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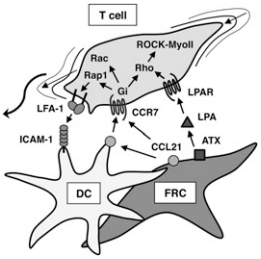
T Cells Taxi with Autotaxin



Within the lymph node (LN), T cells traffic rapidly through the paracortical T zone in search of their cognate Ags. In addition to chemokines and the adhesion molecules LFA-1/ICAM-1, other molecules, such as the lysophosphatidic acid (LPA)-producing ectoenzyme autotaxin (ATX), have been implicated in T cell intranodal migration. Katakai et al. (p. 617) examined the involvement of ATX in this process in depth, first determining that LN stromal cells, particularly fibroblastic reticular cells expressing CCL21 in the T zone, produced ATX and displayed it on the cell surface. Live imaging of T cell migration in LN tissue slices following treatment with an inhibitor of ATX activity or antagonists of LPA receptors revealed an important role for these molecules in interstitial T cell migration through a mechanism independent of LFA-1 and Gαi signaling. Although the authors were not able to identify the receptor through which it acted, LPA stimulated T cell chemokinesis, not chemotaxis, independently of chemokines and LFA-1. In T cells, LPA could activate the small GTPase Rho, but not Rap1, and Rho activity, along with a downstream pathway involving ROCK and non-muscle myosin II, was necessary for LFA-1-independent interstitial T cell migration. Taken together, these data identify ATX and LPA produced by LN stromal cells as key migratory cues for optimal intranodal T cell motility.

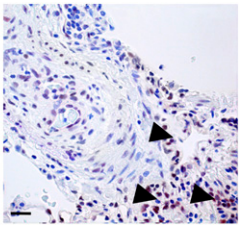
CD70 Modulates MCMV Responses

The interaction of CD27 with CD70, a member of the TNF family, regulates primary and memory antiviral $CD8^+$ T cell responses; however, CD70's function in innate immune responses to viral infection is unclear. To evaluate the role of CD70 in innate viral responses, Allam et al. (p. 871) infected CD70-deficient ($CD70^{-/-}$) mice with murine CMV (MCMV). Although $CD70^{-/-}$ mice had lower viral titers compared with wild-type (WT) mice at early stages of infection, MCMV titers and mortality were higher in $CD70^{-/-}$ mice at later time points. The early response to MCMV in $CD70^{-/-}$ mice was accompanied by a transient burst of cytokine secretion (including IFN-α, IL-12p70, and IL-6) and a transient activation of NK cells. Compared with WT mice, $CD70^{-/-}$ mice at later stages of MCMV infection had reduced numbers of $CD8\alpha^+$ dendritic cells (DCs), which prime naive $CD8^+$ T cells, and this corresponded to a decrease in MCMV-specific $CD8^+$ T cells. MCMV-infected $CD70^{-/-}$ mice had a significant reduction in numbers of regulatory T cells (Tregs) and a diminution in their functional ability to suppress $CD4^+CD25^-$ T cells. In contrast to Treg-depleted MCMV-infected WT mice, which had elevated proinflammatory cytokine levels and increased NK cell activation, MCMV-infected $CD70^{-/-}$ mice did not have altered cytokine production or NK cell activation following Treg depletion. Together, these findings suggest that Tregs play a role in innate immune responses to MCMV and that this role is modulated by CD70.

Editing Ets1 Expression

To avoid autoimmunity, B cell activation and differentiation are regulated by a series of negative signals, including those involving the kinase Lyn, loss of which results in severe autoimmunity. To more thoroughly delineate the pathways that control B cell activity, Luo et al. (p. 909) explored the relationship between Lyn and the transcription factor Ets1, which has been shown to inhibit plasma cell differentiation. B cells lacking either Ets1 or Lyn differentiated into plasma cells more readily than did wild-type B cells, and although Lyn expression was unaffected by Ets1 deficiency, Ets1 expression was decreased in $Lyn^{-/-}$ B cells, suggesting that Ets1 expression was controlled downstream of Lyn. In wild-type B cells, Ets1 was downregulated following BCR stimulation, and this depended upon the actions of Btk and PI3K, but not Akt. In addition, inhibitors of either IKK2 or JNK prevented BCR- or TLR-induced downregulation of Ets1, indicating the involvement of NF-κB and MAPK pathways in Ets1 modulation. Analysis of phosphatases recruited to Lyn-activated inhibitory receptors revealed that SHP1 had a major role in controlling Ets1 expression, and the inhibitory receptors CD22 and SiglecG were also found to be involved. Restoring Ets1 expression in Lyn- or SHP1-deficient B cells downregulated plasma cell differentiation, confirming the importance of Ets1 in this process. This study identifies signaling pathways in B cells downstream of the BCR and TLRs that coordinate to control Ets1 expression and thereby limit plasma cell differentiation.

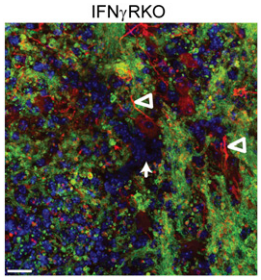
Fibroblasts Modulate Macrophages



Persistent inflammation and tissue remodeling in pulmonary arterial hypertension (PAH) are accompanied by the recruitment of alternatively activated macrophages (AAMs) into adventitial compartments by proinflammatory fibroblasts. Although the current paradigm of alternative macrophage activation is based on IL-4/IL-13 signaling, evidence in pulmonary hypertension (PH) animal models suggests that IL-6-STAT3 signaling may play a role. To better understand the mechanisms driving changes in macrophage phenotype and activation in PH, El Kasmi et al. (p. 597) initially demonstrated in various models

of PH and in the pulmonary artery of patients with PAH an accumulation of macrophages expressing STAT3-regulated markers CD163 and CD206 that are associated with a vascular remodeling phenotype. Coculture of naive bone marrow–derived macrophages with conditioned media from cultured human or bovine PH-adventitial fibroblasts (PH-Fibs) led to increased CD163, CD206, and SOCS3 expression, indicating that fibroblasts modulate macrophage activation. However, activation of AAMs in these cultures did not occur through the traditional IL-4/IL-13 pathway, but rather through IL-6/STAT3 signaling. The authors demonstrated that paracrine PH-Fib-derived IL-6 upregulated STAT3-regulated genes *Hif1* and *Vegfa* in macrophages, whereas genetic ablation in mice of *Hif1* and *C/ebpβ* reduced expression of STAT3-regulated genes in macrophages. Together, these results identify a mechanism by which alternative activation of macrophages is mediated by fibroblasts, through an IL-6/STAT3 signaling pathway, and suggest a pathway to be targeted for therapeutic treatment of PH.

Migration May Matter in MS



The clinical outcome of multiple sclerosis (MS) is determined in part by the spatial distribution of lesions in the CNS. In this issue, two articles analyzed leukocyte recruitment to the brain and spinal cord in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, to assess how preferential migration might occur and influence disease. Conventional EAE manifests with a phenotype of ascending paralysis, characterized by inflammatory demyelination of the spinal cord, whereas an atypical form of EAE can also develop that is characterized by inflammation in the brain. Previous studies have implicated IL-17 in promoting, and IFN-γ in protecting against, brain inflammation, but the mechanisms for these effects are not clear.

In the first article, Stoolman et al. (p. 564) transferred Th1-polarized myelin oligodendrocyte glycoprotein (MOG)–specific $CD4^+$ T cells into C57BL/6 wild-type (WT) or $IFN\gamma R^{-/-}$ hosts to induce conventional or atypical EAE, respectively. Development of atypical EAE in $IFN\gamma R^{-/-}$ mice was associated with the accumulation of neutrophils and upregulation of neutrophil-attracting chemokines including CXCL2 in the brainstem, whereas conventional EAE, as expected, was associated with the accumulation of monocytes/macrophages and donor T cells in the spinal cord. Although IL-17 induces CXCL2 expression, atypical EAE in this system developed independently of IL-17. However, CXCL2 was important for the development of atypical EAE, as atypical (but not conventional) disease was severely attenuated following blockade of the CXCL2 receptor, CXCR2, in $IFN\gamma R^{-/-}$ mice. Conversely, conventional EAE was reduced in mice lacking CCR2, the receptor for the monocyte-attracting chemokine CCL2. Thus, the differential production of chemokines that preferentially attract either neutrophils or monocytes appears to play a major role in determining the course of EAE.

Simmons et al. (p. 555) analyzed the relative roles of IL-17 and IFN-γ in EAE development in C3Heb/Fej mice, which develop atypical disease following adoptive transfer of MOG-specific Th17 cells. $CD4^+$ T cells from MOG-immunized mice were transferred into WT mice or recipients lacking IL-17RA, IFNγR, or both. These experiments supported the notion that IL-17 promoted atypical EAE, whereas IFN-γ promoted spinal cord inflammation and conventional EAE development. Although all recipient strains showed similar monocyte infiltration, neutrophil recruitment into the brain was decreased in $IL\text{-}17RA^{-/-}$ mice and increased in $IFN\gamma R^{-/-}$ mice, relative to WT. In contrast, neutrophil recruitment into the spinal cord was decreased in $IFN\gamma R^{-/-}$ mice but unaltered in $IL\text{-}17RA^{-/-}$ mice. Levels of CXCL2 production, predominantly by astrocytes, were strongly correlated with neutrophil infiltration in both the brain and spinal cord. Interestingly, depletion of neutrophils or administration of a CXCR2 antagonist in WT recipient mice dramatically reduced the development of atypical EAE without affecting conventional disease. The results of these two studies strongly support a pathogenic role for the CXCR2-mediated attraction of neutrophils to the brain in the development of atypical EAE. Treatment of MS patients therefore might benefit from approaches tailored to their patterns of CNS inflammation.

ERAPs Enhance Epitopes

Epitope generation from endogenous peptides entering the endoplasmic reticulum (ER) for efficient Ag presentation on MHC class I (MHCI) is thought to require aminoterminal trimming by human ER aminopeptidase (ERAP) 1 and ERAP2. Efficient generation of a final epitope from a precursor peptide requires the complementary specificities of both of these enzymes. Although evidence suggests ERAP1 and ERAP2 may form dimers, the functional significance of these heterodimers on peptide trimming is unclear. To evaluate ERAP1/2 dimers in situ, Evnouchidou et al. (p. 901) engineered leucine zipper-tagged ERAP proteins, in which both peptidases were tagged with Jun/Fos transcription factor zipper domains and an insect gp64 signal peptide for secretion from baculovirus-infected insect cells. In the supernatants of these cells, ERAP1Jun and ERAP2Fos almost exclusively heterodimerized, whereas ERAP1Fos and ERAP2Fos did not form dimers, due to repulsion by homologous zippers. Dimerization of ERAP1Jun and ERAP2Fos led to almost complete conversion of several peptides into epitopes after 1h, whereas very little epitope conversion was detectable in the presence of the ERAP1Fos-ERAP2Fos enzyme mix. Analysis of the Michaelis–Menten parameters to evaluate substrate binding affinity of each enzyme suggested that the increased efficiency of the heterodimers was due to an increase in ERAP1 activity during its interaction with ERAP2. This study explores the functional significance of ERAP1/2 dimerization on peptide trimming and demonstrates that ERAP1/2 heterodimers have enhanced peptide trimming efficiency, which is likely to improve MHCI presentation.