Molecular Basis of Homing of Intravenously Transplanted Stem Cells to the Marrow

By Mehdi Tavassoli and Cheryl L. Hardy

Homing of hematopoietic progenitors can be defined as the set of molecular interactions that mediates the recognition of hematopoietic stem cells and other progenitor cells by marrow stroma after the progenitor cells are intravenously introduced into a recipient. Through this recognition mechanism, circulating progenitor cells bind selectively to the marrow stroma and begin the process of proliferation and differentiation. It is owing to the homing mechanism that bone marrow transplantation can be carried out by simple intravenous infusion of marrow cells, rather than requiring a surgical operation, as is the case with the transplantation of other organs such as kidney, heart, or liver.

Homing is mediated by membrane interactions between progenitor cells and stromal cells. A homing molecule on the surface of progenitor cells recognizes and binds to a corresponding molecule on the surface of stromal cells. With maturation, the progenitor cell may stop the synthesis and, consequently, can lose its homing molecule, which may be diluted as a result of frequent cell divisions without being replaced. Mature cells, therefore, do not possess the homing molecules and can be released into the circulation.

Investigation into the molecular mechanism of homing was precluded by the lack of necessary molecular probes that would permit exploration of the specificity of this interaction. Because the nature of interaction was unknown, investigators did not know where to start to unveil the phenomenon. There was no direction to the field. The development of appropriate probes during the past decade has permitted determination of the specificity of this interaction. Early evidence, derived from studies with lectin mitogens, suggested that the molecular basis of recognition and homing may involve an interaction between a membrane lectin and appropriate configuration of certain carbohydrate groups of membrane glycoconjugates, leading to the selective seeding of stem cells to the marrow. This suggestion gave a direction to the research in this area and led to the development and synthesis of a number of probes that facilitated the identification of the homing molecule. Parallel work on the trafficking of lymphocytes indicated that a similar mechanism may also be involved in regulating the selective entry of lymphocytes into certain lymphoid organs and, probably, the regulated delivery of mature blood cells from marrow into the circulation.

This article is a critical review of information that has emerged concerning the homing of hematopoietic progenitor cells. Molecular mechanisms regulating the traffic of lymphocytes will also be briefly discussed.

SYNTHETIC NEOGLYCOPROTEIN PROBES

To probe the molecular mechanism of homing, a group of neoglycoproteins was synthesized by covalently linking the p-aminophenyl derivative of various biologically active monosaccharides in pyranose form to bovine serum albumin (BSA). This generates large molecules that are not diffusible into the cell and are capable of being labeled with tritiated thymidine. Biologically active monosaccharides in glycoproteins are few in number (N-acetylglucosamine, mannose, galactose, fucose in addition to the terminal sialyl residue, and all can be obtained or synthesized in p-aminophenyl derivative form, which retains the pyranose (ring) form of the sugar that is necessary for lectin binding. There are other theoretical considerations in the synthesis of these reagents that have been described elsewhere in detail. The carbohydrate moiety of these probes can now compete with naturally occurring glycoconjugates in their interactions with membrane lectins. BSA moiety is inert since it does not have specific receptors on most cells. The competition offered by the carbohydrate moiety prevents interactions of membrane lectin-glycoconjugate and the biologic consequences that may arise from them.

It should be mentioned that an essential characteristic of these probes is their lack of toxicity. Not only are they harmless to animals when injected intravenously, but they also lack toxicity in cell culture systems and, in particular, they lack inhibitory and stimulatory activities in hematopoietic cell systems. This lack of toxicity makes them ideal for...
probing the molecular basis of homing of hematopoietic progenitor cells.

INHIBITION OF HOMING

To investigate the molecular basis of homing of intravenously transplanted stem cells to the marrow, mice are subjected to lethal doses of whole body irradiation. They are then transplanted intravenously with $10^6$ isologous marrow cells. Transplantation is done in the presence or absence of various synthetic neoglycoproteins in inhibiting concentrations. Concentrations of stem cells (CFU-S) and progenitor cells (CFU-GM) are then measured in the tibia at 2 hours, 24 hours, and then weekly after transplantation. It is found that among the biologically active sugars, only galactose- and mannosyl-specific probes (galactosyl-BSA [G-BSA] and mannosyl-BSA [M-BSA]) inhibit homing of stem cells to the marrow. Consequently, the seeding efficiency of stem cells (defined as the concentration of CFU-S in the bone marrow, 2 hours and 24 hours after transplantation) declines. Reconstitution of hematopoiesis (as defined by the cellularity of marrow and concentrations of CFU-S and CFU-GM a week after transplantation) is prevented, and the dose-dependent survival of animals is thereby compromised. This type of observation not only supports previous suggestions that homing is mediated by a lectin-carbohydrate interaction but further assigns the specificity of carbohydrate to galactosyl and mannosyl moieties. Neoglycoproteins with other specificities affect neither homing nor long-term reconstitution of hematopoiesis.

Similar results have been obtained in vitro as well using long-term marrow cultures. In this system, an adherent stromal layer is first established. After 3 weeks, a second inoculum of fresh marrow is added as the source of hematopoietic progenitors that binds to the stromal layer. Proliferation and differentiation of hematopoietic cells, mostly of the granulocytic lineage, occur within this stromal layer; and, upon maturation, cells are released into the supernate. Weekly cell production can be quantified when the medium is changed. Proliferating cells that are bound to the adherent stroma form distinct foci (cobblestones) that can be enumerated by phase microscopy. CFU-S and CFU-GM concentrations can also be measured in the adherent stroma by killing some of the cultures. Since the binding is in a steady state of equilibrium, mature cells as well as CFU-S and CFU-GM are then released into the supernate, where they can also be measured weekly. This system lends itself to the exploration of homing, because cultures can be grown in the presence of neoglycoproteins that inhibit the binding of progenitor cells to stromal cells without altering proliferative characters of progenitors.

In this system, it is also found that not only total cell production, but also the production of CFU-S and CFU-GM declines in the presence of synthetic neoglycoproteins with galactosyl and mannosyl specificities. The system remains unaffected by neoglycoproteins of other specificities. These studies not only confirm in vivo observations, but also define homing essentially as the binding of stem cells and other progenitors to the stroma via a carbohydrate-lectin system of galactosyl and mannosyl specificities. Removal of the inhibiting reagents readily re-establishes the proliferative capacity of the culture with the slope of cumulative cell production being parallel to the initial slope, indicating reversibility of the process and further confirming the lack of toxicity of reagents (Fig 1).

The use of combined probes (both galactosyl and mannosyl) in equal concentrations necessary for inhibition does not afford any additive or synergistic effect. This finding, in the context of overall studies, suggests that a configuration of carbohydrate, probably the glycan chain of a glycoconjugate, is involved in the binding, and that both galactosyl and mannosyl residues are necessary for this interaction. Therefore, if either of the two is inhibited, the binding is entirely

![Fig 1. Murine long term marrow cultures in the absence of neoglycoprotein give a cumulative cell production curve that is linear as a function of time (control). In the presence of galactosyl-BSA (lower curve), cumulative cell production halts after 2 to 3 weeks and the curve becomes a plateau. However, if the inhibiting neoglycoprotein is removed after a few weeks (5 weeks in Exp. 2), cell production resumes with the slope parallel to that of control, indicating the probe has not altered the functional potential of supporting stroma. Experiment 1 shows hematopoietic progenitors first "conditioned" for a week or more with neoglycoproteins and then seeded onto normal stroma. Their cumulative cell production is no different from the control. Reprinted with permission.](http://www.bloodjournal.org)
inhibited. Moreover, obtaining similar results by using combined probes strongly suggests that the same lectin (and not two different ones) is involved in the binding of both carbohydrate residues. This conclusion can be confirmed by ligand binding to cloned hematopoietic cells and by purification of the lectin (vide infra).

The in vitro system can be simplified further by the use of cloned progenitor cells binding to cloned stromal cells. The adherent stromal cell clone, D2X, is grown to near confluence. The progenitor cell FDCP-1, a bipotential clone analogous to CFU-GM, is then used for adherence to the stroma. Binding may be quantitated by labeling the progenitor cells with $^{35}$Cr and measuring the amount of radioactivity firmly bound to the dish after a 2-hour period of contact between stem cells and stromal cells in the presence or absence of neoglycoproteins. Here again, the system reproduces the results of previous observations with consistency: Synthetic neoglycoproteins of galactosyl and mannosyl specificities, but not those of other specificities, inhibit the binding of cloned progenitor cells to cloned stroma.

ASSIGNMENT OF LECTINS TO PROGENITOR CELLS

At this point, one may only conclude that binding of progenitor cells to stromal cells involves an interaction between a membrane lectin on one side of the equation and a membrane glycoconjugate on the other side. However, it is not clear on which side of the equation membrane lectin (herein referred to as "homing protein") is located. To study this question, selective agglutination of progenitor cells was attempted. In this technique, cells possessing the lectin are crosslinked by neoglycoproteins and then pelleted centrifugally. The cells that do not possess the lectin do not agglutinate. Crosslinking is done in whole marrow cell suspensions containing all types of progenitor cells.

These experiments permit the assignment of homing lectin to progenitor cells, since both CFU-S and CFU-GM could be selectively agglutinated by neoglycoproteins of galactosyl and mannosyl but not other specificities. This conclusion has been subsequently confirmed in two factor-dependent cloned progenitor cell lines, B6SUT and FDCP-1, by use of $^{125}$I-labeled G-BSA and M-BSA in standard binding assays. Membrane lectins with these specificities, but not those of other sugars, are found on the surfaces of these cells. In the cell line B6SUT, Scatchard analysis indicates a dissociation constant kd of $2.3 \times 10^{-7}$ mol/L and $1.0 \times 10^{-7}$ mol/L for G-BSA and M-BSA, respectively, with receptor numbers being $1.21 \times 10^{6}$ for G-BSA and a total of $1.36 \times 10^{6}$ per cell for M-BSA.

M-BSA actually has two binding components, but total receptor number is comparable with the receptor number for M-BSA. The in vitro system can be simplified further by the use of cloned progenitor cells binding to cloned stromal cells. The adherent stromal cell clone, D2X, is grown to near confluence. The progenitor cell FDCP-1, a bipotential clone analogous to CFU-GM, is then used for adherence to the stroma. Binding may be quantitated by labeling the progenitor cells with $^{35}$Cr and measuring the amount of radioactivity firmly bound to the dish after a 2-hour period of contact between stem cells and stromal cells in the presence or absence of neoglycoproteins. Here again, the system reproduces the results of previous observations with consistency: Synthetic neoglycoproteins of galactosyl and mannosyl specificities, but not those of other specificities, inhibit the binding of cloned progenitor cells to cloned stroma.

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M-BSA actually has two binding components, but total receptor number is comparable with the receptor number for G-BSA. The double component is not only apparent but real, since it can be confirmed by Hill analysis. However, the fact that receptor numbers are comparable for both ligands strongly suggests that the origin of double component is in heterogeneity of the synthetic ligand rather than the receptor. It must be emphasized that in these studies the synthetic ligands (G-BSA and M-BSA) do not really represent their natural counterparts. Natural ligands are glycan chains in which carbohydrate residues are heterogeneous, and they are linked by glycosidic linkage of 1 → 4 or 1 → 6 in a chain form. This is diagrammatically shown in Fig 2. Synthetic ligands used in these studies contain a core protein to which a single species of hexose is covalently attached, not by a glycosidic linkage, and certainly not in a chain form. The number of carbohydrate residues per protein and their overall three-dimensional configuration may vary, leading to heterogeneity of the molecules. This type of ligand is not expected to present exactly the same kinetics as the natural ligand; only its specificity is reproduced. This difference may also explain the relatively low affinity of ligand for the lectin: most ligands (eg, complement system or antigen-antibody system) have affinities that are higher by several orders of magnitude.

Comparable data are also obtained for FDCP-1. In both B6SUT and FDCP-1 cell lines, the binding of $^{125}$I-ligand is inhibited with excess cold ligand. This inhibition can be obtained with both homologous (eg, $^{125}$L-G-BSA with excess cold G-BSA) and heterologous ligand (eg, $^{125}$I-G-BSA with excess cold M-BSA). Similarly, the displacement of bound radiolabeled ligand can also be obtained with excess homologous or heterologous ligands.

$^{125}$I-labeling and binding assays also indicate that stromal cells lack this lectin. The absence of membrane lectin on stromal cells can be confirmed not only in the adherent stroma of long-term marrow culture, which may be heterogeneous in nature, but also in cloned stromal cell line D2X.

Consequently, one may assign the membrane lectin (homing protein) to progenitor cells with stromal cells providing the corresponding carbohydrate moieties (glycoconjugate) for the binding. The nature of this glycoconjugate is not yet known. Its structural analyses require purification by affinity column techniques, using the lectin as the affinity vehicle. However, there is evidence that its presence is necessary for the binding of progenitor cells to the stroma. This is evident.
from the observation that the sequential treatment of cloned stromal cell membrane with galactosidase and mannosidase abolishes the binding (homing) of cloned progenitor cells, although it has not been shown that the restitution of these carbohydrate moieties restores homing.

It is interesting that the presence of homing protein appears to be developmentally regulated. It is present on carbohydrate moieties restores homing.

Splenic circulation may provide another homing mechanism. It is also of interest that this molecular recognition mechanism appears to be limited to the bone marrow. Homing of stem cells to the spleen is not mediated by this molecular mechanism. Splenic stromal cells may lack the appropriate configuration of glycoconjugate necessary for binding. Alternatively, the open system of splenic circulation may provide another homing mechanism not available in the bone marrow. This topic deserves further exploration.

**POTENTIAL STRUCTURE OF RECOGNIZED CARBOHYDRATE**

Although it is not known if stromal glycoconjugate is a glycolipid or a glycoprotein, one can make the reasonable assumption that a glycoprotein is involved. At any rate, the glycan chain is likely to be independent of its carrier (either membrane protein or membrane lipid). Here, some familiarity with this glycan structure usually seen on the cell membrane may be necessary (Fig 2). The glycan chain begins with two N-acetylgalactosamine (NAG) of which the second one is connected to a mannosyl residue that branches to link to two other mannosyl residues. Each of these latter residues is linked to a NAG residue and, subsequently, to a galactosyl residue that may be exposed or covered by a sialyl residue. The homing protein recognizes and binds to galactosyl and mannosyl residues. The only galactosyl residue available is in the penultimate position. The only mannosyl residues that may be the candidates are at the branching site, either before or after a NAG residue.

Although the structure of glycoconjugate has not been studied and must await its purification, available data from inhibition studies permit certain extrapolations regarding the structure of the glycan that is being recognized.

1. Several pieces of evidence indicate that the presence of both residues is necessary for binding. (a) The inhibition of each residue equally abolishes the binding. (b) The inhibition by either residue is not additive or synergistic: combining neoglycoproteins of two specificities neither enhances nor diminishes the inhibitory effect. (c) The binding of 125I-labeled probes can both be inhibited and displaced by homologous as well as heterologous ligands. The inhibition or displacement by heterologous ligand is a clear indication that both residues must be available for adherence to occur.

2. Moreover, enzymatic treatment of stromal cell surface in vitro indicates that the removal of sialyl residue is necessary for the binding. Therefore, the galactosyl residue can bind only when it is exposed, while this is not a requisite for mannosyl residue, which cannot be exposed at the branching site. Galactosyl residue, therefore, behaves here very similarly to asialoglycoproteins binding to hepatocyte receptors as described by Ashwell and Morell and others.

3. The binding is calcium-dependent.

**PURIFICATION OF HOMING PROTEIN**

Availability of cloned progenitor cells has now permitted purification of the homing protein. Although the technique is cumbersome and the yield is low, a high degree of purification can be attained. Cell membrane fraction is obtained from B6SUT or FDCP-1 cells and solubilized, and all membrane proteins are labeled with 125I. An affinity column is constructed with either galactosyl or mannosyl groups covalently bound to CNBr-activated Sepharose 4B. Membrane proteins are eluted first with buffer only. Subsequent competitive elution with buffer containing either galactose or mannose (but not other sugars) leads to the recovery of a sharp peak of radioactivity. The sharpness of the peak indicates that only a single molecular species is involved. Again, elution of this peak could be obtained not only with the homologous, but also with the heterologous sugar (eg, galactosyl-containing buffer on mannosyl column), suggesting that the glycoconjugate ligand also involves a single molecular species and contains both galactosyl and mannosyl grouping. It is the combined configuration of the two sugars in the molecule that is being recognized by the lectin. This information may be helpful in purifying the glycoconjugate.

When the peak obtained by affinity chromatography is subjected to polyacrylamide gel electrophoresis under nonreducing conditions, followed by autoradiography, a major band with molecular weight (mol wt) of 110,000 and two lesser bands with mol wt of 87,000 and 23,000 are seen. Only the latter two bands are seen under reducing conditions. Experiments with endoglycosidase F treatment indicate 5% carbohydrate content that is N-linked. Thus, it can be concluded that the hematopoietic homing protein is a glycoprotein heterodimer, disulfide-bonded, with two chains of 87 Kd and 23 Kd for a total mass of 110 Kd (Fig 3).

This finding is quite consistent with the wealth of recent information on membrane lectins with biologic recognition functions. A large number of these lectins are calcium-dependent in their interaction with carbohydrates (as is the hematopoietic homing protein). These are known as C-lectins (reviewed in references 90 and 91). They are glycosylated components of the cell membrane, disulfide-bonded, and their recognition function depends on this bonding. In these characteristics, homing protein is in the mainstream of...
C-lectins. Similarities also exist between this homing protein in hematopoietic progenitors and the homing receptor in lymphocytes. Although the carbohydrate specificity is somewhat different, it would be interesting if the homing protein proved to be related to LEC-CAM or the selectin family of C-type lectins that have been shown to be involved in lymphocyte- and neutrophil-endothelial cell interactions.

ANATOMICAL CONSIDERATIONS

The studies thus far reviewed relate to the molecular basis of binding of progenitor cells to the hematopoietic stroma. Anatomical considerations of bone marrow structure indicate that homing of progenitor cells to the marrow may be somewhat more complex: bone marrow is a highly compartmentalized organ, with its two major compartments being vascular and extravascular ones. At least in mammalian systems, hematopoiesis occurs entirely in the extravascular compartment. Cellular exchange between the two compartments occurs at the level of specialized vessels known as sinusoids. The wall of these vessels forms a barrier between the two compartments (bone marrow-blood barrier) and consists of several components, all with well-defined functions. Its most consistent component is a layer of endothelium that controls the nature and magnitude of cellular and molecular exchange between vascular and extravascular compartments. Such substances as iron-transferrin complex do not enter the marrow at the interendothelial junction. They are taken up by endothelium on the luminal side through a receptor-mediated mechanism, traverse the endothelial cytoplasm via a vesicular transport system, and are selectively extracted and bound to lineage-specific stromal cells that support their proliferation and maturation. The studies thus far reviewed only pertain to this second step in homing. Less is known of the first step, the recognition and interaction of progenitor cells with endothelium. Available evidence suggests that here, too, a membrane lectin-glycoconjugate interaction may be involved. However, here the process is reversed: luminal surface of endothelium provides the lectin and progenitor, the carbohydrate moiety. Also, carbohydrate specificity of recognition may be different and be limited to the galactosyl moiety. Evidence for this postulate is derived from the observation that after intravenous infusion of galactosyl-containing neoglycoproteins, the highest uptake per gram of tissue occurs in the bone marrow, not, as expected, in the liver, whose hepatocytes are known to possess galactosyl receptors. Similarly desialated glycoproteins, of which the penultimate galactosyl residues are exposed, are rapidly removed by bone marrow. Since bone marrow circulation is closed (ie, endothelium forms a barrier between the circulatory space and the hematopoietic compartment), it is reasonable to assume that the uptake occurs in the endothelium. In fact, electron microscopic observations, in which labeled neoglycoproteins are perfused in the regional circulation of bone marrow, have confirmed this assumption. Endothelium takes up galactosyl probes, but not mannosyl or fucosyl probes. In contrast to the homing protein that mediates the binding of progenitor cells to stromal cells and is not internalizable, the binding of galactosyl probe to endothelium is followed by internalization and subsequent externalization on the abluminal side of endothelium. Of course, this is expected, because the endothelium must mediate the transport from circulation into the hematopoietic compartment.

Certainly a more systematic approach to endothelial recognition and transport of progenitor cells is needed. To provide some coherence into the fragmentary evidence reviewed here, the following conclusions may be helpful. (1) Endothelial recognition of progenitor cells appears to be mediated by a lectin system with galactosyl specificity. (2) It is in the reverse of stromal-progenitor cell recognition, with endothelium providing the lectin and progenitor, the carbohydrate moiety. (3) The adhesion is followed by internalization and then externalization on the abluminal side (transport).

THE ROLE OF EXTRACELLULAR MATRIX

Proteoglycans (PG) are another group of matrix substances that have been implicated in the regulation of hematopoiesis and, in particular, the homing phenomenon. In this role, proteoglycans may provide, if not high specificity (as is the case with the homing protein), at least strengthening of the bond between progenitor cells and stroma.

Structurally, PG consist of a core protein to which a repeating sequence of usually sulfated glycan structures is attached. The core protein has several well-defined domains. The glycan group is different in classes of PG. Proteoglycans are synthesized by cells of hematopoietic tissues, mostly by stromal cells, transferred to the cell membrane where they may remain for a variable period and then are enzymatically cleaved and released into the extracellular space. They have recently received considerable attention, because emerging evidence indicates that some of them are involved in regulation of growth and differentiation, particularly in hematopoietic system. For instance, it has been shown that one class of PG, heparan sulfate, can selectively extract and bind hematopoietic growth factors, presumably presenting them to progenitor cells. This latter class is preferentially associated with the cell membrane, rather than with the extracellular space. Another fairly well-defined protein called hemonectin has been shown to be involved in the lineage-specific binding of granulocytic precursors during both postnatal and fetal development. Erythroid maturation has been shown to be associated with a developmentally regulated change in fibronectin receptors, an observation strongly suggesting that the fibronectin component of the matrix may regulate the maturation of erythroid cells.

This class of membrane-associated PG has received relatively little attention, if only because the bulk of PG are in the...
extracellular space and only a small proportion is membrane-associated. However, in the course of studying PG produced by hematopoietic progenitors, it was found that these cells synthesize a considerable amount of chondroitin sulfate (CS) that is first associated with the membrane, but subsequently is released into the extracellular space.\textsuperscript{117} When these progenitor cells are layered on top of stromal cells so as to bind (home) to them, membrane-associated CS is stabilized and the life span of these molecules on the membrane is prolonged.\textsuperscript{117} Recent work in our laboratory has indicated that membrane-associated CS can also mediate the binding of progenitor cells to stromal cells. At the molecular level, this binding appears to occur via the interaction between membrane-associated CS on progenitor cells and membrane-associated fibronectin (FN) on stromal cells. It is this interaction that subsequently stabilizes membrane-associated CS. The presence of FN on the stromal cell membrane has been well documented.\textsuperscript{114-116,140-141,146-147} The possibility that the membrane-associated CS may be involved in the binding of progenitor cells is derived from studies where enzymatic removal of CS abolishes the binding. Binding of CS to FN may occur via the cell binding domain of fibronectin, which has as its essential structural feature a repeating sequence motif RDG (Arg-Gly-Asp). Evidence for the involvement of this particular domain is concluded from experiments in which inhibition of binding can be obtained by the synthetic pentapeptide GRGDS (Gly-Arg-Gly-Asp-Ser). This pentapeptide competitively inhibits the binding of progenitor cells to the tripeptide sequence motif RDG. Alternatively, the binding of CS to FN may occur via the glycosaminoglycan domain of FN.\textsuperscript{112}

Figure 4 shows how the two systems of homing protein and CS-FN can be involved in the binding of progenitor cells to stromal cells, the first system providing recognition specificity and the second one strengthening the bond.

**LYMPHOCYTE HOMING RECEPTORS**

In recent years considerable interest has centered on the molecular basis that regulates the traffic of lymphocytes, and may provide certain parallelism with hematopoietic progenitor cell homing. Owing to the development of several monoclonal antibodies, a number of molecules involved in homing have been identified on the surface of both murine and human lymphocytes, generally known as lymphocyte homing receptors. Because purified lymphocyte preparations can easily be obtained, these homing receptors have been better characterized with respect to their distribution and function than their counterparts in hematopoietic progenitors. The most intense area of research is in the initial interaction between the lymphocyte homing receptor and its ligand, which resides on post-capillary venules bearing specialized high-walled endothelium (HEV). Several recent reviews have treated this subject,\textsuperscript{95,96,148,150} and, consequently, this area will be alluded to only briefly here.

The area of lymphocyte homing evolved from the finding of Marchesi and Gowans\textsuperscript{14} that lymphocyte passage in post-capillary venules of lymph nodes occurs through the HEV rather than between endothelial cells. This finding, which was debated for many years,\textsuperscript{111-113,115-117} subsequently led to exploration of the mechanism whereby lymphocytes bind to endothelium.

Very early in these studies, using a homing assay developed by Stamper and Woodruff,\textsuperscript{132} it was found that lymphocytes display organ specificity for endothelium.\textsuperscript{153-157} For instance, lymphocytes bearing a peripheral lymph node (PLN)-specific receptor will not bind to the endothelium of mucosal lymphoid tissue (MLN). Conversely, lymphocytes with an MLN-specific homing receptor will not recognize and bind PLN. It was this specificity that allowed the generation of several monoclonal antibodies. Table 1 summa-
A human lymphocyte surface antigen, designated as gp90 Hermes, mediates the interaction between lymphocytes and mucosal HEV. Expression of this antigen is not restricted to lymphocytes, suggesting a broader function than lymphocyte adhesion to HEV. This molecule has also been termed “H-CAM” (homing-associated cell-adhesion molecule) and is identical to CD44, ECMR III, P80, and Pgp-1. Recently, the cloning of this receptor has been reported by several groups. Its cDNA sequence is distinct from the antigen recognized by MEL-14 and predicts that a portion of this protein bears homology to the link protein of the cartilage and a related segment of proteoglycan core protein, both parts of a complex of proteins in the cartilage. Functional implication of this structural homology is not known.

Other lymphocyte surface molecules that participate in lymphocyte adhesion to endothelium are the integrins LFA-1 (lymphocyte function antigen) and LPAM-1 (lymphocyte Peyer’s patch HEV adhesion molecule). LPAM-1 binds to the HEV of Peyer’s patches while LFA-1 is less restricted in specificity for HEV. These receptors on lymphocytes have their ligand counterpart on HEV to which they bind. These ligands are known as vascular addressins. However, this area is still in its infancy, and a critical evaluation is difficult.

**CONCLUSION**

Homing is likely to be a complex phenomenon involving multiple interactions at the molecular level. Membrane-associated molecules, as well as extracellular matrix, may be involved. Each of these molecules may act additively or synergistically (positive or negative) with others, providing a complex regulatory system. This complexity is more evident in lymphocyte homing compared with the hematopoietic system. However, from the studies reviewed here we can conclude that the 110 Kd homing protein molecule is responsible for selective recognition of progenitor cells by stroma. Interaction of CS and FN is too commonplace to provide for selectivity of the recognition. Nonetheless, this interaction can serve to strengthen and stabilize the binding that has occurred via the homing protein.

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