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Outside Influence: TLRs Direct Hematopoietic Cell Fates

Toll-like receptors (TLRs) modulate immune responses indirectly by promoting the efficacy of antigen presentation. In this issue of *Immunity*, Nagai et al. (2006) demonstrate that TLR signals also bias hematopoietic progenitor cells toward myelopoiesis directly by replacing cytokine and differentiative cues.

Hematopoietic progenitor cells produce the leukocytes of both the innate and the adaptive immune systems. The common ancestor of all these cells, the hematopoietic stem cell (HSC), has the capacity for self-renewal and the ability to produce by asymmetric division more differentiated, nonrenewing multipotent progenitors (MPPs). Progeny of the MPPs subsequently lose developmental plasticity to establish committed lineages of progenitor cells known as common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). CLPs generate all classes of lymphocytes, and CMPs produce granulocytes, monocytes, erythrocytes, and megakaryocytes. Whereas the lymphoid and myeloid differentiation pathways are generally thought to be mutually exclusive, there is evidence for alternative, bipotent pathways, and, curiously enough, both CLP and CMP appear able to generate dendritic cells (DCs) (Kondo et al., 2003) (Figure 1).

Normally, this leukopoiesis is thought to take place in specialized bone marrow microenvironments known as stem cell niches (Wilson and Trumpp, 2006). These niches protect HSCs—and perhaps their more differentiated progeny—from environmental perturbations that might activate quiescent cell pools or affect developmental fate decisions. HSC niches shelter stem cells from the *sturm und drang* of the peripheral lymphoid tissues and provide relief from exogenous signals that might interfere with the normal cell differentiation plan.

How very surprising it is then, that Yoshinori Nagai and his colleagues (Nagai et al., 2006) show in this issue of *Immunity* that the source of virtually all immunological storm and stress, Toll-like receptors (TLRs), are expressed on HSCs and their progeny, especially CLP and granulocyte/macrophage progenitors (GMPs).

TLRs interact with specific microbial components and are abundantly expressed on many mature leukocytes, including macrophages and DCs, B lymphocytes, and some T cell subsets. TLRs are expressed on the cell surface or within endosomal compartments; TLR ectodomains comprise multiple leucine-rich repeats that bind microbial protein, carbohydrate, or nucleic acid structures, and the intracytoplasmic domains bear a characteristic Toll IL-1 receptor resistance (TIR) signaling motif (Akira et al., 2006). TLR genes are present in fish, amphibians, and birds, and mammals express ten to twelve TLRs that can be organized into six families that are also represented in lower vertebrates; indeed, the *Drosophila* Toll9 gene is sufficiently similar to mammalian TLR genes to indicate an evolutionary origin that predates the divergence of insects and vertebrates (Beutler, 2005).

TLR ligands induce receptor dimerization and conformational changes that recruit adaptor molecules to the cytoplasmic TIR domain. Four adaptors are currently known: MyD88, TIRAP (Mal), TRIF (TICAM1), and TRAM. MyD88 is critical for TLR signaling, and it recruits the secondary kinases, IL-1R-associated kinase 4 (IRAK-4) and IRAK-1, to the TIR motif. TIRAP is required for recruiting MyD88 to activated TLR4, and this MyD88 TIRAP signal pathway leads to the expression of proinflammatory cytokines. TRAM-TRIF signaling is distinct from the MyD88 pathway and elicits IFN-β and IFN-inducible gene expression (Akira et al., 2006).

It can scarcely have escaped anyone's attention that TLR signaling initiates acute inflammatory responses by induction of inflammatory cytokines and chemokines. In addition, TLR signaling is an important component of DC maturation and activation; without TLR signaling, DCs are unable to function as effective antigen-presenting cells (APCs) for naive T helper cells. Activated DCs also release inflammatory cytokines that mitigate suppression by T regulatory cells. More recently, TLR signaling in B cells was reported to be necessary for the maintenance of serum IgM and IgG amounts as well as for the induction of IgG1 and IgG2 antibody by T-dependent antigens (Pasare and Medzhitov, 2003). This claim is, however, the subject of vigorous debate (Nemazee et al., 2006; Pasare and Medzhitov, 2006).

It is within this inflammatory context that the findings of Nagai et al. (2006) are so remarkable. Populations of HSCs, MPPs, and more differentiated progenitors express TLR2 and/or TLR4 and their associated accessory molecules. In vitro, LPS and Pam_3CSK_4, ligands for TLR4 and TLR2, respectively, drove normal but not MyD88-deficient HSCs and MPPs to proliferate and greatly increased their output of differentiated progeny. Even more remarkably, GMP—and, to a lesser extent, CMP—cultures containing LPS no longer depended on the M-CSF and GM-CSF growth and differentiation factors for cell survival and differentiation. LPS and Pam_3CSK_4 promoted CLPs to generate increased numbers of $CD11c^+$ DC. In vivo, LPS administered by intravenous or intraperitoneal injection quickly found its way to the bone marrow and modulated the TLR4 receptor complex on HSCs and MPPs. TLR ligands act on hematopoietic progenitor cells to bias hematopoiesis toward production of monocytes and macrophages with inflammatory phenotypes (Figure 1).

Nagai et al. (2006) conclude that TLR ligands are cues for hematopoietic cell proliferation and fate determination and that this interaction constitutes an innate response to microbial pathogens. Leukocyte progenitor cells are direct sensors of inflammation and respond accordingly by increasing the production of myeloid leukocytes to control infection. The links between TLRs and immunity now extend from the secondary lymphoid tissues to the sites of primary leukopoiesis.

While surprising, the observations of Nagai et al. (2006) are not without some precedent. Previously,

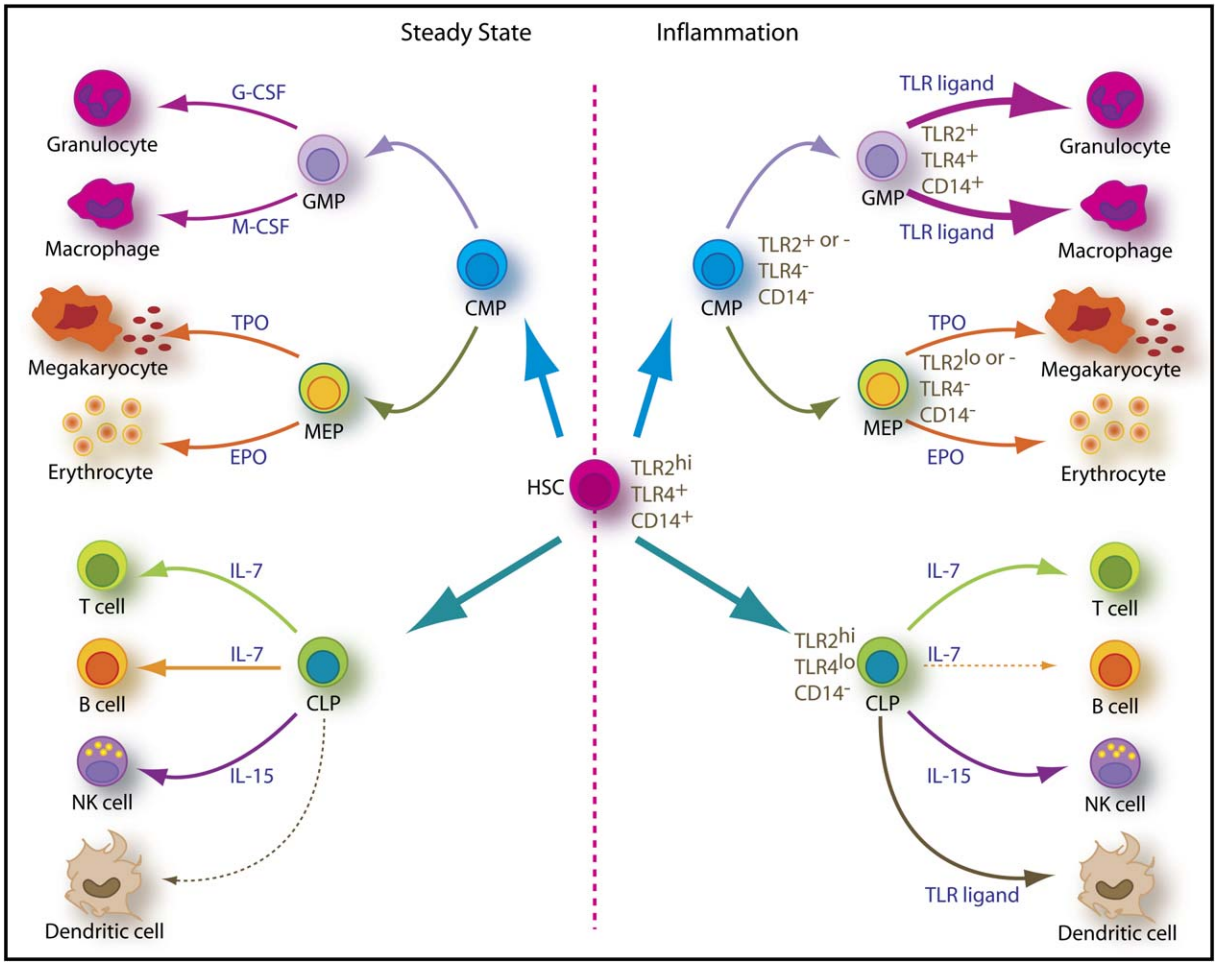

Figure 1. Hematopoiesis under Steady-State and Inflammatory Conditions

All hematopoietic lineages arise from a common ancestor, the hematopoietic stem cell (HSC). In turn, the self-renewing HSC gives rise to progenitor cells with more limited developmental plasticity through asymmetric division. Committed myeloid progenitors (CMPs) and committed lymphoid progenitors (CLPs) are multipotent cells incapable of self-renewal but under normal, steady-state conditions (left) generate all differentiated myeloid and erythroid (CMP) or lymphoid (CLP) cell types. CLPs differentiate to B or T lymphocytes under the influence of IL-7, to NK cells with IL-15, or to DCs. CMPs produce even more differentiated granulocyte and macrophage progenitors (GMP) that respond to granulocyte colony stimulating factor (G-CSF) or macrophage colony stimulating factor (M-CSF) by differentiating to granulocytes or macrophages, respectively. Megakaryocyte and erythroid progenitors (MEPs) are driven by thrombopoietin (TPO) or erythropoietin (EPO) to form, respectively, megakaryocytes or erythrocytes. In the presence of the TLR ligands LPS and Pam_3CSK_4 (right), HSCs and more differentiated progenitors bearing TLR2 and/or TLR4 respond by altering hematopoietic output. GMPs become capable of producing granulocytes and macrophages in the absence of G-CSF or M-CSF, and while lymphocyte production by CLPs is reduced, DC output becomes increased. Generation of megakaryocytes and erythrocytes by MEPs is little affected.

Yoshihiro Ueda and his colleagues (Ueda et al., 2004, 2005) demonstrated that LPS and various other inflammogens profoundly and rapidly affect bone marrow hematopoiesis by the mobilization of lymphoid progenitors to the blood and spleen and the consequent expansion of central myelopoiesis. These effects could be mimicked by recombinant TNFα and IL-1β and were minimized in TNF receptor-deficient mice. Ueda also observed that this inflammatory redirection of leukopoiesis correlated with reductions in bone marrow CXCL12 and stem cell factor amounts and demonstrated that CMPs were less sensitive to these growth factors than were CLPs.

Ueda's observations complement the present work of Nagai et al. (2006), and together these reports suggest an intimate and highly regulated link between primary leukopoiesis and inflammation that acts to expand the production of granulocytes and monocytes in response to infection. During infection, acute monocyte and/or granulocyte responses are crucial for host protection, and, in contrast to lymphocytes, most mature monocytes and granulocytes are short lived and incapable of mitosis. TLR signaling in leukocyte progenitors and the modification of the bone marrow's generative niches may represent a natural form of "just in time manufacturing" that maximizes innate responses to microbial pathogens while minimizing the deleterious effects and risks of chronic inflammation. This notion is an attractive possibility and one that highlights the ancient role of Toll genes in development (Ferrandon et al., 2004).

The tantalizing possibility that (at least some) TLR ligands can replace the endogenous cytokines normally

required for the survival, proliferation, and development of hematopoietic progenitors (Nagai et al., 2006) merits widespread attention and will surely be the object of further investigation. Does TLR engagement drive progenitor cell development by a molecular mechanism that parallels the normal, cytokine-signal pathway(s) or via some unknown receptor crosstalk? If the latter, where and how might the signaling pathways of TLR and growth factor receptors intersect? Given that LPS and Pam_3CSK_4 acted as cytokine surrogates only in MyD88-sufficient cells, a starting point for these questions is clear, even if the answers are not. Regardless of how TLRs influence hematopoietic fate decisions, the possibility that HSCs listen and respond to environmental cues requires a new appraisal of just how innate immunity and acquired immunity are intertwined.

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Pre-T Cell Receptor's *clashing* Signals: "Should I Stay or Should I Go"

In this issue of *Immunity*, Kersh and colleagues (Xi et al., 2006) investigate the regulatory network that permits two otherwise clashing cellular processes—proliferation and gene rearrangement—to occur at temporally distinct periods following the formation of the pre-T cell receptor (pre-TCR) complex.

At a critical time during T cell development, known as β selection, immature $CD4^-$ $CD8^-$ double-negative (DN) thymocytes expressing a pre-T cell receptor (pre-TCR; a productively rearranged TCR-β paired with a pre-Tα) receive a set of signals that mediate cellular survival, proliferation, differentiation to the $CD4^+$ $CD8^+$ double-positive (DP) stage, cessation of TCR-β gene rearrangement, and initiation of TCR-α gene rearrangement (von Boehmer and Fehling, 1997). β selection is driven by the pre-TCR and encompasses the first checkpoint of T cell development, and, as such, cells that fail to generate a TCR-β chain do not proceed along the αβ-lineage differentiation pathway. At the center of β selection lie two incompatible cellular processes, i.e., induction of proliferation and initiation of gene rearrangement.

Expression of the pre-TCR triggers a complex differentiation program (Michie and Zúñiga-Pflücker, 2002). However, the signaling pathways and genetic regulatory networks that mediate the various aspects of β selection have not been fully elucidated. Among the earliest transcriptional changes induced by pre-TCR signals is the expression of the zinc finger transcription factor, early growth response gene-3 (Egr3) (Xi and Kersh, 2004a, 2004b). In previous studies, Kersh and Xi demonstrated that Egr3 expression is rapidly and transiently induced in β-selected cells. Using a transgenic overexpression system, they investigated the effect of sustained expression of Egr3 past the β selection checkpoint and noted increased apoptosis of DP cells and altered TCRα rearrangement. These effects resulting from ectopic expression of Egr3 in DP thymocytes were due to a reduced expression of $Bcl\text{-}x_L$ and the thymic isoform of the retinoic acid receptor-related orphan receptor-γ (RORγt), which are necessary for DP survival (Sun et al., 2000). These initial findings begged the question of whether Egr3 regulates RORγt directly or indirectly. The answer appears to be yes, as both effects seem to be in operation (Xi et al., 2006).

In the present work, Kersh and Xi take advantage of a variety of experimental model systems, including a DP cell line to examine the function of RORγt expression in blocking proliferation and to analyze RORγt promoter regulation by Egr3. They also make use of ChIP-mediated cloning to uncover a new RORγt transcriptional target, mCPEB4, an RNA binding protein that appears to inhibit cell division. Additionally, Egr3-transgenic mice and Egr3-deficient mice are employed to demonstrate an inverse correlation between Egr3 and RORγt expression, which they show to be due to Egr3-induced expression of the E protein inhibitor Id3 that prevents E2A (E12/E47)-dependent RORγt expression. Of interest, they also report a direct association