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# IN THIS ISSUE

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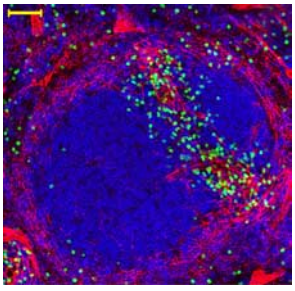
THE JOURNAL OF IMMUNOLOGY

# In This Issue

## Marginal Zone Highways



Lymphocyte trafficking within lymph nodes has been more thoroughly studied than that in the splenic white pulp (WP). In the lymph node, fibroblastic reticular cells (FRCs) guide lymphocyte motility, leading Bajénoff et al. (p. 3947) to analyze whether FRCs might play a similar role in the spleen. FRCs were localized specifically to the marginal zone bridging channels linking the T cell zone of the WP (the periarteriolar lymphoid sheath, or PALS) to the marginal zone, suggesting that FRCs might serve as a conduit between the WP and the marginal zone. To address this hypothesis, the migration of adoptively transferred T cells in chimeric mice expressing GFP in nonhematopoietic cells was analyzed using two-photon microscopy in bisected spleens. T cells were found to actively migrate along paths laid out by the FRC network in the PALS. In support of the hypothesis that the FRC network directs the migration of T cells from the marginal zone to the PALS, further imaging analysis clearly showed that T cells entered the WP almost exclusively by way of the FRC-rich marginal zone bridging channel regions. B cells were also observed to migrate along the FRC network to gain access to the splenic WP. This clear visualization of lymphocyte migration along FRC "roads" in the spleen provides important insights into the nonrandom nature of cellular migration in lymphoid organs.

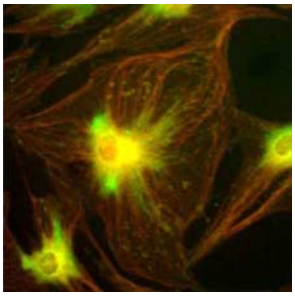
## Arrested Development

The roles that the large family of Wnt ligands and their frizzled receptors play in hematopoiesis are both extremely complex and quite controversial. To reconcile conflicting previous reports, Malhotra et al. (p. 3955) used a novel coculture system to specifically assess the effects of canonical vs noncanonical Wnt signaling pathways on lymphopoiesis. Canonical signaling involving Wnt3a was found to suppress hematopoiesis in both an autocrine and a paracrine manner. Further in vitro experiments demonstrated that Wnt3a expression on stromal cells strongly inhibited hematopoietic stem cell (HSC) differentiation into B cells and plasmacytoid dendritic cells, whereas noncanonical signaling induced by Wnt5a augmented B cell lymphopoiesis. Only HSCs and other very primitive progenitors were fully sensitive to these effects of Wnt ligands. Interestingly, uncommitted lymphoid progenitors were destabilized in response to Wnt3a signaling and underwent a degree of dedifferentiation that resulted in cells with stem cell characteristics and increased myeloid and erythroid potential. Taken together, these data suggest that the opposing effects of the Wnt3a and Wnt5a pathways regulate early B lymphopoiesis and begin to clarify the roles played by Wnt family proteins in immune cell development.

## Becoming a Killer

In response to viral infection, Ag-specific naive $CD8^+$ T cells proliferate and differentiate into effector CTLs. Jenkins et al. (p. 3818) studied the acquisition of effector molecule expression at the single-cell level to determine whether CTL activity is linked to cell division. CFSE-labeled naive OT-1 transgenic T cells were adoptively transferred into naive mice, which were then infected with an influenza A virus expressing OVA. OT-1 cells were then sorted based on the number of cell divisions they had undergone, and the authors found that the acquisition of CTL lytic activity correlated directly with cellular division. Analysis of granzyme and perforin expression in these cells by single-cell RT-PCR showed that effector molecule mRNA transcription also increased with cell division and resulted in heterogeneous expression profiles, with granzymes B and K expressed at consistently higher frequencies than perforin or granzyme A. The OT-1 CTLs were further divided into $CD62L^{low}$ and $CD62L^{high}$ populations, of which the $CD62L^{low}$ cells were found to be more potent effectors with greater expression of granzyme B. These data describe a heterogeneous but cell division-dependent acquisition of virus-specific CTL effector function and introduce new questions regarding at what stage a $CD8^+$ T cell can accurately be designated as an effector.

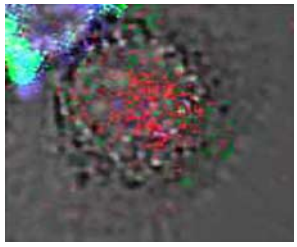
## TAO and Receptor Complexes



In Graves' disease (GD), autoantibodies target the thyrotropin receptor (TSHR). These Abs are also linked to the pathogenesis of thyroid-associated ophthalmopathy (TAO), the orbital manifestation of GD, but the roles that they and TSHR play in TAO remain unclear. Because TSHR signaling has been linked to insulin-like growth factor 1 receptor (IGF-1R) signaling, Tsui et al. (p. 4397) assessed the possible activities of these two receptors in TAO. Expression analysis demonstrated that TSHR levels were much lower in orbital fibroblasts than in thyrocytes. In contrast, IGF-1R was expressed at much higher levels on orbital fibroblasts from TAO patients than on those from control donors, and treatment of these TAO-derived fibroblasts with IGF-1 induced proliferation. Confocal microscopy demonstrated specific patterns of TSHR colocalization with IGF-1R$\alpha$ and IGF-1R$\beta$ in orbital fibroblasts, and immunoprecipitation experiments showed that TSHR and IGF-1R$\beta$ formed a tight physical complex. A functional consequence of this association was suggested by the demonstration that blockade of

IGF-1R could prevent thyrotropin-mediated signaling. The indication that TSHR and IGF-1R may form a functional complex suggests a mechanism by which low levels of TSHR in orbital fibroblasts might have a significant impact on the pathogenesis of TAO.

## Sharing Antigens



B cells function as Ag-specific APCs, but their Ag-presenting activity is short-lived and other professional APCs dominate as the immune response progresses. It has been shown that murine B cells can transfer Ag to macrophages, but the mechanism responsible for this transfer is not known. Harvey et al. (p. 4043) determined that human B cells could also directly transfer Ag to macrophages and delineated a mechanism by which this might occur. They found that human macrophages rapidly and directly acquired Ag from human B cells through a mechanism that required cell contact and often involved transfer of portions of B cell membrane and/or cytosol. This transfer did not involve phagocytosis but was instead mediated by the class A scavenger receptor, which has been shown to be involved in the exchange of membranes between APCs. In conditions of limiting Ag concentrations, macrophages could collect greater amounts of Ag in the presence of specific B cells than in their absence. The discovery that B cells can transfer Ag to macrophages suggests a mechanism by which B cells may amplify or edit an ongoing immune response in preference to a specific Ag. These data have broad implications for the understanding of B cell Ag presentation and its relationship to autoimmunity and other T cell-mediated immune responses.

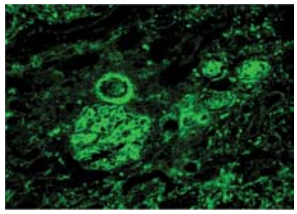
## A Nod to Tolerance

Pattern recognition receptors, including cell-surface TLRs and intracellular Nod proteins, mediate innate immune responses to infection. Macrophages can be rendered hyporesponsive to bacterial stimuli by pretreatment with the TLR4 ligand LPS and the Nod2 ligand muramyl dipeptide (MDP). In this issue, Kim et al. (p. 4340) analyzed the function of Nod2 in this tolerization process through the use of Nod2-deficient macrophages. LPS plus MDP-induced tolerance was impaired in these cells, as assessed by responses to stimulation with either pathogenic or commensal bacteria. This reduced tolerization was accompanied by increases in TNF-$\alpha$ and IL-6 production and NF-$\kappa$B and MAPK activity and was found to be dependent upon the presence of Nod1. Additional experiments using wild-type macrophages suggested that Nod1 and Nod2 agonists could induce cross-tolerance such that Nod1 agonists tolerized cells to Nod2 stimuli and vice versa. In support of this idea, Nod2-deficient macrophages pretreated with LPS and MDP were hyperresponsive to Nod1 stimulation, and this could be prevented by pretreatment with a Nod1 agonist. These data suggest that Nod1 and Nod2 activities are interrelated, and a perturbation of the balance between these pattern recognition receptors may be relevant to conditions, such as Crohn's disease, that are exacerbated by a loss of Nod2.

## RAG Selectivity

Omenn Syndrome (OS) is a severe primary immunodeficiency caused by mutations in *RAG1* and *RAG2* that result in a restricted TCR repertoire. It has been suggested that these mutations uniformly reduce recombinase activity, but Wong et al. (p. 4124) hypothesized that specific *RAG* mutations might selectively affect the TCR repertoire by targeting specific coding regions. To address this idea, the authors analyzed the mouse R972Q RAG1 mutant, which is found in a spontaneous mouse model of OS and corresponds to R975Q in human RAG1, which has been observed in OS patients. The R972Q mutation specifically impaired recombination at the hairpin formation step on substrates containing an AC coding flank, an effect the authors designated "coding flank hypersensitivity." Further, R972Q, in significant contrast to wild-type RAG1, showed a strong preference for a few coding flank sequences. No other OS RAG1 mutations studied demonstrated this coding flank hypersensitivity, suggesting that RAG mutants may cause OS through either uniform inhibition of recombination or selective coding flank hypersensitivity. In addition, these data support the idea that specific mutations in the *RAG* genes can affect lymphocyte repertoire diversity by distinct mechanisms.

## Progressive Tolerance



The induction of tolerance to MHC-mismatched allografts is an important goal in clinical transplantation. Previous studies showed that treatment with the immunosuppressive drug FK506 could induce tolerance to fully mismatched renal allografts in inbred miniature swine. Griesemer et al. (p. 4027) extended these observations by asking whether linked suppression could occur in these animals. First, tolerance to a fully mismatched kidney was confirmed by successful transplantation of a second kidney that was MHC-matched to the primary graft. The tolerant animals were found to possess a suppressive cell population that did not express CD25 but was able to specifically inhibit antidonor responses. These suppressive cells appeared to require TGF-$\beta$2 for their activity and, interestingly, could also mediate linked suppression to third-party Ags on cells coexpressing donor MHC. The possibility of linked suppression in vivo was analyzed by the transplantation of kidneys coexpressing a third-party MHC and the MHC of either the primary donor or the recipient into the tolerized animals. Animals that had previously accepted a second donor-matched kidney transplant demonstrated tolerance to a third transplant coexpressing third-party MHC and donor MHC but not to one expressing third-party MHC and self MHC. These data suggest that linked suppression can occur in a large animal model, likely via the activity of suppressive cells that must be restimulated in vivo by re-exposure to donor Ags.

**Summaries written by Jennifer Hartt Meyers, Ph.D.**