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the authors did observe loss of myeloid engraftment at 16 weeks. The explanation for the discrepant results appears to be the capacity of wild-type competitor cells to "rescue" the early transplant defect of SCL-deleted cells. Also in the current issue, Kunisato and colleagues (page 3336) used a retroviral HSC transduction strategy to overexpress SCL or to inhibit it with a dominant-negative (DN) SCL construct. Overexpression of SCL increased short-term contribution to the myeloid lineage, whereas DN-SCL enhanced the contribution to lymphoid lineages without affecting long-term HSC proliferation. This was confirmed using fetal thymic organ cultures where single-sorted DN-SCL cells generated high numbers of T-committed cells and low numbers of myeloid-committed cells. Some effects were seen on erythroid (increased immature cells) but not on megakaryocytic differentiation in animals with DN-SCL that underwent HSC transplantation. The discrepancy between this and most other studies that do not show enhancement of lymphopoiesis could be related to DN-SCL interfering with bHLH motif–containing transcription factors other than SCL. A direct comparison in a competitive repopulation assay of HSC differentiation with SCL knocked down by DN-SCL, or excised by cre/lox, would be informative.

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HEMATOPOIESIS

Clearance receptor controls death and differentiation

Fetal macrophages associate intimately with developing erythroid cells and thymocytes, removing discarded nuclei and apoptotic cells in fetal liver and thymus. The phosphatidyl serine receptor (PSR) originally defined by Fadok et al[1] plays an essential role in ingestion of corpses, as shown by Kunisaki and colleagues (page 3362) in this issue of *Blood*. PSR$^{-/-}$ mice die perinatally, with severe blocks in definitive erythropoiesis and thymocyte development. The

authors hypothesize that phagocytic clearance may be reciprocally linked to cell differentiation and programmed death, as also suggested by recent studies in *Caenorhabditis elegans*[2] and in murine lung and brain.[3]

It is not generally appreciated that resident macrophages perform poorly understood trophic functions during hemopoiesis in the adult as well as in the fetus.[4] Stromal macrophages in bone marrow, for example, express nonphagocytic adhesion molecules for erythroblasts and developing neutrophils, whilst avidly ingesting extraded erythrocyte nuclei and apoptotic leukocytes. Their potential ability to express and secrete factors that control hemopoietic cell growth and differentiation has not been sufficiently studied. It is not clear from the published studies whether macrophage development is also defective in PSR knockout mice, although diminished phagocytosis of apoptotic cells by existing macrophages is clearly evident. Conversely, macrophages can express death-inducing surface receptors and secretory products. The present study shows that excessive production of proinflammatory cytokines such as tumor necrosis factor α (TNF-α) is unlikely to contribute to the mortality of PSR-deficient mice.

An important question that is not fully addressed in the present paper is whether defective apoptosis accompanies diminished clearance and abnormal differentiation. Whilst the emphasis here is on hemopoiesis, other studies suggest the interactions between developing cells, apoptosis, and phagocyte removal are more widespread in tissues, although not universal.[3]

Many plasma membrane receptors have now been shown to contribute to binding and ingestion of apoptotic cells by macrophages, although in cases such as scavenger receptor A deficiency, the resultant phenotype is less marked, with minimal impact, if any, on apoptosis and differentiation[5] but more on inflammation. The role of PSR in cell-cell interactions is of a special nature, which should teach us more about the linkage between death and differentiation on the one hand and clearance of apoptotic cells on the other.

—Siamon Gordon
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HEMOSTASIS, THROMBOSIS, AND VASCULAR BIOLOGY

VAP-1: a new anti-inflammatory target?

Polymorphonuclear leukocyte (PMN) migration through the endothelium to sites of

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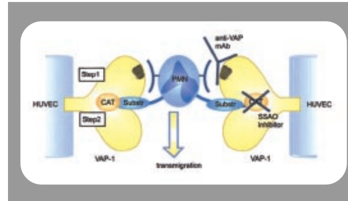
tissue inflammation is regulated by a complex network of signals involving adhesion molecules and chemotactic cytokines. The first stage of the extravasation cascade involves leukocyte tethering to the endothelial surface, followed by rolling, events largely mediated via selectins. If additional activation signals are received, the leukocytes proceed to the second stage and firmly adhere. This phase is mediated by a variety of cell adhesion molecules. Leukocytes then transmigrate through the endothelial layer, and it is this third stage of the extravasation cascade that is perhaps the least well understood. In this issue of *Blood*, Koskinen and colleagues (page 3388) provide new insight into how leukocyte transmigration may be regulated. Vascular adhesion protein-1 (VAP-1) is unique amongst endothelial cell–expressed adhesion molecules because it possesses semicarbazide-sensitive amine oxidase (SSAO) activity. Until now it has not been clear whether the enzymatic activity of VAP-1 contributes to its actions as an adhesion molecule. Koskinen and colleagues provide the first evidence that VAP-1 SSAO activity is required for leukocyte rolling and transmigration through the endothelium.

The plethora of signals regulating the extravasation cascade of leukocytes can complicate assessment of the contributions made by individual molecules. The studies of Koskinen et al were facilitated by the fact that human umbilical vein endothelial cells (HUVECs) are normally VAP-1–negative, enabling expression of wild-type VAP-1 or catalytically inactive (Y471F) VAP-1 without the complication of endogenous VAP-1. The effects of SSAO inhibitors and anti–VAP-1 antibodies on rolling, firm adhesion, and transmigration of PMNs on the transduced HUVECs were quantified using a laminar shear flow capillary model designed to recapitulate the conditions of shear stress in postcapillary venules. BTT-2027, a newly described SSAO inhibitor, reduced leukocyte rolling on HUVECs expressing wild-type VAP-1, as did the prototypical SSAO inhibitors semicarbazide and hydroxylamine. Although the features of BTT-2027 have yet to be fully reported, this is the first evidence linking the enzymatic activity of VAP-1 to a role in mediating PMN migration. More striking and perhaps



significant is the demonstration that VAP-1 SSAO activity is also required for leukocyte transmigration. Remarkably, BTT-2027 reduced transmigration through wild-type VAP-1–transduced HUVECs by almost 50%, quite surprising considering the wide range of cell adhesion molecule interactions occurring between PMNs and endothelial cells. The authors propose a sequential model for adhesion and SSAO activity in facilitating transmigration, but how the adhesive and enzymatic properties of VAP-1 collaborate to facilitate transmigration remains enigmatic. Clearly, further investigations will be required to determine whether one or more of the products of VAP-1 enzymatic activity are required to facilitate transmigration and, if so, their mechanism of action. Identification of molecules that interact with and activate VAP-1 are also important future goals. Critically for this study, preadministration of BTT-2027 reduced PMN extravasation in an in vivo model of acute inflammation. This "proof of principle" identifies VAP-1 SSAO activity as a potential new target for anti-inflammatory therapy, a particularly attractive prospect since VAP-1 is one of the few molecules currently known to possess SSAO activity in humans.

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HEMOSTASIS, THROMBOSIS, AND VASCULAR BIOLOGY

Of men; why not mice?

Platelet glycoprotein (GP) VI and integrin $\alpha_2\beta_1$ cooperate to mediate effective adhesion of platelets to collagens. When platelets engage collagen, it is thought that GPVI predominantly mediates outside-in signaling leading to activation of $\alpha_2\beta_1$ that then mediates a more stable adhesion. While debate remains as to the relative contribution of each receptor, it is clear that in mice the genetic elimination of either receptor alone does not completely inhibit collagen-induced platelet responses.[1,2]

The independent function of $\alpha_2\beta_1$ and its potential role in collagen-induced signal transduction may be underestimated. Recently, Inoue et al[3] looked more carefully at the role of $\alpha_2\beta_1$ in adhesion and spreading of platelets on a collagen-coated surface. A key observation was that the intracellular signaling cascade used by $\alpha_2\beta_1$ shares many of the features of the GPVI signaling cascade, including participation of Src kinases, Syk, SLP-76, and PLCγ2. The only component that is apparently not involved is LAT. Moreover, engagement of $\alpha_2\beta_1$ initiates signals through an independent pathway involving FAK, PMCA, and Ca^{2+} mobilization that is not detectable when collagen binds to platelets in suspension.

For several years, it has been known that platelet $\alpha_2\beta_1$ levels in humans can vary by roughly 4-fold due to the inheritance of 1 or more of 3 major human *ITGA2* haplotypes.[4] Haplotype 1 (T_{807}; G_{1648}) is associated with the highest density of $\alpha_2\beta_1$; haplotype 2 (C_{807}; G_{1648}) confers the lowest density; and the low-frequency haplotype 3 (C_{807}; A_{1648}) is associated with intermediate receptor density. The molecular basis for differences in expression associated with each of these haplotypes remains to be precisely determined. In addition, 2 single nucleotide polymorphisms (SNPs) within the promoter region of human *ITGA2* can decrease binding of the transcription factor Sp1, resulting in decreased transcription of *ITGA2* and further reductions in densities of platelet $\alpha_2\beta_1$.[5]

Until now, these genetic differences in $\alpha_2\beta_1$ expression had been thought to be unique to humans. However, Li and colleagues (page 3396) in this issue of *Blood* describe yet another experiment of nature

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