In This Issue

J Immunol 2010; 185:1-2; ;
doi: 10.4049/jimmunol.1090048
http://www.jimmunol.org/content/185/1/1

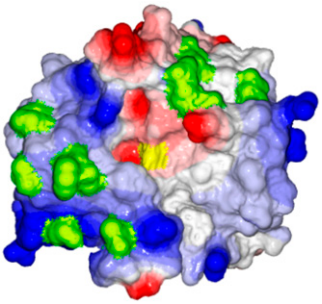
The Journal of Immunology is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

THE JOURNAL OF IMMUNOLOGY

In This Issue

Mix-Matching for Ab Elucidation



Anti-neutrophil cytoplasmic Abs (cANCA) are autoantibodies specific for proteinase 3 (PR3) and are believed to contribute to the development of the vasculitis observed in Wegener's granulomatosis (WG). The presence of cANCA in the sera of WG patients is commonly used to diagnose WG. However, the results from currently developed diagnostic tests can be inconclusive, as the knowledge of cANCA binding specificities is incomplete. Many previous unsuccessful strategies have relied on linear peptides to identify PR3 epitopes. In this issue, Kuhl et al. (p. 387) describe a new strategy to reduce nonspecific binding and identify conformational PR3 epitopes. To remove the known major linear binding sites from human PR3, human and gibbon *PR3* genes were recombined, and key human residues were substituted with gibbon-specific residues. When mouse mAbs representing three nonoverlapping groups of PR3 specificities were tested, the authors identified three discontinuous surfaces as common cANCA determinants. The testing of sera from WG patients revealed similar major antigenic determinants to the mAbs, including the binding site of α-1 protease inhibitor. Collectively, these findings demonstrate that the construction of functional PR3 hybrids is a useful strategy for mapping the conformational PR3 epitopes of cANCA sera from WG patients.

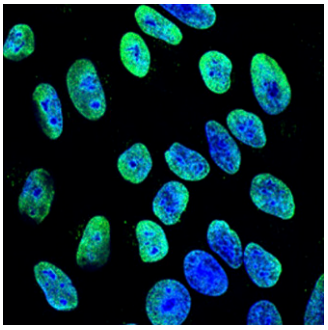
Waning c-Kit Marks Commitment

At the $CD4^{-}CD8^{-}$ double negative (DN) 2 stage, thymocytes retain the potential to become non-T lymphoid cells, including NK cells and dendritic cells. Interestingly, there is also evidence that thymocytes become committed to the T lymphoid lineage during the DN2 stage. In this issue, Yui et al. (p. 284) reconcile these observations. At the DN2 stage, thymocytes express both high and intermediate levels of the stem cell-associated marker c-Kit, whereas DN3 stage lymphocytes are homogenously $c\text{-}Kit^{low}$. The downregulation of c-Kit expression during the DN2 stage suggested a correlation of its expression with thymocyte maturation. Thus, to facilitate studies investigating the shift of DN2 cells from multipotency to T cell committed, adult DN2 thymocytes were separated into $c\text{-}Kit^{high}$ (DN2a) and $c\text{-}Kit^{intermediate}$ (DN2b) fractions. When cultured on stromal cells that provided Notch ligands required for T cell development, both fractions showed a similar potential to generate T cell lineage precursors. In the absence of Notch ligands, however, cultured DN2b cells gave rise to virtually no NK cells or dendritic cells, compared with DN2a cells. Correspondingly, DN2b cells showed decreased levels of the myeloid differentiation transcription factor PU.1. These data reveal that c-Kit expression levels define two groups of cells that exhibit distinct differences in their ability to differentiate into non-T lymphoid cells or claim T cell lineage commitment.

Stimulating Macrophage Polarization

Studies of tumor-associated macrophages (TAMs) and neoplastic growth suggest a link between extensive macrophage infiltration and tumor metastasis. Reciprocally, the tumor milieu also appears to instruct monocyte differentiation, preferentially polarizing monocytes into M2 macrophages. To better understand the manipulative role of the tumor environment on monocyte differentiation, Solinas et al. (p. 642) studied the effect of tumor-conditioned supernatants obtained from various human cancer cell lines, including colon, ovarian, and pancreatic cancers, on human monocyte differentiation. Of the 16 cell lines tested, only two promoted monocytes to differentiate into macrophages. Global gene profiling revealed that both of these supernatants upregulated the expression of several genes that are also expressed by TAMs and M2-polarized macrophages, but not M1 macrophages. These included migration stimulating factor (MSF), a fetal isoform of fibronectin that is aberrantly expressed in cancer epithelial and stromal cells. MSF derived from tumor-conditioned macrophages was shown to induce tumor cell migration. Taken together, these findings ascribe a role for MSF as a marker for TAM and M2 macrophages and reveal that TAM-expressed MSF can induce tumor cell migration.

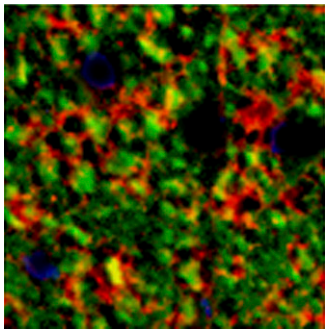
When BCRs Are Allowed To Loiter



The lysosomal protein LAPTM5 has been shown to negatively regulate cell surface TCR expression through its promotion of CD3ζ-chain degradation. In this issue, Ouchida et al. (p. 294) investigated a similar role for LAPTM5 in the regulation of BCR cell surface levels. In *Laptm5*$^{-/-}$ mice, B cell development appeared to be normal. However, on quiescent B cells BCR cell surface expression levels were increased compared with wild-type B cells. After BCR cross-linking, this disparity increased, and *Laptm5*$^{-/-}$ B cells exhibited a more activated phenotype. In contrast, other B cell markers, including CD19 and CD40, were expressed at normal levels, suggesting that BCR molecules were a specific target for LAPTM5 activity. In vivo, Ag immunization produced greater numbers of Ag-specific

B cells in *Laptm5*$^{-/-}$ mice compared with wild-type mice and also generated increased levels of Ag-specific IgM and IgG1 Ab. Further studies revealed that LAPTM5 physically interacted with the BCR complex under lysosome-dependent conditions and LAPTM5-mediated degradation of the BCR occurred independently of Ag-induced BCR internalization. Collectively, these findings reveal a role for LAPTM5 in the negative regulation of BCR cell surface expression and B cell activation.

More to Mast Cells than Histamine



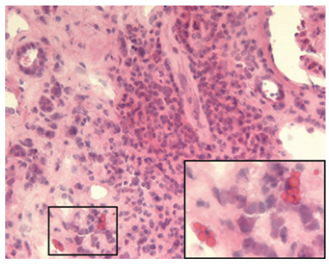
In this issue, two articles investigate the interactions of mast cells with two cell types not commonly associated with mast cell function: astrocytes and NK cells. In the CNS, mast cells are observed colocalizing with astrocytes. To determine if astrocytes can activate mast cells, Kim et al. (p. 273) cocultured mast cells and astrocytes and studied key signaling pathways of mast cell activation. In cocultures, mast cell intracellular calcium $[Ca^{2+}]_i$ levels significantly increased. Astrocytes express CD40, the receptor for the mast cell-expressed TNF family member CD40L. Pretreatment of astrocytes with an anti-CD40 Ab decreased mast cell coculture-induced $[Ca^{2+}]_i$ levels. In addition, anti-CD40 Ab treatment inhibited coculture-induced mast cell secretion of proinflammatory mediators, cytokines, and chemokines. The activation of other important signaling components of mast cell activation was also inhibited by CD40 blockade and/or CD40 siRNA, including the Rho family GTPases, various PKC isoforms, MAPKs, and the transcription factors NF-κB and AP-1. Finally, the prevalence of mast cells in experimental allergic encephalitis mouse brains was found to be 5-fold increased over control mouse brains. Collectively, these data reveal that astrocytes can activate mast cells through engagement of CD40L and suggest a role for astrocyte–cell interactions in multiple sclerosis/experimental allergic encephalomyelitis pathogenesis.

In the second article, Vosskuhl et al. (p. 119) investigated bone marrow-derived cultured mast cell and splenic NK cell interactions in response to TLR stimulation. When cocultured with LPS-stimulated mast cells, NK cells secreted increased levels of IFN-γ compared with NK cells cultured with LPS alone. In contrast, LPS-induced expression of TNF-α by mast cells was not influenced by the presence of NK cells. Coculture neither affected CD69 surface expression on NK cells nor altered NK cell-mediated toxicity, even in the presence of LPS. Treatment of mast cell–NK cell cocultures with TLR3 and TLR9 ligands also resulted in significantly amplified NK cell-derived IFN-γ production. Cell–cell contact was found to be essential for mast cell-mediated effects upon NK cell IFN-γ expression. Mast cells also express the TNF family member OX40L, and NK cells express its receptor. When LPS-stimulated cocultures were pretreated with an anti-OX40L Ab, NK cell secretion of IFN-γ was significantly reduced. In vivo, NK cells in mast cell-deficient mice i.p. injected with LPS showed decreased IFN-γ production compared with NK cells in wild-type mice. Together, these data show a role for mast cells in modulating the NK cell response to infection.

Directing DC Traffic from the Skin

Upon invading the skin or mucosa via cuts or abrasions, herpes simplex virus (HSV) encounters γδ T cells and various subsets of dendritic cells (DCs). To better understand how HSV modulates the primary cutaneous immune response, Puttur et al. (p. 477) studied the influence of HSV infection on Langerhans cells (LCs) and dermal DC (dDC) subsets during disease progression. Compared with noninfected controls, HSV infection induced increased levels of DC migration from whole-skin explants during the first 3 d of infection. Closer analysis revealed that DC subsets were dissimilarly affected by HSV infection, as the first wave of emigrants was largely uninfected LCs, followed by infected langerin$^+$ dDCs and uninfected langerin$^-$ dDCs. Notably, infected LCs exhibited impaired migration; infected LCs upregulated CCR7, but large numbers of LCs failed to migrate from the skin, apparently owing to a failure to downregulate E-cadherin and a propensity to undergo apoptosis. Finally, time-lapse confocal microscopic monitoring of a GFP-tagged strain of HSV revealed that infection of keratinocytes and γδ T cells preceded the infection of LCs. These findings reveal a temporal aspect to HSV infection of epidermal cells and disparate effects upon the migration of DC subsets from the skin to draining lymph nodes.

The Ontogeny of Rejection



Under conditions of chronic inflammation, inflammatory cells appear to organize into structures that morphologically resemble secondary lymphoid tissues. This lymphoid neogenesis is observed in chronically rejected renal allografts. To determine if the development of these structures recapitulated the embryonic ontogeny of lymphoid organogenesis, Thaunat et al. (p. 717) analyzed kidneys obtained from failed allografts. Based on the expression patterns of key lymphoid organogenesis genes, graft tissues grouped into four clusters (C1–C4), with C4 appearing to be the most developed. The C1 cluster comprised grafts explanted owing to non–immune-mediated failure and lacked evidence of lymphoid neogenesis. C3 and C4, but not C1 and C2, contained what appeared to be ectopic germinal centers (eGCs). eGCs produced an active local humoral response, with the highest number of IgG-producing memory B cells in C4 samples. The cluster sequence also appeared to correlate with worsening clinical outcomes: C2 grafts had survived, on average, 7.8 years before chronic rejection, whereas C4 grafts had lasted only an average of 3.5 years. These data reveal that intragraft lymphoid neogenesis resembles embryonic lymphoid organogenesis and suggest that therapeutic intervention of this process may improve the clinical outcome of renal allograft transplantations.

Summaries written by Meredith G. Safford, Ph.D.