Stem cell homing: Rolling, crawling, and nesting

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Hematopoiesis is the best-studied stem cell system; the progenitor hierarchy has been defined along with the cytokine requirements and transcriptional regulators. The lymphohematopoietic stem cell also has been characterized morphologically, and cell surface antigens have been identified. Hematopoietic stem cell transplantation is one of the major advances in cancer therapy that gave rise to a Nobel prize.

The article by Frenette et al. (1) represents an important step in defining the homing of hematopoietic stem cells to recipient marrow. Stem cell motility, directed movement, and homing represent the most important new frontier in hematopoietic stem cell biology. Although the hematopoietic stem cell homing field is still in its infancy, its basis was created by studies defining a wide variety of adhesion receptors and other ligands that mediate cell-to-matrix and cell-to-cell interactions. These include the selectins, the integrins, the Ig family, and a grab bag of "others." In seminal studies investigating lymphocyte trafficking, scanning electron microscopy was used to demonstrate lymphocyte binding to the high endothelial venules of lymph nodes (2, 3). From this important visual research, phenomenology at its best, the understanding of the roles of various adhesion receptors and cellular homing has evolved (4, 5).

L-selectin was defined as the major homing receptor for naïve T cells for entry into lymphoid organs (6), and subsequent work revealed a family of selectins (L, P, and E) that function as lectin and adhesion molecules; all mediate leukocyte adhesion to endothelial cells, along with other activities. L-selectin is expressed by leukocytes, E-selectin is expressed by endothelial cells, and P-selectin is found in intracellular granules in platelets (α granules) and endothelial cells (Weibel-Palade bodies) (7, 8). L-selectin gained its notoriety as the receptor responsible for the initial tethering and "rolling" function of lymphocytes on endothelium before engagement of the integrins. It later was shown that αβ also could mediate such attachment and rolling under physiologic flow (9).

All of the different classes of cell adhesion receptors appear to play a role in anchoring of hematopoietic cells within the marrow or the promotion of differentiation (5, 10). Intercellular adhesion molecule (ICAM-1) in the Ig family, very late antigen 4 (VLA-4), an integrin, L-selectin, and CD44 (other) are examples of receptors that play a role in the adhesion of marrow cells.

The integrin class of adhesion receptors are heterodimers of the noncovalently associated α and β chains. There are at least 18 different α and 8 different β chain types (4). The integrins are grouped according to the β chain types. The system is complex, in that several α chains can associate with more than one β chain type and vice versa, integrins may associate with more than one ligand and vice versa, and several subunits have alternatively spliced cytoplasmic domains, including β1, α3, and α6. The integrins have specific binding sites on the extracellular matrix (ECM) ligands. Activation and inactivation of integrin function occur, possibly by conformational changes or phosphorylation. The β1 chain associates with α1 through α5 to form the VLA antigens (VLA-1 through VLA-6). αβ1 is the classical fibronectin receptor, and αβ1 is a laminin receptor. Fibronectin αβ1 can bind to the CS-1 alternatively spliced domain of Fn or to a vascular cell adhesion molecule (VCAM-1). αβ1 may function as a receptor for collagen, laminin, or fibronectin. αβ1 is a homing receptor for lymphocytes to the Peyer's patches of the intestine.

The article by Frenette et al. (1) addresses the issue of hematopoietic progenitor cell homing using P- and E-selectin knockout mice and VCAM-1 blocking antibodies. Homing studies to date have been limited because of the rarity of the hematopoietic stem cell and the associated difficulty in isolating a large enough number of stem cells for detection in murine marrow after i.v. infusion. These investigators used a simple, but clever, approach to circumvent these difficulties—they measured surrogate stem cells (clonal progenitors assayed in vitro) 14 hours after marrow infusion into lethally irradiated mice. With this approach, they showed defective homing to P-and E-selectin knockout mice, which correlated with increased radiation mortality of these mice. Perhaps most striking were the observations that, although administration of anti-VCAM-1 had little effect on 14-hour progenitor homing in wild-type mice, it caused a dramatic further decrease in homing when administered to the P- and E-selectin-deficient mice. This observation suggests a multistep process involving L-selectin and VLA-4 and fits with in vitro and in vivo observations implicating VLA-4 as a critical homing integrin (11–14). It is also consistent with in vitro observations indicating that pre-exposure to fibronectin was necessary for cytokine-induced migration of cell line hematopoietic cells (FDC-P1mix) (15). That this process is probably a good deal more complex is indicated by studies implicating these and other adhesion receptors in stem cell binding or homing.

Previous work has shown the presence of α4 and/or α5 on immature blasts, erythroid progenitors, monocytes, and CD34+ cells, and, in general, expression of α4 appears to decrease with maturation (16). There also appears to be differential binding of hematopoietic cells to different ECM components: erythroid cells bind to fibronectin (17, 18), and CFU-GM and BFU-E bind to collagen (19).

Papayannopoulou et al. (20) demonstrated decreased engraftment (at 3 hours after transplant) in the bone marrow but increased uptake by the spleen of bone marrow cells when the cells were pre-incubated with antibody to VLA-4 or when the recipients were injected with antibody to VCAM-1. In separate studies, another group showed that antibodies to VLA-4, VCAM-1, or CD44 blocked marrow engraftment in mice, but only anti-CD44 blocked splenic homing in a 3-hour transplant model (21). The timing of evaluation is important in homing studies, and several groups have shown that most stem cells are proliferative within 12 hours to several days after marrow infusion (22, 23), and, thus, at longer time intervals, any result will be determined not only by immediate homing but also by cellular phenotypes. The companion to this Commentary begins on page 14423 in issue 24 of volume 95.

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PECAM increased the functional activity of VLA-4 on or antibody to a platelet adhesion receptors. It has been demonstrated that ligands for receptor expression on hematopoietic progenitor cells is summarized by cytokine incubation. In addition, hematopoietic progenitor cells can bind to thrombospondin (28, 29), interleukin 3 (IL-3), and c-Kit ligand (29), and these cytokines bind to heparan sulfate proteoglycan (29). Adhesion receptor expression on hematopoietic progenitor cells is summarized in Table 1.

Another important consideration is the functional activity of adhesion receptors. It has been demonstrated that ligands for or antibody to a platelet/endothelial cell adhesion molecule (PECAM) increased the functional activity of VLA-4 on human CD34+ progenitors (30). Steel factor was found to activate and then inhibit the function of \( \alpha \beta_1 \) integrins in MO7e cells by activating the high affinity form of \( \beta_1 \) integrin (31). IL-3 and GM-CSF also have been found to activate \( \beta_1 \) integrin on MO7e cells (32).

A number of studies have been performed with mice deficient in L-, P-, or E-selectin, or combinations of these, with or without deficiency of other receptors. Redundancy in selectin function was suggested by studies in murine knockouts of E-selectin in which P-selectin could compensate for the E-selectin deficiency (33–35). Likewise, the effect of P-selectin deficiency on leukocyte rolling can be compensated for by L-selectin (36, 37), and rolling in L-selectin-deficient mice can be taken over by P-selectin (37). However, L-selectin-deficient mice also have markedly decreased lymphocytes within peripheral lymph nodes (38) and cannot mount delayed-type hypersensitivity responses (39). A combined defect of P-selectin and ICAM-1 leads to reduced neutrophil accumulation in a peritonitis model (40) in which both the P-selectin and LFA-1 (\( \beta_2 \) integrin receptor for ICAM-1) pathways are obliterated. In the combined E- and P-selectin knockout, there is a severe defect in leukocyte rolling and, consequently, mucocutaneous infections (41). These mice also have leukocytosis (4-fold increase), granulocytosis (16-fold increase), splenomegaly (2-fold increase), increased blood levels of IL-3 (40-fold) and GM-CSF (5-fold), and increased BFU-E and CFU-GM in the spleen, with extramedullary hematopoiesis in the liver (42). Primitive human hematopoietic progenitor cells express PSGL-1, a P-selectin ligand, and adhere to P-selectin, so deficiency could well be expected to lead to disrupted hematopoiesis (43). Thus, there is a profound disruption of both immune function and hematopoiesis in the combined E- and P-selectin knockout. In the model presented by Frenette et al. (1), the deficiency of E- and P-selectin will eliminate stem cell rolling on the endothelium, and the addition of VCAM-1 blockade will impair stem cell binding to endothelium and stroma. Thus, the end result is a marked impairment of stem cell homing and engraftment.

Stem cell biology has focused on long-term engraftment as the defining characteristic of a hematopoietic stem cell, and homing is the first step in this engraftment process. In general, the role of homing per se in long-term engraftment has not been separated from proliferation and/or differentiation, in part because of the rare cell problem noted above. Recent work has tied the engraftment phenotype of primitive stem cells to cell cycle transit with defective or normal engraftment associated with late S/G2 and G2, respectively (44). This loss of engraftment is reversible, and, thus, the engraftment phenotype of the stem cell is quite plastic. Further work investigating adhesion receptor expression on normal stem cells and stem cells transiting cell cycle has demonstrated expression of a relatively large number of adhesion receptors (45). The expression of multiple receptors is modulated with cytokine incubation and cell cycle transit, including a decrease in expression of VLA-4 (45), suggesting that fluctuations in long-term engraftment are probably secondary to fluctuations in adhesion receptor expression. Various cytokine receptors also modulate with cell cycle transit and also probably are involved in adhesive homing interactions.

The homing process of course involves both the lymphohematopoietic stem cell and the presumptively sessile marrow stromal cells in the company of ECM proteins, proteoglycans, and growth factors to which these cells home, termed by some as “niches.” Previous dogma, which has now clearly lost its bite, was that “space” had to be cleared for stem cells to engraft. However, this appears not to be the case. Cytotoxic therapy damages marrow and actually appears to impair homing (23). A series of experiments has shown quantitative engraftment at the stem cell level in nonmyeloablated mice and indicated that the final engraftment phenotype is determined simply by the ratio of host to donor stem cells (46–50). This was confirmed by studies showing that stem cell toxic (nonmyeloletic) treatments probably increased donor phenotype in syngeneic transplants (51). All together, this suggests that no host treatment is necessary for engraftment and that engraftment in irradiated mice is probably abnormal. The relative ease of engraftment is particularly impressive, given the sequential tissue cellular compartments through which the stem cell must travel.

The present and evolving observations remind us that the stem cell is not the dormant, sluggish creature envisioned by many but is a truly remarkable and highly functional cell. The stem cell migrates from the aorta-gonad-mesonephros (AGM) (52) to yolk sac to fetal liver and finally to marrow during adult life cycles and is easily mobilized with infection, cytokine, or cytotoxic treatment. The stem cells resident in the human cord blood repopulate a human infant (and probably adult) in conventional ablative marrow transplant approaches. In concert with the tremendous renewal, proliferative, and differentiated potential of this cell, it is a highly functional cell with a capacity to rapidly traverse marrow endothelial barriers and marrow space and set up residence in marrow niches. Given the appropriate experimental conditions, it has been established that a single stem cell can travel in the blood stream, traverse endothelial barriers, enter the marrow cavity, find the niche, and eventually differentiate and proliferate until all host lymphohematopoietic cells have derived from a single cell (53). The capacity and adventures of this cell are quite extraordinary; it is anything but dormant.

The article by Frenette et al. (1) presents impressive data implicating selectins and VLA-4 as critical homing receptors. Several caveats should be mentioned; these studies were carried out in irradiated hosts, and, as mentioned above, that

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**Table 1. Adhesion receptor expression by hematopoietic progenitor cells**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligands</th>
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<tbody>
<tr>
<td>VLA-4</td>
<td>Alternatively spliced fibronectin (CS-1); VCAM-1</td>
</tr>
<tr>
<td>VLA-5</td>
<td>Fibronectin (RGD)</td>
</tr>
<tr>
<td>VLA-6</td>
<td>Laminin</td>
</tr>
<tr>
<td>PECAM</td>
<td>Sulfated glycosaminoglycans</td>
</tr>
<tr>
<td>L-Selectin</td>
<td>Sialyl Lewis X, Sialyl Lewis a, CD34, GlyCAM-1</td>
</tr>
<tr>
<td>CD44</td>
<td>Hyaluronic acid, collagen, mucosal addressin</td>
</tr>
</tbody>
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indicates that damaged marrow is being assessed. Whether these results can be extrapolated to normal animals is an open question. In addition, knockout mice were used for these studies. Knockouts are extremely valuable for developmental studies and may give great insight into various aspects of biology. However, a knockout mouse could be termed a compensated mouse. Clearly, many events occur in the knockout that compensate for the absent protein, enzyme, etc. This is well illustrated by studies in the op/op mouse, which is a natural CSF-1 knockout in which there is progressive correction of the phenotypic abnormalities over time (54). Thus, the actual impact of P- and E-selectin in normal marrow stem cell physiology may be even greater than is indicated by the paper by Frenette et al. (1). These data clearly show that P- and E-selectin and VCAM-1 are necessary for homing; however, these findings do not negate a role for a number of other adhesion receptors. All together, this evolving field suggests a complex series of adhesive interactions mediating stem cell migration, movement, and homing. The details defining which integrins, which receptor pairs, and which interacting cell populations are involved should be the focus of exciting future research with obvious application to the clinical areas of gene therapy and hematopoietic cell transplantation.