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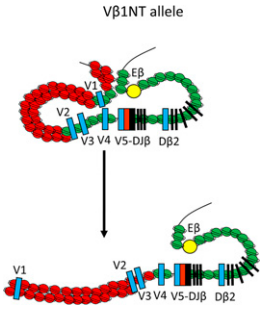
De-Emphasizing Decontraction

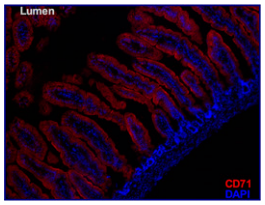


Precise transcriptional and conformational regulation of AgR gene expression and V(D)Jβ recombination during thymocyte development is essential for the development of a diverse, allelically excluded AgR repertoire and to help prevent detrimental chromosomal translocations that could lead to the development of lymphoma or leukemia. During the double-negative (DN) thymocyte developmental stage, the TCRβ gene locus (*Tcrb*) contracts to facilitate recombination between Vβ (*Trbv*s) and DβJβ gene segments. Successful recombination events promote DN thymocyte proliferation and differentiation into double-positive (DP) thymocytes, causing *Tcrb* locus decontraction, which is believed to facilitate the discontinuation of V(D)Jβ recombination and contribute to allelic exclusion. In this issue, Majumder et al. (p. 1262) closely examined the regulatory roles that conformational changes play in V(D)Jβ recombination in thymocytes transitioning from the DN to the DP stage. Using chromosome conformation capture techniques, they found that decontracted *Tcrb* loci in DP thymocytes still remain closely associated with recombination centers and that only the distal portion of the *Trbv* cluster becomes conformationally segregated from these interactions, suggesting that spatial separation may not be the primary means by which allelic exclusion is enforced. The authors also determined that dissociation of distal *Trbv*s occurred independently of the proliferative burst that precedes the DN to DP thymocyte transition and completion of functional VβDβJβ rearrangement, suggesting that this event is independent of V(D)J rearrangement status. These data indicate that the spatial segregation of AgR gene segments in DP thymocytes may not play as crucial a role in allelic exclusion as previously believed, and that this process may instead be more dependent on transcriptional regulation of these loci.

Metalloproteinase Mischief in TB

Tuberculosis (TB) incidence and drug resistance are becoming increasingly prevalent, highlighting the need for better understanding of the host factors involved in immune responses to this disease. Secreted matrix metalloproteinases (MMPs) have been identified as key elements in driving TB pathology; however, the role of membrane-bound MMPs in this disease has not been extensively studied. To investigate the functional role of membrane-type 1 MMP (MT1-MMP), a membrane-bound MMP integral to leukocyte migration and collagen destruction in many inflammatory settings, Sathyamoorthy et al. (p. 882) quantified the gene expression of MT1-MMP in sputum samples from TB patients and healthy controls. They found that MT1-MMP gene expression was increased only in the sputum of TB patients, which correlated with inflammatory infiltrates into the lung tissue. Using an MT1-MMP–specific Ab to stain lung biopsy samples from TB patients with active disease also revealed the presence of this protein in granulomas and multinucleate giant cells. To explore the involvement of MT1-MMP in *Mycobacterium tuberculosis* (Mtb)-induced pathology, the authors infected human primary monocytes with Mtb and found that MT1-MMP expression was upregulated on the cell surface. In a collagen degradation assay, Mtb-infected monocytes degraded ~43 times more collagen than did uninfected controls, an outcome that could be counteracted by the addition of an MT1-MMP–specific inhibitory Ab. MT1-MMP was also found to be integral to monocyte migration, and both this function and collagen degradation were dependent on p38 MAPK and chemokine signaling. Together, these data suggest a central role for membrane-bound MT1-MMP in TB disease pathogenesis.

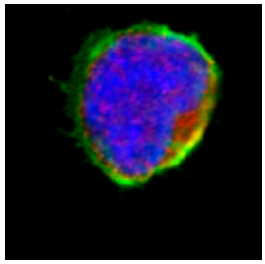
Enigmatic Erythroid Splenocytes in Neonates



A recent report indicated that depletion of neonatal $CD71^+$ erythroid splenocytes led to enhanced pathogen clearance following bacterial challenge, suggesting that these cells are immunosuppressive and could be targeted for sepsis therapy in newborns. In this issue, Wynn et al. (p. 1064) further investigated the impact of these cells, which are found in neonatal hematopoietic tissues such as the spleen, bone marrow, and liver, in models of sepsis and bacterial clearance. Ex vivo coculture of murine neonatal $CD71^+$ erythroid cells suppressed TNF-α production by $CD11b^+$ cells from adult bone marrow following stimulation with heat-killed *Listeria monocytogenes*. However, TNF-α production was also blunted following coculture of $CD11b^+$ bone marrow cells with neonatal or adult bone marrow, suggesting that immunosuppression is a global effect of hematopoietic tissues rather than a unique property of neonatal $CD71^+$ erythroid splenocytes. Adoptive transfer of neonatal $CD71^+$ erythroid splenocytes into mice in endotoxin challenge or polymicrobial sepsis models did not reduce mortality, questioning the notion that these cells are inherently immunosuppressive. In the initial studies spurring these investigations, Ab-mediated depletion of neonatal $CD71^+$ erythroid cells resulted in reduced bacterial load. To follow up on these findings, neonatal mice were treated with anti-CD71 Ab, and in half of the mice $CD71^+$ erythroid splenocytes were replenished by adoptive transfer before induction of nonlethal sepsis. Intriguingly, the

authors found no difference in bacterial load between the two groups and went on to show that the previously reported results were likely due to disruption of the gut epithelium by the anti-CD71 Ab, leading to an immune priming effect from exposure to commensal bacteria. To tie these findings to the potential use of $CD71^+$ cells in the clinic, the authors characterized $CD71^+$ cells from human cord blood. These cells were largely $CD235^+$, indicating that they were reticulocytes, which was confirmed by morphological examination after flow-cytometric enrichment. As reticulocytes rapidly decrease following birth, targeting this cell population likely would not improve newborn survival of sepsis. Results from these bacterial infection models suggest that neonatal $CD71^+$ erythroid splenocytes may not be as immunosuppressive as originally thought; further study of this population is warranted before the development of sepsis treatments that use $CD71^+$ cells.

ANXA1 Attracts Monocytes



Neutrophils are one of the first lines of immune defense against infection, but are also capable of causing immunopathology if they are not appropriately cleared by phagocytic cells during the resolution of an immune response. McArthur et al. (p. 1139) delineated a novel pathway for monocyte recruitment in response to Annexin A1 (ANXA1), a protein factor found in inflammatory exudates that can bind the promiscuous ALX/FPR2 receptor and is known to induce neutrophil apoptosis and phagocytic uptake by macrophages. In the Boyden chamber assay, human peripheral blood monocytes migrated toward human recombinant ANXA1 (hrANXA1), a process dependent on cytoskeletal rearrangement mediated by a p38 MAPK/calcium-independent phospholipase A_2/lysophosphatidic acid (LPA) axis. $CD14^{high}CD16^-$ classical monocytes were the primary responders to ANXA1, likely due to their expression of the receptor ALX/FPR2, which was not detected on other monocyte subsets. LPA inhibitor and small interfering RNA studies indicated that LPA_2 was likely the primary LPA receptor involved in ANXA1-mediated chemotaxis. To investigate the role of ANXA1 in monocyte recruitment in vivo, wild-type (WT) and alx/fpr2/$3^{-/-}$ mice deficient in the murine ortholog of ALX/FPR2 were injected i.p. with hrANXA1. Twenty-four hours later, monocytes were found in the peritoneum of WT but not alx/fpr2/$3^{-/-}$ mice. Similar results were obtained with two models of inflammation, zymosan-induced peritonitis and the cecal ligation and puncture model of sepsis. To emulate the resolution of inflammation, apoptotic neutrophils were injected into dorsal air pouches in mice, inducing two times more ANXA1-dependent monocyte recruitment to these areas in WT compared with alx/fpr2/$3^{-/-}$ mice. Importantly, exposure of human monocytes to supernatant from apoptotic human neutrophils induced chemotaxis, but not when the apoptotic neutrophils had been treated with a neutralizing anti-ANXA1 Ab. These results define an important pathway in neutrophil clearance and resolution of inflammation.

Clec9A Ab Targets T_{FH} Response

Targeting Ag to dendritic cell (DC) receptors is a promising vaccine strategy for generating efficient and robust pathogen-specific immune responses. Previous studies have shown that targeting Ag to Clec9A, a receptor expressed on $CD8\alpha^+$ DCs that recognizes damaged cells, induces robust humoral immunity in both mice and nonhuman primate models, even in the absence of immune response–boosting adjuvant or CD40 coreceptor stimulation. These responses were also accompanied by the expansion of a $CD4^+$ follicular helper T cell (T_{FH})-like subset, which is associated with the production of effective humoral immunity. To investigate more closely the T_{FH}-like properties of these cells in engineered Clec9A Ab–triggered immunity, Kato et al. (p. 1006) adoptively transferred transgenic $CD4^+$ T cells specific for either OVA or HSV-1 into wild-type mice, followed by challenge with a Clec9A-targeting or isotype control Ab linked to the appropriate $CD4^+$ T cell epitopes for these Ags. They found that immunization with either Clec9A-targeting Ab, but not isotype control Abs, resulted in the proliferation of transferred $CD4^+$ T cells, many of which exhibited hallmark characteristics of T_{FH}, including high cell surface expression of CXCR5 and PD-1 and expression of the transcription factor Bcl-6. Using adoptive transfer into wild-type mice of OT-II $CD4^+$ T cells expressing an IL-21 GFP reporter, the authors also found that these cells produced IL-21 upon immunization of adoptive hosts with the anti–Clec9A-OVA Ab. T_{FH} induced by Clec9A-targeted Abs were also able to transition into long-term memory T_{FH} that could respond to Ag in secondary recall responses and augment the formation of $GL7^+$ $CD95^+$ germinal center B cells. These data identify Clec9A targeting as a viable means to induce humoral immune responses in the absence of adjuvant, and suggest this strategy as a candidate for use in vaccine development.