challenge with *S. chartarum* stimulated IL-17 production in the lungs of wild-type mice, which was significantly reduced in TLR9^{-/-} mice. Ab-mediated neutralization of IL-17 suppressed lung inflammation and the activation and accumulation of lung CD4⁺

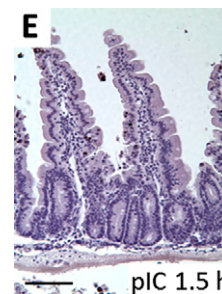
T cells. CD4⁺ T cells and $\gamma\delta$ T cells were found to be cellular sources of IL-17 production in this model. *S. chartarum* stimulation also induced the production of IL-23 from dendritic cells (DCs), which was impaired in TLR9-deficient DCs. *S. chartarum*-induced IL-17 production by T cells required DC-derived IL-23, and intratracheal administration of IL-23 restored granulomatous inflammation and T cell infiltration and activation to wild-type levels in the lungs of TLR9^{-/-} mice. This study presents, in a model of HP, a link between TLR9, DC-mediated IL-23 production, and Th17-mediated lung inflammation that may have implications for the treatment of granulomatous lung disease.

A Novel Role for Mac-1

Macrophage-1 Ag (Mac-1) is a surface receptor integrin with several aliases, including CD11b/CD18, complement receptor 3 (CR3), and $\alpha_M\beta_2$. As suggested by its many names, this pattern recognition receptor recognizes a number of ligands, including LPS from gram negative bacteria, aggregated β -amyloid, and high mobility group box 1 (HMGB1). Wide expression of Mac-1 on cells of the innate immune system indicate its varied participation in adhesion, chemotaxis, phagocytosis, and cellular activation. Zhou et al. (p. 115) have added to this extensive roster of roles by demonstrating that Mac-1 acts as a receptor for dsRNA, which is an important signaling molecule in viral infection. Importantly, this interaction is independent of TLR3, a known receptor of dsRNA. Mac-1 (predominantly the CD11b subunit) was shown to bind dsRNA on the surface of macrophages and internalize it. This was mediated through Mac-1 activation of PI3K signaling. In addition, Mac-1 dsRNA interactions synergized with macrophage TLR3-dependent activation of interferon regulatory factor 3. The dsRNA:Mac-1 interaction was also necessary for intracellular reactive oxygen species production by the NADPH oxidase (NOX2) and downstream activation of MAPK and NF- κ B. Taken together, these data present an exciting new role for Mac-1 as a surface receptor of the extracellular dsRNA found during viral infection and in activating the innate immune response.

Small Intestinal Survival

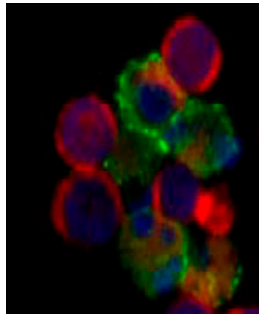
Signaling induced by the TLR3 ligand dsRNA during viral infection can result in damage to the small intestinal mucosa. To identify the signaling pathways responsible for this damage, McAllister et al. (p. 418) analyzed the effects of in vivo administration of the viral nucleic acid mimic polyinosinic:polycytidylic acid (pIC) on intestinal epithelial cells (IECs). In the



proximal small intestine, pIC administration rapidly caused IEC apoptosis followed by villus shortening, which was accompanied by luminal fluid accumulation and diarrhea. TLR3 and its downstream adaptor TRIF were required for these effects. However, mice lacking other RNA sensors were not protected against dsRNA-induced IEC apoptosis. pIC induced much higher levels of IEC apoptosis than other TLR ligands, and this apoptosis did not require TNF, IL-6, or IL-1 β , although these cytokines were upregulated by pIC treatment. In addition, pIC-induced IEC apoptosis occurred independently of IL-15, NK1.1⁺ cells, type I IFN signaling, and IRF3. IEC apoptosis was also unchanged from that in wild-type mice in mice lacking RAG1 or dendritic cells, or in bone marrow chimeras with TLR3^{-/-} hematopoietic cells. NF- κ B signaling, particularly that involving IKK β , protected IECs in the small intestinal crypts from apoptosis but did not affect IEC apoptosis in the villi. Caspase 8 in IECs was required for pIC-induced apoptosis but was also necessary to prevent villus destruction, small intestinal epithelial loss, and death. Thus, dsRNA-mediated signaling through a TLR3–TRIF–caspase 8 pathway may both cause IEC apoptosis and represent an antiviral host protective mechanism in the intestinal mucosa.

Immunotherapeutic α -CD20s

B chronic lymphocytic leukemia (B-CLL) can be difficult to treat, especially in cases of chemotherapy resistance. The addition of an immunotherapeutic to the treatment regimen can enhance patient outcomes. In the case of fludarabine refractory B-CLL, the immunotherapeutic human anti-CD20 mAbs ofatumumab and rituximab can be used. Considering the importance of these biologic therapies, Bologna et al. (p. 231) compared their efficacy against B-CLL targets under laboratory conditions that mimicked physiological conditions as closely as possible. They compared the relative lysing of B-CLL targets in whole blood taken from patients with B-CLL. In 24 h, ofatumumab was found to have a mean lysis of B-CLL targets of 56% versus 16% with rituximab. The lysis mediated by ofatumumab was shown to be completely complement dependent, as there was an almost complete inhibition of lysis with the administration of anti-C5



(eculizumab). In addition, blocking the complement inhibitors CD55 and CD59 increased ofatumumab-mediated lysis. Like rituximab, the efficacy of ofatumumab-mediated lysis was dependent on CD20 expression levels. The authors also tested the lytic ability of ofatumumab in the presence of the chemotherapeutic agents mafosfamide and fludarabine and found a synergistic effect. Once again, ofatumumab was more effective at lysing B-CLL targets under these conditions than rituximab. Thus, the authors conclude that under physiologic conditions, ofatumumab is responsible for effective complement-mediated lysis, giving further support for its addition to the anti-B-CLL armamentarium.

FeTImaternal Tolerance

A host of immune effects are necessary to maintain the tolerance that leads to a successful pregnancy: induced regulatory T cells, a Th2 cytokine profile, innate immune cells, and decidual dendritic cells (DCs). However, the role of T cell immunoglobulin mucin-3 (TIM-3), a pattern recognition receptor constitutively expressed on some macrophages and DCs, has yet to be examined at the feto-maternal interface. Chabtini et al. (p. 88) found that TIM-3 expression on innate immune cells was vital for maintenance of a normal allogeneic murine pregnancy. The authors found that TIM-3 expression was increased on uterine and draining lymph node DCs in the mid-gestation phase of pregnancy, and varied profiles of TIM-3 expression were found on uterine macrophages and myeloid-derived suppressor cells (MDSCs). The use of an anti-TIM-3 blocking Ab caused the accumulation of both macrophages and granulocytes expressing inflammatory markers at the uteroplacental barrier, as well as a reduction in uterine macrophage phagocytosis of apoptotic bodies. Blocking the effects of TIM-3 also increased the expression of IFN- γ and TNF- α . A reduction in litter size and resorption of fetal mice was increased with the use of the anti-TIM-3 treatment when compared with syngeneic pregnancies or untreated pregnant controls. In response to the increased inflammatory environment, Ly-6C^{hi}G^{neg} monocytic MDSCs expressed inducible NO synthase and arginase but these were unable to block the inflammatory response. Increased levels of IFN- γ and TNF- α found after TIM-3 blockade at the maternal-fetal interface led to a “breaking” of tolerance to the allogeneic pregnancy and ultimately to the rejection of the fetus. Taken together, the data demonstrate a vital role for TIM-3 in promoting the tolerance necessary at the maternal-fetal interface for a pregnancy to successfully come to term.