# Regulation of hematopoiesis through adhesion receptors

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**Abstract: Normal steady-state hematopoiesis takes place in the bone marrow microenvironment. Soluble factors as well as contact interactions between the hematopoietic cells and the marrow microenvironment dictate the fate of hematopoietic stem cells and progenitors. Over the last decade it has become clear that cell-cell and cell-extracellular matrix interactions through adhesion receptors play a major role in the hematopoietic process. They are required for the residence of stem cells and progenitors in the marrow, as well as for homing of stem and progenitor cells to the marrow in the setting of stem cell transplantation. Furthermore, adhesion receptors play an important role in regulation of cell behavior, either through direct activation of signal pathways important for cell survival, cell growth, and cell fate decision-making processes, or by modulating responses to growth factors. Insights in the abnormalities seen in these interactions in diseases of the hematopoietic system will help to develop better therapeutic strategies based on the pathogenesis of these diseases. *J. Leukoc. Biol.* 69: 307–316; 2001.**

***Key Words:*** *bone marrow · stem cells*

## INTRODUCTION

Hematopoiesis is a complex process in which hematopoietic stem cells self replicate and differentiate to generate all the different mature blood cells. Under steady-state conditions, stem cells and hematopoietic progenitor cells reside in the bone marrow (BM) medullary cavity in contact with the bone marrow microenvironment [1]. Most, if not all, the factors required for the orderly development of stem cells are present in the BM microenvironment [2]. These include mesenchymal cells as well as other hematopoietic cells that secrete hematopoietic cytokines and extracellular matrix components. More than 40 different growth factors, cytokines, and chemokines interact with stem and progenitor cells through specific receptors and regulate proliferation, differentiation, and cell fate [3]. Hematopoietic growth factors are produced by mesenchymal cells and hematopoietic cells and are present in cell-bound forms, bound to the extracellular matrix (ECM) or in solution [4–7]. Stem and progenitor cells express adhesion receptors that provide specific cell-cell and cell-ECM interactions [8–11]. Aside from retaining stem and progenitor cells in the BM, engagement of adhesion receptors on stem and progenitor cells by BM stromal ligands also plays an important role in the regulation of the hematopoietic process [12–15] (**Fig. 1**). Adhesive interactions serve as growth or survival signals but also may modulate growth factor-dependent signals [16]. In addition, cytokines and growth factors affect adhesive interactions between stem and progenitor cells and their adhesive ligands in the BM, providing a further level of regulation of hematopoiesis [17]. A number of studies have demonstrated that diseases of the hematopoietic system are characterized by abnormal adhesive interactions that result in abnormal regulation of growth, differentiation, or survival [18, 19].

More than 20 different adhesion receptors have been identified on stem and progenitor cells [20–24]. Based on domain structure and function, adhesion molecules can be divided into integrins, cadherins, selectins, members from the mucin-like family, and members of the immunoglobulin family of receptors [10]. $\beta_1$- and $\beta_2$-integrins are responsible for interactions between cells and extracellular matrix components, such as fibronectin, collagen, laminin, and thrombospondin, or cell surface-expressed adhesion molecules, including vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM) [20, 21, 24–27]. Progenitors also express the sialomucins CD34, CD43, CD45-RA, P-selectin ligand-1 (PSGL-1), CD164, and podocalyxin-like protein-1 (PCLP-1) [28]. Although there is evidence that sialomucins play a role in hematopoiesis, the ligands for these receptors are not all known [15]. $CD34^+$ cells express cell adhesion molecules of the immunoglobulin family, including platelet-endothelial-(PE)CAM-1 [29], and express L-selectin [30]. The CD44 receptor, which supports adhesion to hyaluronate, is expressed on virtually all cells of hematopoietic origin [31] and participates in the adhesion of hematopoietic cells with the extracellular matrix in cooperation with the $\beta_1$ integrins [32] (**Table 1**).

## ROLE OF ADHESION RECEPTORS IN TRAFFICKING OF HEMATOPOIETIC CELLS IN THE BONE MARROW

Although stem cell transplantation has successfully been done for more than 30 years we do not know how stem cells infused

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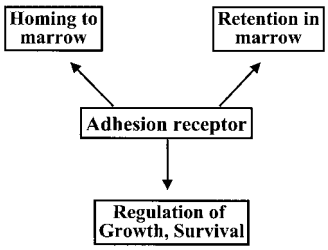


**Fig. 1.** Role of adhesion receptors in hematopoiesis. Adhesion molecules expressed by HSC participate in regulation of homing and retention of hematopoietic progenitors within the BM and regulation of growth and survival through interactions with their ligands.

intravenously home to the marrow microenvironment. It is believed that adhesion molecules contribute to retention of stem and progenitor cells in the BM and are essential for homing of stem and progenitor cells to the BM. Except perhaps for lectins [33], no adhesion receptor has been identified that is exclusively present on stem cells. Likewise, no adhesive ligand, except perhaps for hemonectin [34], has been identified that is exclusively present in the BM microenvironment. Extravasation of mature leukocytes has been extensively studied. This requires tethering of neutrophils to endothelial-expressed addressins via selectins, firm integrin-mediated attachment, and subsequent integrin-mediated migration through the endothelium and the extracellular space, a series of events that are enhanced significantly by chemotactic agents [35–40]. It is thought that similar mechanisms guide entry of stem cells in the marrow space and that one of the chemotactic agents that attracts stem cells to the BM space is the marrow-specific chemokine stromal cell derived factor-1α (SDF-1α) [41–43]. Even less well understood is the finding that a number of adhesion receptors thought to be instrumental in homing of stem and progenitor cells to the BM are also thought to be responsible for mobilization of stem and progenitor cells in the blood. Which receptors or what signals provide specificity to the direction of either entering or leaving the BM space is not known (**Fig. 2**).

## Integrins

*In vivo* experiments have shown a dominant role for $\beta_1$-integrins in the retention of stem and progenitor cells in the BM and for trafficking of stem and progenitor cells between the blood and BM in the adult [23, 24, 42, 44, 45] (Fig. 2). Absence of stem and progenitor cells in the fetal liver of $\beta_1$-integrin$^{-/-}$ mice indicates that $\beta_1$-integrins are important for trafficking of stem cells between the different hematopoietic organs during development [46]. This was confirmed further by a recent study showing that $\beta_1$-integrin$^{-/-}$ stem cells fail to engraft but are sequestered in the circulation [47]. A number of studies have shown that the interaction between $\beta_1$-integrins and their ligands (VCAM-1 and fibronectin) participates in the mobilization of progenitor cells into the blood [11, 23, 48, 49]. Ligands for $\beta_1$-integrins are also expressed in a number of tissues outside of the BM. Therefore, other ligand-receptor pairs must provide the specificity of BM-stem and progenitor cell interactions.

Some studies have implicated interactions between $\beta_2$-integrins and their ligands, ICAM-1 and ICAM-2, in mobilization of hematopoietic progenitors [50, 51]. However, unlike mature neutrophils evidence for a role of LFA-1 or other $\beta_2$-integrins in HSC homing is lacking. Patients with leukocyte adhesion deficiency, due to mutations in $\beta_2$-integrin, do not have obvious defects in the hematopoietic stem cell pool, and mobilization of stem and progenitor cells in the blood can be achieved [52].

## Selectins

Selectins are a family of glycoproteins that mediate leukocyte-endothelial cell interactions. Known ligands for the selectins include sulfated oligosaccharides, especially those rich in fucose residues, as well as glycosaminoglycans (GAGs). Stem and progenitor cells express L-selectin (Fig. 2). The bone marrow endothelial ligand for L-selectin is unknown, although MECA79-positive ligands [53], such as CD34 and PCLP-1, have been found on high endothelial venules. Moreover, a MECA79-negative but sialic acid-dependent ligand for L-selectin on CD34$^+$ has been recently identified [54]. A recent study showed that the ligand on CD34$^+$ cells may represent that hematopoietic cell L-selectin ligand is a specialized glycoform of CD44, which requires sialofucosylated N-linked glycans and is sulfation-independent [55]. The role of L-selectin on CD34$^+$ cells is not known. Selectin-deficient mice have varying degrees of leukocytosis, and P- and E-selectin-deficient mice have an increased propensity of infections, due to decreased rolling of neutrophils over endothelium [56]. However, there is no good evidence that L-selectin$^{-/-}$ mice have obvious defects in the hematopoietic stem cell compartment [57]. Sulfated fucans, branched and linear, mobilize stem and progenitor cells [58]. However, mobilization of stem and progenitor cells was similar in L-selectin-deficient mice (and P$^{-/-}$E$^{-/-}$ or L$^{-/-}$P$^{-/-}$E$^{-/-}$ mice) and wild-type controls, suggesting that fucan-dependent mobilization is not caused by blocking selectin-mediated binding/migration via sulfated fucans. A clinical study has suggested a role for L-selectin in homing and engraftment, as the level of L-selectin expression on CD34$^+$ cells predicted faster recovery of platelet counts after transplantation [59].

E-selectin and P-selectin are expressed on endothelial cells, and may serve as ligands for stem and progenitor migration. As is true for L-selectin$^{-/-}$ mice, E-selectin$^{-/-}$ and P-selectin$^{-/-}$ animals do not display an obvious hematopoietic progenitor

TABLE 1. Normal CD34$^+$ Cells

| Adhesion Receptors | Ligand | Function |
|---|---|---|
| Integrins | | |
| Beta-1 | ECM, VCAM | Homing, growth, survival |
| Beta-2 | ICAM | Homing |
| L-selectins | ? | Homing |
| Sialomucins | | |
| CD34 | ? | Homing? differentiation? |
| CD43 | ? | Growth, survival |
| CD162 (PSGL-1) | P-selectin | Homing?, growth, survival |
| CD164 | ? | Growth, survival |

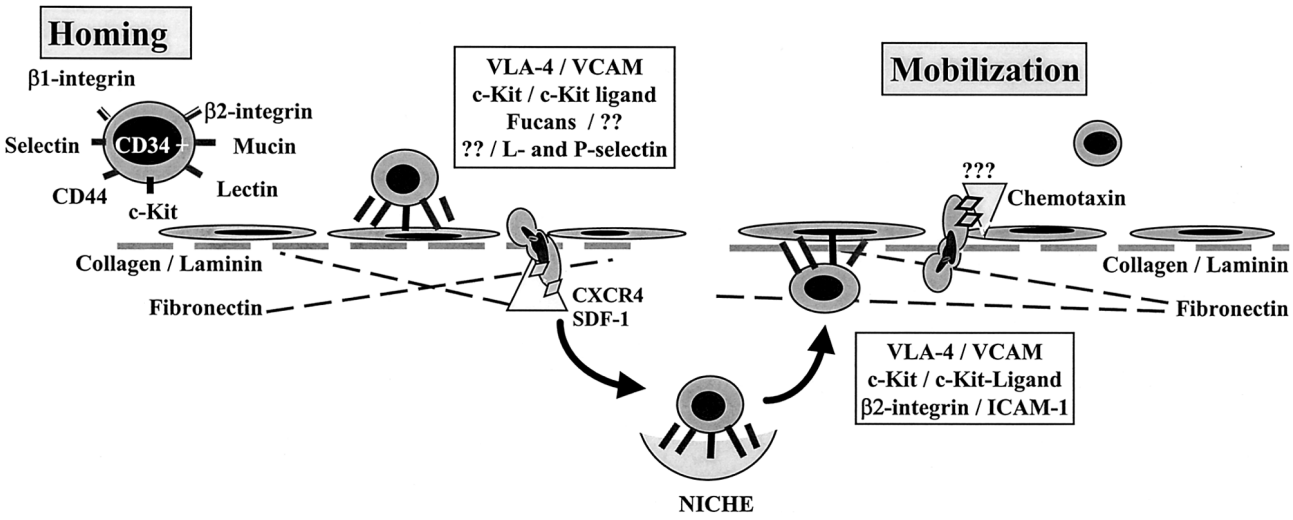


**Fig. 2.** Role of adhesion receptors implicated in homing and mobilization of hematopoietic progenitors. HSC/HPC express a number of adhesion receptors on their surface that allow them to interact with a large number of adhesive ligands. In the setting of transplantation, it is thought that hematopoietic cells tether to ligands on endothelial cells via selectins and fucans. This then results in the activation of integrins, which allow firm adhesion and transmigration through the endothelium. The chemokine SDF-1α that binds to the CXCR4 receptor has been implicated in providing specificity of this activation process. Adhesion to fibronectin and possibly VCAM-1 through $\beta_1$-integrins participate in more permanent anchoring of hematopoietic cells to the BM niche. The mobilization process requires de-adhesion of progenitors from the BM. $\beta_1$, $\beta_2$, and c-kit are implicated in mobilization of hematopoietic cells through an unknown mechanism.

defect [56, 60, 61]. However, a recent study has suggested a possible role for E- and P-selectin expressed in the BM microenvironment in stem cell homing [62] (Fig. 2). Homing and engraftment of stem cells in lethally irradiated P- and E-selectin-deficient mice were significantly reduced, and were further compromised when a function-blocking anti-VCAM-1 antibody was added. This suggests not only that selectins play a role in homing but also that there may be cooperation between different adhesion receptors in progenitor trafficking, as has been shown for neutrophil migration into tissue [39, 40]. Again, as E- and P-selectin are expressed in multiple tissues, it remains unclear what co-receptors are responsible for the specificity of stem and progenitor cell-BM interactions.

## Sialomucins

Sialomucins represent an emerging family of glycoproteins expressed by stem and progenitor cells that may play a role in the interactions between stem and progenitor cells and BM [15]. The CD34 antigen was the first sialomucin to be described on progenitors, and is also expressed on the majority of stem cells [63–66]. Some studies have provided evidence that CD34 may play a role in progenitor adhesion. However, the exact role of CD34 in stem and progenitor cell interaction with the BM is still unclear [65]. The subpopulation of murine and human stem cells that does not express CD34 antigens on the cell surface, is capable of engrafting [66, 67]. This suggests that CD34 may not be required for homing and engraftment of stem and progenitor cells to the BM.

CD43 is a transmembrane sialomucin expressed on the majority of progenitor cells, including myeloid and lymphoid-committed and primitive progenitors [68, 69]. Its role in adhesion of progenitor cells to stroma is unknown. CD162, or the P-selectin glycoprotein ligand (PSGL-1), is the sole receptor for P-selectin on immature progenitors and contributes significantly to adhesion of HPC to endothelial cells, suggesting a potential role in the initial steps of homing [70].

Finally, CD164 is expressed both on $CD34^+$ progenitor cells and on BM stromal cells [71]. Three different epitopes of CD164 have been described, with class-I and -II being expressed on hematopoietic cells at different stages of differentiation [72]. For instance, lymphocytes express class-I epitopes, whereas endothelium in high-endothelium venules are class II-positive, suggesting reciprocal homing functions in this tissue [73, 74]. Such ontogeny-dependent, differentiation-dependent, and tissue-specific expression pattern of the different isoforms of this receptor may provide a certain degree of specificity in homing of HPC, although *in vivo* studies will be needed to clarify this [71–74].

## Chemokines

Approximately 10% of stem cells home to the BM microenvironment [75]. The other 90% of cells are likely retained in other vascular beds. As pointed out above, few if any of the adhesion receptors and their ligands are expressed exclusively on progenitor and stem cells or in the BM microenvironment. One would therefore expect even lower levels of homing/engraftment in the BM.

Chemokines are 70- to 100-amino acid-long polypeptides, containing four cysteine residues [76]. The family has diverged into two groups: the C-X-C or α chemokines in which the first two of the four cysteine residues are separated by an additional amino acid and the C-C or β chemokines in which the first two cysteine residues are adjacent to each other. Originally, they were considered as inducible mediators of inflammation, but in recent years, several chemokines have been identified that are expressed constitutively and function in the physiological traffic and homing of leukocytes as well as stem and progenitor cells [77]. The chemokine, SDF-1, and its receptor, CXCR-4

[78], have been implicated in the homing and mobilization of human $CD34^+$ cells (Fig. 2). The chemokine SDF-1α, which binds to the CXCR4 receptor, increases cell migration: SDF-$1\alpha^{-/-}$ or $CXCR4^{-/-}$ mice have hematopoiesis in the fetal liver, but stem cells are unable to migrate to the BM. Therefore it is believed that SDF-1α is the major chemoattractant for stem and progenitor cells in the BM [79, 80]. This can be measured *in vitro*, where migration of normal progenitor cells through endothelium or fibronectin-coated transwells is significantly enhanced when SDF-1α is present in the compartment opposite to the cell compartment [41, 43, 81]. Furthermore, there is now solid evidence that SDF-1α is responsible for homing of stem and progenitor cells to the BM microenvironment when human $CD34^+$ cells are transplanted into NOD-SCID mice [42]. SDF-1α affects the function of a number of adhesion receptors, including $\beta_1$- and $\beta_2$-integrins allowing stem and progenitor cells to roll over and eventually adhere to the endothelium expressing SDF-1α and extravasate into the BM space [36, 42, 81].

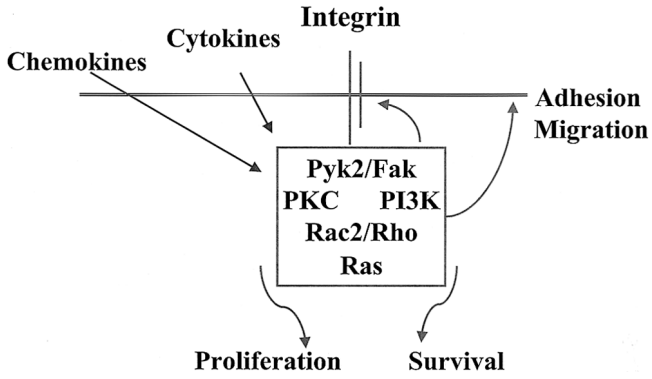


**Fig. 3.** Signaling through integrins. Integrins are non-tyrosine kinase receptors that transmit signals into the cell by recruiting non-receptor kinases. Integrins activate the focal adhesion kinase (FAK) or the related kinase, Pyk-2, forming focal adhesion contacts that serve to bind and activate a number of Src-homology domain (SH)2 and SH3 containing adaptor proteins and activate the phosphoinositide 3-kinase (PI3K) pathway as well as small GDP/GTPases such as Rho or Rac. Activation of signal transduction pathways regulate adhesion and migration, proliferation (cell cycle), and survival (apoptosis). Other signals produced by cytokines and chemokines regulate via outside-in mechanism the affinity and avidity of integrins, thus providing a further level of control.

## REGULATION OF PROLIFERATION AND SURVIVAL OF PROGENITORS BY ADHESION RECEPTORS

Besides their role in trafficking and location, adhesion receptors transmit signals from the extracellular milieu into the cell. Signaling via integrins has been most extensively studied in biological systems other than the hematopoietic system [25, 26, 82, 83]. Because integrins, like all adhesion receptors, do not have intrinsic kinase activity, activation of signal pathways requires recruitment of non-receptor kinases [84–86]. Integrins activate the focal adhesion kinase [85] or the related kinase, Pyk-2 [87, 88], which serve to bind and activate a number of Src-homology domain (SH)2 and SH3 containing adaptor proteins [89–92], and activate the phosphoinositide 3-kinase pathway and the Ras/mitogen-activated protein kinase (MAPK) pathway [93, 94], both of which mediate growth and survival regulatory signals (**Fig. 3**). Less is known about signaling that occurs via other receptors, including CD44, selectins, and sialomucins.

Direct evidence for a role of adhesive interactions between hematopoietic progenitors and ECM components has been provided by studies showing that proliferation of committed progenitors as well as long-term culture-initiating cells [12, 14, 95] is inhibited when $CD34^+$ cells are cultured in contact with stroma. When direct contact between progenitors and stromal cells is prevented, increased proliferation of hematopoietic progenitors is observed [95]. The mechanism(s) involved in adhesion-mediated proliferation inhibition are not completely understood. A number of studies showed that integrins, selectins, and mucins may all participate in contact-mediated effects on progenitor proliferation, differentiation, and survival.

### Integrins

Adhesion of progenitor cells to BM extracellular components through $\beta_1$-integrins inhibits cell proliferation [13, 96] by inhibiting $G_1$/S progression, when tested in the presence of serum and low concentrations of cytokines, conditions that may mimic the physiological BM environment. $\beta_1$-dependent adhesion of $CD34^+$ cells is associated with up-regulation of the cell cycle inhibitor $p27^{Kip}$, decreased expression of cyclin E, and decreased cyclin E-cdk2 activity [16, 97]. Growth inhibition seen in the hematopoietic system differs from what has been observed in most other biological systems, as adhesion is a requirement for cell cycle progression for most adherent cell types [98]. $\beta_1$-mediated cell cycle inhibition and increased $p27^{Kip}$ expression is antagonized by addition of higher (nanogram rather than picogram) concentrations of cytokines, which may be required *in vivo* to accelerate hematopoiesis when there is an increased need for mature blood elements [16, 17] (**Fig. 4**). Furthermore, addition of cytokines and other growth factors present in sera affects progenitor adhesion [17, 99]. Thus, both outside-in (growth inhibition) and inside-out signaling (cytokine-mediated changes in adhesion) occurs via $\beta_1$-integrins. Therefore, the behavior of stem and progenitor cells in a complex microenvironment is influenced by the combined effects of cytokine and adhesion receptor-mediated interactions. All studies described here were done using *in vitro* assays for primitive and more committed progenitors. Whether integrin stimulation also affects hematopoietic stem cell proliferation is not yet known and will require enumeration of transplantable cells cultured with or without integrin stimulation.

Yet other studies have shown that engagement of integrins on stem and progenitor cells participates in the prevention of apoptosis of $CD34^+$ cells [100]. This observation may explain the improved engraftment of stem and progenitor cells after transduction with retroviral vectors in contact with stroma or fibronectin compared with transduction without stroma or fibronectin [101, 102].

Which signal transduction pathways are implicated in $\beta_1$-dependent regulation of cell cycle progression, and survival in

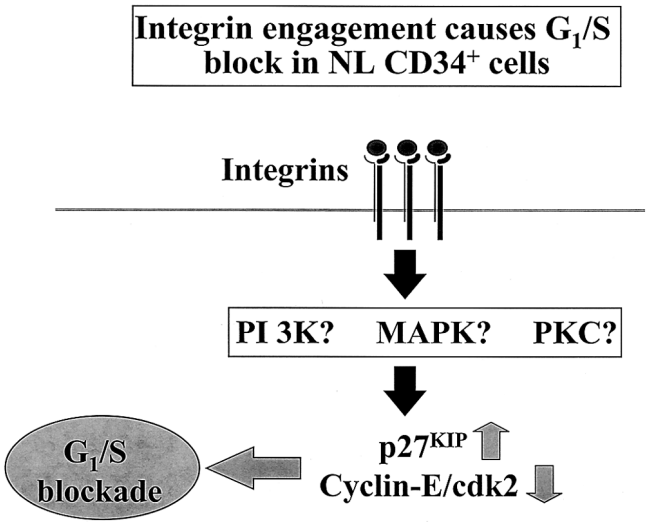


**Fig. 4.** Cell cycle regulation in hematopoietic progenitors by integrins. In normal hematopoietic progenitors engagement of $\beta_1$-integrins induces a cell cycle blockade at $G_1$/S phase, which is associated with increased expression of $p27^{Kip}$, and down-regulation of cyclin E/cdk2 activity. Which signal transduction pathway is implicated in adhesion-mediated cell cycle control is unknown but may involve protein kinase C, PI3K/AKT, or MAPK.

hematopoietic stem cells and progenitor cells still needs to be determined.

### Sialomucins

Cell adhesion through members of the sialomucin family of receptors can also regulate progenitor proliferation, apoptosis, and differentiation. Engagement of CD164 on $CD34^+$ cells prevents recruitment of quiescent progenitor cells into cycle when stimulated with a cocktail of cytokines and induces apoptosis of a fraction of $CD34^+CD38^-$ cells [71]. Engagement of P-selectin via antibodies or with immobilized or soluble ligand for PSGL-1 has marked growth-inhibitory effect of HPC, implicating P-selectin in regulation of HPC cell cycle [70]. Likewise, engagement of CD43 inhibits $CD34^+$ cell growth and induces death of committed but not primitive progenitors [68, 103]. What effects these receptors have on hematopoietic stem cell proliferation is not yet known and will require transplantation of stem cells cultured with or without mucin-receptor simulation *in vivo*.

Another example of mucin-mediated signaling in progenitors is prevention of terminal differentiation of myeloid cells seen after enforced expression of CD34 [104]. Consistent with this finding, $CD34^{-/-}$ mice showed a significantly decreased number of progenitors in the yolk sack or fetal livers, possibly related to premature terminal differentiation [105].

Like integrins, mucin cytoplasmic tails lack tyrosine kinase activity, so interactions through other signaling and adaptor proteins are required [15, 106]. The nature of downstream signaling from mucins in progenitors is still unclear, even though there is recent evidence that mucins interact with the cytoskeleton through the actin-binding proteins ezrin and moesin and that stimulation of mucin receptors induces activation of protein tyrosine kinases, the phospholipase C/phosphoinositides, and G proteins signaling pathways [69].

## CONTRIBUTION OF ABNORMAL EXPRESSION AND FUNCTION OF ADHESION RECEPTORS TO HEMATOLOGICAL MALIGNANCIES

There is a significant body of evidence that abnormal expression or function of cell adhesion molecules contributes to the aberrant behavior observed in a number of hematological diseases. For instance, the type of adhesion receptors expressed on leukemic cells often differs from that on non-leukemic cells at the same stage of differentiation [18, 107–111]. It has been postulated that this may allow leukemic cells to exit the marrow prematurely and to migrate in and be supported by non-hematopoietic microenvironments [18, 107].

### Chronic myelogenous leukemia (CML)

We and others have shown that although $\beta_1$-integrins are present on CML $CD34^+$ cells, integrin function is defective [18, 19, 99, 112]. CML $CD34^+$ cells adhere significantly less well to stromal feeders and to fibronectin [18, 107] than their normal counterparts. In contrast to normal progenitors, engagement of $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins on CML $CD34^+$ cells does not affect the growth of CML progenitors. $\beta_1$-integrin engagement in CML $CD34^+$ cells does not affect $p27^{Kip}$ expression and function [16], likely due to the fact that $p27^{Kip}$ is located in the cytoplasmic compartment of the cell instead of in the nucleus where it is unable to inhibit the cell cycle. Of note, treatment of CML $CD34^+$ cells with agents known to induce hematological and sometimes cytogenetic remissions is associated with normalization of $\beta_1$-integrin function [113]. For instance, treatment of CML $CD34^+$ cells with interferon-$\alpha$ *in vitro*, improves the adhesive [19] and signaling [114] function of $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins. Likewise, treatment of CML $CD34^+$ cells with BCR/ABL specific tyrosine kinase inhibitors [115], which also results in hematological responses in most patients and in some patients in cytogenetic responses [116, 117], restores $\beta_1$-integrin-dependent adhesion and adhesion-mediated growth regulation. In normal hematopoiesis, adhesion to the bone marrow microenvironment is required to prevent stem cell and progenitor apoptosis. As the $p210^{BCR/ABL}$ inhibits apoptosis [118, 119], CML progenitors, but not normal progenitors may be capable of surviving in the absence of contact with the microenvironment. This has led to the thesis that defective adhesion through $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins in CML is in part responsible for the abnormal premature circulation of CML $CD34^+$ cells in the blood [120, 121]. Lack of integrin-mediated regulation of CML progenitor growth and increased ability of CML $CD34^+$ cells to survive in the absence of interactions with the BM microenvironment may contribute to the massive expansion of the malignant stem and progenitor cell population in CML. Finally, there is also evidence that abnormal expression of chemokine receptors as well as abnormal responses to chemokine stimulation may contribute to aberrant circulation and growth of the leukemic cells in CML [122].

As sialomucins are a second set of adhesion receptors known to affect cell growth and cell survival [64, 66, 67, 99], it is possible that defects in signaling from this class of receptors may also be involved in the abnormal proliferation of CML progenitors. Preliminary studies by Levesque and Simmons

suggest that normal growth inhibition seen after engagement of PSGL-1 may not occur in CML [Levesque and Simmons, personal communication].

A number of other adhesion receptors, including CD44 [31, 123, 124] and L-selectin [125] have also been implicated in abnormal trafficking and growth of CML leukemic blasts. However, it is much less well understood how differences in expression or function of these receptors contribute to the abnormal hematopoietic process in CML than what is currently known for integrins.

## B cell acute lymphocytic leukemia (ALL)

Adhesive defects have also been described in B-lineage acute lymphoblastic leukemia [126, 127]. Normal B cell development occurs in the BM microenvironment. $\beta_1$-integrins play an important role in regulating growth and survival of immature B cell precursors. In a recent study, expression and function of $\beta_1$- and $\beta_2$-integrins was studied in 20 patients with B-lineage acute ALL [126]. Even though $\alpha_4\beta_1$ and $\alpha_L\beta_2$ were uniformly expressed in $CD10^+$ ALL blast, adhesion was severely impaired in 85% of the patients. Furthermore, phorbol 12-myristate 13-acetate was not able to activate LFA-1- or VLA4-mediated adhesion in 10 and 7 patients, respectively. Thus, as has been seen in CML, the mutations underlying development of B cell ALL may obviate the need for integrin-mediated interactions required for normal growth and survival of B cell precursors. Indeed, a number of studies have shown a role for integrin-mediated interactions in abnormal regulation of proliferation and apoptosis in ALL [127]. For instance, the B-ALL-derived cell line, BLIN-2, can be cultured on VCAM-1-negative fibroblast, suggesting that $\alpha_4\beta_1$/VCAM interactions are not required for BLIN-2 growth or survival.

Other studies have shown that interactions between $\alpha_4\beta_1$ and its ligand, VCAM, may play a role in chemotherapy resistance [128]. B-ALL cells undergo cell cycle arrest in G1 phase of the cell cycle and eventually go into apoptosis when incubated with cytarabine or etoposide. However, contact with stroma prevented both cell cycle arrest and apoptosis of B-ALL cells [128]. Interaction with fibronectin did not enhance survival of ALL cells during chemotherapy exposure. However, interaction with the other ligand of the $\alpha_4\beta_1$ integrin, VCAM, provided maximal protection from cytarabine and etoposide-induced cell death. Although the molecular mechanisms of this effect are unknown, these observations suggest a role for the BM microenvironment in modulating the response of B-lineage ALL cells to chemotherapy.

## B cell chronic lymphocytic leukemia (CLL)

B-CLL-leukemic cells express $\beta_1$- and $\beta_2$-integrins, including $\alpha_3$, $\alpha_4$, and $\alpha_5$. They also express LFA-1 (receptor for ICAM-1, ICAM-2, and ICAM-3), L-selectin, and CD44 [129–131], even though expression varies significantly between patients [132]. Differences have been associated with the clinical behavior of the disease [132]. B-CLL associated with the 11q deletion is characterized by extensive lymphadenopathy, rapid disease progression, and short progression-free survival. In comparison with B-CLL leukemic cells from patients that do not have the 11q deletion, expression of the $\beta_2$ integrins CD11a/CD18, CD11c/CD18, CD31, CD48, or LFA-3 was significantly reduced in patients with B-CLL with the 11q deletion and low-level expression of the $\alpha_4$ integrin was correlated with a decreased overall survival.

B-CLL cells are characterized *in vivo* by their prolonged survival and resistance to apoptosis. *In vitro*, however, B-CLL cells undergo rapid apoptosis. Recently it has been demonstrated that adhesion to BM stroma prevents apoptosis in B-CLL but does not rescue normal B cells from undergoing apoptosis in *in vitro* cultures [133]. Apoptosis is prevented at least in part through interactions between the $\alpha_4\beta_1$ integrin and fibronectin and is associated with an elevated Bcl2/Bax ratio [134]. Adhesive interactions between $\beta_1$ integrins and fibronectin may also contribute to chemotherapy resistance in B-CLL [Garcia-Pardo, personal communication]. Whether B-CLL cells from patients with the 11q deletion, that express less $\alpha_4$ integrins required for B cell-stromal interactions [132], have the capacity to survive in the absence of interactions with the BM microenvironment is not known.

## Multiple myeloma

The last disease, again B cell in origin, for which there is ample evidence for a role of BM stroma in the development and progression of diseases as well as development of drug resistance, is multiple myeloma. Excellent reviews have been recently published [135, 136] and these will be briefly summarized here. Differences in expression of adhesion molecules between normal plasma cells, multiple myeloma cells, and plasma cell leukemia have been used for diagnostic purposes. For instance, acquisition of CD56, CD58, and the receptor for hyaluronan-mediated motility (RHAMM), as well as loss of CD11a is associated with malignant transformation [137]. As is true for other hematopoietic malignancies, there is evidence that adhesion receptor-mediated interactions contribute to the pathogenesis of myeloma. The $\alpha_4\beta_1$ integrin supports plasma cell adhesion to VCAM-1 and fibronectin, providing anchoring for plasma cells to the BM [138, 139]. The interaction between $\alpha_4\beta_1$ and VCAM-1 enhances the production of osteoclast-stimulating [140] and protects plasma cells from drug induced apoptosis [138, 139]. Prevention of drug-induced apoptosis by integrin engagement is associated with $p27^{Kip}$ up-regulation [138]. Interactions via CD40 on plasma cells and CD40-L expressed in the BM results in the production of interleukin-6 [141], which is required for the survival and growth of the plasma cells. Thus, as is the case in other malignancies, adhesion receptor-mediated interactions are not only important for the localization of plasma cells in the BM microenvironment, but also affect growth and survival of plasma cells.

# CONCLUSION

In summary, cell-cell and cell-extracellular matrix interactions through adhesion receptors plays a major role in the hematopoietic process. Not only are adhesive interactions responsible for the residence of the primitive hematopoietic cell pool in the marrow, but they are also responsible for "homing" of stem and progenitor cells to the marrow in the setting of stem cell

transplantation. Because no bone marrow-specific ligands have been identified and no hematopoietic stem and progenitor cell-specific receptors are known, the specificity of interactions between progenitors and marrow elements must be mediated by yet to be identified receptor ligand pairs. Alternatively, the specificity may be imparted by additional signals from for instance cytokines or chemokines, such as SDF-1α that are expressed specifically in the marrow and influence activation status of adhesion receptors in the marrow microenvironment. Aside from anchoring cells in a given microenvironment, adhesion receptors also play an important role in regulation of cell behavior, either through direct activation of signal pathways important for cell survival, cell growth, and cell fate decision-making processes, or by modulating responses to growth factors. Future studies characterizing the signals arising from cell adhesion receptors on hematopoietic progenitors will contribute to a better understanding of the mechanisms that govern the tightly regulated process of normal hematopoietic cell proliferation and differentiation. Furthermore, insights into the abnormalities seen in diseases of the hematopoietic system will help to develop better therapeutic strategies based on the pathogenesis of the disease.

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