MINIREVIEW

Homing of Hemopoietic Progenitor Cells to the Marrow (43201)

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Abstract. **The recognition of hemopoietic stem cell after intravenous transplantation of marrow cells occurs initially by a lectin moiety on the surface of marrow sinus endothelium. The cell is then transported across the endothelial cytoplasm much in the way that a soluble ligand, such as transferrin, is transported. In the extravascular compartment, the cell binds to lineage-specific stromal cells. This mechanism, known as homing, is mediated by a lectin-glycoconjugate interaction, the lectin being on the surface of progenitor cell with specificity for galactosyl and mannosyl residues. The binding is subsequently stabilized by membrane-bound proteoglycans, integrin-like receptors, and fibronectin.** [P.S.E.B.M. 1991, Vol 196]

The concept of homing can best be perceived in the context of organ transplantation: Organs such as kidney or heart can only be transplanted orthotopically or heterotopically by surgical techniques, providing for their connections with those of the recipient's body. These are well-differentiated organs, structurally well organized, and their structural organization must be retained in the recipient if their function is to be retained.

Bone marrow is an exception. Its diffuse stromal organization and vasculature, within the frame of bone, are not easily susceptible to damage and they possess a remarkable potential for repair (1, 2). Thus, transplantation of marrow involves merely the introduction of a source of stem cells and progenitor cells (3, 4) which, supported by the preexisting stromal organization, differentiate and mature into functional blood cells. Introduction of progenitor cells into the recipient is done by intravenous infusion of marrow cell suspensions.

Homing can then be defined as molecular interactions that lead to recognition and selective seeding of hemopoietic stem cells onto the recipient's bone cavities (5).

It has been shown that intravenously transplanted marrow cells pass through most tissues and organs (6). Most differentiated cells among them undergo a few divisions, forming small and *transient* intravascular hemopoietic colonies, particularly in the lungs. But *sustained* hemopoiesis occurs only in the extravascular spaces of the bone marrow (6). This requires recognition and selective seeding of circulating stem cells to lineage-specific stroma of marrow (5).

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Cellular Recognition

Evidence for a Lectin-Sugar Recognition. Since hemopoiesis in the bone marrow is an extravascular event (7–10), transplanted cells must first interact with the luminal surface of marrow endothelium to penetrate it and enter the extravascular space. This occurs in special vessels, known as sinuses (10), whose wall is remarkably simplified, consisting of an attenuated endothelial layer and an inconsistent adventitial layer. At a molecular level, the interaction between progenitor cells in the lumen (transplanted or otherwise circulating) and the endothelium appears to involve a lectin with galactosyl specificity (11, 12). This view is supported by the following lines of evidence:

1. A lectin with galactosyl specificity has been identified on the luminal surface of sinus endothelium (11). It has not been, however, well characterized.

2. Infusion of radiolabeled galactosyl-containing molecules in the regional circulation of bone marrow

results in their uptake which can be inhibited by simultaneous infusion of excess unlabeled molecules (11, 12).

3. Distribution of intravenously infused radiolabeled synthetic galactosyl probes is highest in the bone marrow, when calculated as per gram tissue (13).

4. Treatment of the cell surface by neuraminidase reduces hemopoietic colony formation in spleen [colony-forming unit-spleen (CFU-S)] by marrow cell suspensions (14). This treatment exposes the penultimate galactosyl residues of membrane glycoconjugates. One interpretation of this observation is that the exposition of galactosyl residues shifts the cellular uptake to the bone marrow in preference to the spleen, thus reducing spleen colonies. Treatment with other glycosidases does not affect colony formation.

5. Galactosyl-containing probes infused with transplanted marrow cells can reduce the seeding efficiency of these cells (11, 15).

Thus, it appears that a galactosyl moiety on the membrane of hemopoietic progenitors recognizes a galactosyl-specific lectin molecule on the luminal surface of endothelium. As is the case with many other ligands such as transferrin (16) (except that with transplanted cells, the ligand is not soluble, but membrane-bound) binding leads to internalization and then transport of the cell to the abluminal side. This internalization of the ligand, not seen in the second step (see below) is essential for cellular passage into the hemopoietic compartment.

Endothelial-Progenitor Interactions. Cellular transport across the sinus endothelium is, thus, transendothelial and not interendothelial. A similar mode of migration has been documented for mature cells that exit from the marrow (17–20). With this mode of transport, endothelium apparently can control the type of cells and the magnitude of cellular traffic across the wall (7).

This mode of traffic probably operates in normal state and maintains a gradient of stem cells between marrow and blood. In marrow transplantation, however, a new set of factors operates that may help this transendothelial phase of homing in the marrow (21). Marrow transplantation is generally preceded by conditioning regimens, such as radiation and/or chemotherapy. These regimens can alter the permissiveness of endothelium through an effect on cell membrane. The consequence of this conditioning is that the endothelium no longer provides an effective barrier between blood and marrow. Cells within the lumen can easily gain access to the hemopoietic compartment. This is evident from the presence of mature red blood cells in the extravascular space (22). Normally, only reticulocytes are present in this compartment.

Thus, conditioning regimens given before marrow transplantation, in addition to the purpose they fulfill in the treatment of underlying diseases, or modulating the immune system, can also facilitate the homing of transplanted cells.

Stromal-Progenitor Interactions. Once in the hemopoietic compartment, progenitor cells adhere to stromal cells, usually by a lineage-specific mechanism. There is now a large body of evidence that this adherence is necessary for the subsequent phases of hemopoiesis (proliferation, differentiation, maturation). As these lines of evidence have recently been reviewed (23), they will not be repeated here.

Adherence to the stroma is the second step in homing and probably a complex one. It provides for the seeding selectivity of bone marrow after intravenous transplantation of marrow cell. This selectivity is exerted by a system involving membrane homing protein expressed on the surface of progenitor cells, and interacting with specific configuration of a glycoconjugate on the stromal cell membrane (15, 24, 25). There are other adhesive molecules involved in this adherence, but they probably do not provide selectivity. They merely strengthen the adherence forces (5).

Analysis of these mechanisms of adherence has been facilitated by the development of cloned progenitor cells and stromal cells, permitting dissection of a complex system into its more simplified elements (26).

Homing Protein. Homing protein is a membrane protein that is present on the surface of hemopoietic stem cell (CFU-S) and macrophage-granulocytic progenitors (colony-forming unit-granulocyte macrophage). Differentiation in erythroid lineage is apparently associated with the loss or dilution of this protein (27). Nor is this protein present on the surface of marrow or spleen stromal cells (28). The protein is a heterodimer with M_r of 110,000 (29). The two chains are disulfide bonded and have M_r of 87,000 and 23,000. There is a 5% carbohydrate content. Functionally, a function of homing protein is lectin-like, recognizing and binding to an as yet unknown configuration of membrane carbohydrate, probably the glycan moiety of a glycoprotein. It shows specificity for galactosyl and mannosyl (24, 25, 30). Apparently both residues are necessary for the binding, since competitive inhibition with one residue abolishes the binding altogether (24).

Using cloned progenitor cells and synthetic glycoproteins of known sugar specificities (31), determination of binding characteristics indicates K_d of 2.3×10^{-7} M and 1.0×10^{-7} M, respectively, for galactosyl and mannosyl residues. There are $\sim 10^6$ copies of protein per cell, but this can be increased by short incubation with interleukin 3 and granulocyte-macrophage colony-stimulating factor (32). Interaction of this receptor with its ligand does not lead to their internalization (31). This is , of course, expected, since the ligand in its natural state is membrane bound and part of a glycoconjugate on the surface of stromal cell. The absence

of internalization is in contrast to the endothelial lectin whose internalization of its ligand leads to the transmural transport of progenitor cells.

Evidence for the Involvement of Homing Protein. Evidence indicating that this homing protein is indeed involved in homing of progenitor cells is summarized below:

1. Seeding of intravenously transplanted marrow cells is inhibited competitively by preincubation of cells with synthetic glycoprotein and simultaneous infusion of these glycoproteins (15). Only synthetic molecules of galactosyl and mannosyl, but not fucosyl, specificities inhibit the seeding.

2. Similarly, synthetic molecules of these specificities inhibit the binding of progenitor cells to the feeder layer in long-term bone marrow cultures, thereby inhibiting cell production in these cultures (24, 25).

3. Binding of cloned hemopoietic progenitors to cloned stromal cells are also inhibited by synthetic glycoproteins of these specificities. This binding is a simplified experimental version of homing (26).

4. Treatment of stromal cell surface with neuraminidase followed by galactosidase and mannosidase residues reduces or nearly abolishes the homing of progenitor cells to stromal cells. Treatment in the reverse order has no such effect. This indicates the presence of a glycoconjugate on the surface of stromal cells that can interact with the homing protein in the binding of the two cells.

5. Incubation of hemopoietic progenitors with such growth factors as interleukin 3 and granulocyte-macrophage colony-stimulating factor increases the surface density of homing protein. Concomitantly, the seeding efficiency of these cells in lethally irradiated mice and their adherence to stromal cells increase, suggesting a cause and effect relationship (32).

Other Adhesive Mechanisms in Homing. The homing protein certainly can account for the selectivity of homing of marrow cells after intravenous bone marrow transplantation. But, there are other molecular mechanisms that can strengthen the bond between progenitor cells and their supporting stroma. Through this adhesion they may serve a biologic function as well, to regulate hemopoiesis. In this regard the role of the extracellular matrix should be mentioned.

Extracellular matrix (ECM) is formed from glycoproteins and proteoglycan units, which interact with each other to produce well-organized supramolecular assemblies. The dynamic architecture of the ECM explains its functions as a whole (like support and sieving), but also its specific functions like those related to cell-matrix interactions, which are dependent on the presence of site-specific domains on each of the ECM units (33–35). Cell-matrix interactions influence how cells proliferate, differentiate, and migrate (36–38).

In the bone marrow, the hemopoietic stem cell depend upon interactions with elements of the microenvironment for self-renewal and differentiation. This is a complex environment composed by stromal cells and their products, growth factors, and ECM (39–41).

ECM Complexes. Bone marrow stromal cells produce specific ECM components like fibronectin, laminin, hemonectin, various types of collagens, and proteoglycans (42–45). The assembly of a hematocompetent ECM from its components (as it occurs in nonhemopoietic tissues) probably involves the binding of single biomatrix molecules to themselves (polymerization) as well as to other oligomers to form heterologous complexes (34, 35).

In vitro studies have shown that when such orderly complexes of ECM are formed, they promote a rapid formation of an adherent stromal cell layer which subsequently permits proliferation and differentation of hemopoietic cells (46).

The observation that individual cellular elements of the marrow stroma have only a limited capacity to support hemopoiesis (as compared with whole marrow stromal cells) suggests that the organized matrix also plays a role in facilitating cell lodgement and homing to the marrow (42, 47, 48).

Although little is known about the molecular mechanism involved in the interaction of progenitor cells and ECM, it has been established that the synthesis and deposit of biomatrix molecules in the marrow space are required for hemopoiesis (49). This process which leads to the production of homologous and heterologous ECM complexes is coincident with the onset of hemopoietic cell production. The relationship between the genesis of the biomatrix and the onset of hemopoiesis has been demonstrated by use of compounds like β-xylosides (50, 51) and hydroxyproline derivatives (52), which affect the production of proteoglycans and collagen, respectively. In both cases, the *in vitro* rate of proliferation of progenitors (CFU-S) is markedly modified by the drugs.

Modulation of hemopoiesis, via ECM production, seems also to be under the control of growth factors. Hemopoietic inhibitory lymphokines (53) have been reported to increase the synthesis of biomatrix molecules by stromal cells. Transforming growth factor-β stimulates collagen synthesis and promotes the synthesis of surface proteoglycans (54, 55), and α-interferon increases the synthesis of glycosylaminoglycans by marrow stromal cells (56). In the case of transforming growth factor-β it seems that the cellular response to the cytokine is the enhanced deposition of matrix components together with the augmented expression of their receptors (57).

The regulatory interactions between the hemopoietic microenvironment and progenitor cells seems to be determined at least partially by mutual recognition and adhesive processes. Since a spatial distribution of

progenitor cells occurs in the cytoarchitecture of the marrow stroma (58), it appears that the course of the maturation of hemopoietic cells involves an orderly expression of cytoadhesive properties of stem cells and their progeny. By changes in their cytoadhesive properties, progenitor cells may respond differently to proliferation and differentiation signals and may change their capacity to migrate toward the marrow sinus wall.

Role of Fibronectin. Among the ECM molecules, fibronectin (FN) is the most effective in inducing adhesion, reorganization of cytoskeletal structures, and motility (34). FN is organized into discrete units of aminoacid homologies, termed types I, II, and III. However, other molecular forms may arise from alternative splicing of a single gene transcript (59).

Several marrow stromal cells produce FN and organize it in an insoluble form in the ECM (42). Along with its ability to produce FN, fibroblasts bind soluble forms of FN by high-affinity cell surface sites that recognize a type I repeat, located close to the amino-terminal region of the molecule (34). Endothelial cells show a polarized FN secretion and its assembly occurs adjacent to the basolateral cell surface (60).

Hemopoietic cells of several lineages recognize the locus of the FN-cell attachment domain. This recognition, which involves attachment and adhesion is brought about by a structurally related family of membrane surface receptors, named integrins (61). Integrins have been observed in a variety of hemopoietic cells, including a subset of CD 34-positive precursor cells (62), burst-forming units, erythroid and colony-forming units-erythroid (63), erythroid precursor cell lines (64), monocytes, and T lymphocytes (65).

In erythroid cell development, the FN-adhesion property associated with integrins is lost during differentiation in two sequential stages: the high-adhesion property of FN in colony-forming unit-erythroid is reduced in the transition to proerythroblasts, while a complete loss of the remaining adhesive properties occurs at the time of enucleation (66). The latter may be the underlying cause for the release of end stage cells into the circulation.

Whether integrin-FN recognition mechanisms have other effects in cell proliferation and differentiation is not well understood. Recently, it has been postulated that FN binding provides hemopoietic progenitors with an anchorage mechanism as well as with a proliferative stimulus. The latter seems to be related to the promotion of proliferation or recruitment into cycle of primitive hemopoietic cells by FN (67). This opens the possibility that FN may act as a growth factor alone or in concert with other hemopoietic growth factors.

Other Adhesive Proteins. Hemonectin (HN), a component of the marrow ECM, is a cytoadhesive glycoprotein which shows lineage and organ-specific attachment properties for cells of the granulocytic lineage. The preferential localization of hemonectin in the marrow to the bone lining cells and its specific binding to granulocyte/macrophage progenitor cells have led to the idea that HN localizes granulocytic cells in close proximity to growth factor-producing stromal cells. HN expression and its binding by putative receptors in granulocyte precursors seem also to be under maturation control. Loss of adhesion to HN results in the release of mature granulocytes into the circulation (45, 68).

A collagen-containing matrix is an essential prerequisite for the proliferation and cytoorganization of marrow stromal cells. Besides a mechanical role in hemopoiesis, collagen polymeric forms may also participate in adhesive interaction by binding directly to progenitor cells. It has been well documented that cell surface receptors for collagen exist in a variety of cells, which in most cases are glycoproteins related to the α/β chains of the integrin family of adhesive molecules (69). On the other hand, collagen forms may interact indirectly with hemopoietic cells by acting as anchorage receptors to typical adhesive proteins, which in turn may bind to their own receptor in the progenitor cells (70). The latter may result in the formation of heterogenous matrix complexes with variable adhesive properties. The physiologic significance that these adhesive mechanisms, mediated by collagen polymeric forms, may have in the regulation and differential expression of hemopoiesis is still not well understood.

Proteoglycans. Proteoglycans (PG) produced by marrow stromal cells have been isolated and their molecular characteristics determined (43, 71, 72). Stromal PG or their glycosaminoglycan (GAG) moiety seem to play a major role in hemopoiesis, as evidenced by studies in which the production of PG by stromal cells has been experimentally altered by exposure to β-xylosides. In these studies, the production of stem cells (CFU-S) as well as that of committed progenitors (CFU) was dramatically affected by the xylosides derivatives (50, 51).

Although, the mechanism of action of stromal PG in hemopoiesis is not well understood, several lines of evidence suggest that they contribute to the establishment and reorganization of the ECM through its ability to interact with individual ECM components (73). Stromal PG also seem to be involved in marrow adhesive processes, since changes in GAG content and distribution result in the detachment and exit of early myeloid precursors from the marrow space (74). The ability of PG or its GAG moieties to bind growth factors seems to be another expression of the role of these molecules by directing the proper compartmentalization and molecular orientation of hemopoietic growth factors (75, 76).

Recently, we have provided evidence that, in addition to stromal cells, progenitor cells also synthesize

PG (77). A characteristic feature of progenitor cells is that they produce a unique type of PG known as chondroitin sulfate (CS) which shows a preferential membrane-associated (MA) distribution. This component is not stable and is released from the cell membrane in a time-dependent fashion. However, in the presence of stromal cells, progenitor cells stabilize its MA-PG, a phenomenon that has suggested a MA-CS-PG role in the adhesive interactions between progenitor and stromal cells (77).

Such a role of MA-PG in adhesive hemopoietic interactions is consequent with the observation that the binding of hemopoietic progenitor cells to a fibronectin-containing support is mediated by the CS moiety of the MA-PG. The removal of the GAG portion by chondroitinases or the addition of heparin, free CS, or a monoclonal antibody against the CS portion of the PG can all decrease the binding of the progenitor cell to hematocompetent stromal cell lines or to fibronectin-coated vessels (unpublished results). This role is not without precedents, since several studies have shown that membrane-associated CS-PG from various cellular sources are involved in adhesive processes (54, 78, 79).

The apparent multiplicity of possible forms of MA-CS-PG in different hemopoietic progenitor cells (80) implies that the functions of these compounds may be equally varied; however, the current evidence strongly suggests their involvement in the initial interactions of the progenitor cell with the stromal substratum.

Since binding of hemopoietic progenitor cells to stromal ECM also involves interaction with the cell binding domain in FN, through a putative integrin-like receptor, it seems that interactions of progenitor cells with FN represent a major adhesive phenomenon.

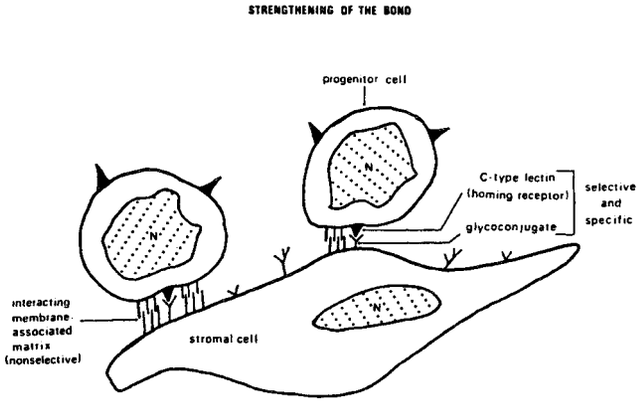

Figure 1. Diagrammatic representation of how homing protein can bring about the specificity of binding of progenitor cells to the stroma. Progenitor cells contain homing protein, which is C-type lectin. It recognizes and specifically binds to a specific sugar configuration in a glycoconjugate on the surface of stromal cells. This interaction provides for selectivity of the interaction. The binding can be further strengthened by interaction between various components of membrane-bound matrix, which provides for strengthening but not selectivity (Reproduced with permission from Ref. 5).

Thus, regulation of the expression of integrins and MA-PG in the progenitor hemopoietic cells may affect their adhesive interactions with the FN-containing stroma. The net result of these interactions can be the stabilization of the binding of the progenitor cell to the marrow stroma, initially brought about by homing proteins (5). While homing proteins are involved in the recognition and initial weak binding ($K_d \sim 10^{-7}$ M) (31) of progenitor cells to stroma, the stabilization of this binding may be a contribution made by membrane-associated proteoglycans, integrin-like receptors, and fibronectin. The later ECM-related molecules show strong interactions (in concert or coupled) with K_d of 10^{-8} to 10^{-10} M which involve reversible interactions as well as covalent stabilization and cross-links (34). Strong interactions, by creating high local concentrations of participant molecules, may allow weaker interactions to operate and to contribute by rearrangement of bonds to the establishment of structures which meet specific functional needs. A working model that fits the existing data and provides a conceptual basis for the homing process is illustrated in Figure 1.

Recently, studies have indicated that in addition to the homing protein and ECM molecules in targeting, adhesion, and trafficking of progenitor cells to and into the marrow stroma, hempoietic-cell adhesion molecule expression is also a factor in the complex network of cellular interactions in the marrow microenvironment (5).

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