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**Getting to the Ncor of HSC emergence**

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In this issue of *Blood*, Wei et al describe how the nuclear corepressor ncor2 helps keep hemogenic gene expression at just the right level necessary for hematopoietic stem and progenitor cell (HSPC) emergence.1 Forming an HSPC requires a Goldilocks touch in that too much or too little signal can inhibit their formation from specialized hemogenic endothelial cells. These results shed light on a critical factor in establishing proper balance during HSPC formation.

Derivation of bona fide HSPCs from pluripotent stem cells could provide a new source of hematopoietic cells for patients in need of bone marrow transplantation, but current protocols for directed differentiation generally produce embryonic blood cells and fail to generate engraftable, definitive HSPCs. During development, HSPCs are born from a subset of endothelial cells within the dorsal aorta (see figure). Hemogenic endothelium induction is a necessary intermediate step for definitive hematopoietic cell generation, yet the transcriptional events needed to specify hemogenic from vasculature endothelium are largely unknown. Two recent studies aimed at direct reprogramming of nonhematopoietic cells to HSPCs demonstrate that Fos is one factor involved in promoting hemogenic endothelium induction.2,3 The current study in *Blood* now sheds light on the endogenous regulation of c-fos during the in vivo endothelial-to-hematopoietic transition and provides insight into other factors important for the conversion.

The nuclear corepressor ncor2 is highly expressed in the dorsal aorta of developing zebrafish at a time in the development when hemogenic endothelium induction occurs. Using genetic approaches in zebrafish, Wei et al define ncor2 and its corepressor, the histone deacetylase hdac3, as essential components for HSPC production. This complex normally represses expression of genes; thus, upon knockdown several transcripts are increased, such as c-fos (FBJ murine osteosarcoma viral oncogene homolog) and vascular endothelial growth factor-D (vegfd). Genetic epistasis experiments showed that vegfd is downstream of c-fos, which is downstream of ncor2 and hdac3 during HSPC formation. Interestingly, a dose escalation study showed that low-level overexpression of c-fos actually enhanced HSPC production consistent with the reprogramming studies, whereas high-level overexpression was inhibitory. Endothelial overexpression of c-fos further demonstrated that there is an optimal level of c-fos needed to promote hemogenic endothelium formation in a cell autonomous manner. One question arising from these studies is “What is the fate of the endothelial cells that no longer turn into blood?” The authors provide data showing an increase in expression of the arterial markers ephrinb2 and dll4 (d-like protein 4), both targets of the Notch signaling pathway. These data imply that ncor2 normally suppresses Notch activity. Consistent with this finding, chemical inhibition of Notch signaling with DAPT restores HSPC levels in ncor2 knockdown embryos, as well as when c-fos or vegfd is overexpressed. Previous studies in mouse and chick also found that sustained and inappropriate Notch activity diminished blood production from the dorsal aorta.4,5 Together, these data suggest that when Notch signaling is imbalanced, hemogenic endothelium will likely remain arterial endothelium.
While this study is a clear step forward in our understanding of hemogenic endothelium induction, many questions remain. ncor2 is expressed throughout the aorta and controls expression of arterial markers throughout the vessel, yet loss of ncor2 only affects hemogenic endothelial formation. What other factors restrict the function of ncor2 within the presumptive hemogenic endothelial cells? Does ncor2 regulate expression of c-fos, vegfd, or Notch targets directly by binding to cell-type specific regulatory elements within these genes? How does ncor2, c-fos, or vegfd alter Notch signaling? vegfd is a prolymphangiogenic molecule, so could the effects observed with vegfd overexpression result from changes to lymphatic vessel formation? Are lymphatic vessel formation and HSCP emergence coordinated? Although lymphatic endothelial cells are derived from venous endothelial cells and not arterial cells, there is evidence that lymphangiogenic factors might have a supportive role for hematopoietic progenitors during development. Could these endothelial fate changes alter the HSCP niche? The work from Wei et al provides novel insight into the delicate balance of endothelial and hematopoietic fate choices during development, offering new directions in the quest to generate functional blood stem cells in vitro for transplantation therapies.

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Marginal zone B-cell dysfunction in ALPS

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In this issue of Blood, Neven et al report that patients with autoimmune lymphoproliferative syndrome (ALPS) have poor production of anti-polysaccharide immunoglobulin (Ig) M antibodies and an elevated risk of pneumococcal sepsis and demonstrate that this is caused by infiltration and disorganization of the splenic marginal zone by the prototypical double-negative T cells.

ALPS is caused by genetic defects that prevent appropriate lymphocyte cell death. Most patients harbor mutations in the FAS (TNFRSF6) death receptor gene and present with the predictable consequences of abnormal lymphocyte removal: chronic splenomegaly, lymphadenopathy, autoimmunity, and increased risk of lymphomas.

Although under normal conditions these patients can handle perfectly well infections by viral, fungal, and bacterial agents, they seem to be exceedingly sensitive to spleen removal. Sepsis by encapsulated bacteria is a well-known complication after splenectomy or during functional or congenital asplenia, but splenectomized ALPS patients have even higher complication rates compared with these populations. In fact, the rate of invasive bacterial infections after splenectomy in ALPS reported here and in a recent publication by the National Institutes of Health ALPS group was as high as 30% and 50%, respectively. Currently, postsplenectomy sepsis is the main cause of death in ALPS and not lymphoma development.

In their article, Neven et al provide a plausible explanation for these findings and incriminate a cell population typically seen in ALPS: the TCRαβ+CD4+CD8-T cells, also termed double-negative T cells (DN-Ts). They report that DN-Ts infiltrate and disorganize the splenic marginal zone (MZ) during periods of disease activity, leading to abnormal MZ B-cell function.

The splenic MZ is a specialized area at the interface between the circulation and the immune system, thought to be a prime site for the generation of first-line low-avidity IgM antibodies against blood-borne pathogens. These antibodies are produced by local MZ B cells, which in humans have a IgM^hiIgD^loCD1c^+CD21^hiCD27^ phenotype and seem to be particularly important for mounting T-cell independent anti-polysaccharide antigen responses. However, for their proper function, MZ B cells have to be correctly placed within the MZ, as antigens entering the spleen through the perifollicular zone are trapped by neutrophil extracellular trap-like structures emanating from unusual B-cell helper neutrophils inside the MZ. The MZ B cells are then exposed to the trapped antigen and initiate antibody production locally, mostly IgM but also IgG or IgA.

Neven et al demonstrate that the spleen MZ in ALPS patients with active disease is packed with DN-Ts, resulting in a paucity of MZ B cells in situ and in the peripheral blood. They go 1 step further by demonstrating that DN-Ts seem to be attracted to and retained within the MZ by the interaction between their αβ integrin and a thick layer of MAdCAM-1 expressed by MZ stromal cells. This disruption of the normal MZ B-cell responses seems to result in a mild B-cell immunodeficiency that is aggravated by the removal of the spleen. These data could also explain findings of low numbers of circulating memory and MZ-like B cells and reduced serum IgM levels occasionally seen in ALPS patients with active disease.

Although not explored here, it is also plausible that the susceptibility of ALPS patients to pneumococcal sepsis after splenectomy may be further enhanced by DN-T infiltration into additional functional reserves of MZ B cells in humans, such as...
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