MINIREVIEW

Adhesive Interactions in the Hematopoietic System: Regulation by Cytokines (44021)

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Five years ago, in collaboration with the late Medhi Tavassoli (1) we wrote a minireview on the adhesive interactions taking place in bone marrow and their functional significance (2). In that review the emphasis was placed on the homing mechanisms of progenitor cells to the marrow stroma.

Although, at that time information was available on other adhesive processes occurring at the progenitor-stroma interphase, the data were mainly descriptive. Recent research has not only expanded the knowledge about the adhesive mechanisms operating in bone marrow, but has resulted in a wider understanding of their role in steady-state and diseased hematopoiesis. Moreover, new data have illustrated the way in which the expression and/or function of several cytoadhesive molecules or their respective ligands is modulated by cytokines. The latter will be briefly reviewed in this article.

Adhesion Molecules in Progenitor and Stromal Cells

Hematopoiesis is a complex process in which stem cells can either self-replicate and/or differentiate into committed progenitors and mature cells. Myelopoiesis and B lymphopoiesis occur in close proximity with cellular elements or with extracellular matrix (ECM) molecules in the bone marrow microenvironment. Within this cytoarchitecture, concerted interactions in time and space among progenitor cells, cytokines and stromal cells or ECM molecules result in ordered, steady-state hematopoiesis (3, 4). *In vitro* studies have shown that stromal cells are tightly entwined with hematopoietic cells, forming aggregates within the marrow. Based on these observations, a model has been proposed for stromal-hematopoietic cell interactions, which predicts that changes in the makeup of these aggregates could be revealing in terms of either stem or stromal cell responses to hematopoietic regulatory signals (5).

Most stromal-hematopoietic cell interactions are mediated by adhesive receptors/ligands, which are located both at the side of the progenitor and stromal cells as well as in the surrounding ECM (6). Signals thus generated play a significant role in the homing, self-replication, differentiation, maturation, and migration of progenitors within the marrow microenvironment and finally in their egress as mature cells into the circulation. The characteristics of adhesive receptors ligands expressed in hematopoietic and stromal cells, as well as those manifested in the associated extracellular matrix, have been extensively reviewed by several authors. For detailed and comprehensive information on these adhesive molecules, the reader is referred to a series of excellent reviews on these topics (4, 7–11).

Apart from these adhesive molecules, we would like to summarize data that have recently emerged demonstrating or suggesting that a novel set of adhesive molecules are present in the hematopoietic system or that membrane-associated molecules which were not considered as having an adhesive function may serve as adhesive receptors or ligands.

Cell Adhesion Molecules (CAM Types). By means of a novel cell-blotting technique, a set of at least nine marrow stromal proteins with properties of

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This work was supported by Grant 1950-238 (FONDECYT, Chile) and P3006-9433 (DTI, University of Chile).

0037-9727/96/2124-0313$10.50/0

cell adhesion molecules (CAMs), has been recently described (12). In this work, the standard procedure to extract and identify cell adhesion molecules has been modified by using lithium dodecyl sulfate-PAGE followed by blotting onto polyvinylidene difluoride membranes. The molecules thus obtained, which are not related to CAMs already described in stromal cells (CD44, CD54, and CD106) interact with differentiation-restricted counterreceptors (not yet defined) on hematopoietic progenitor cells. Another novel adhesive molecule which mediates the heterotypic adherence between lymphoblastic and stromal cells has been recently described (13). The adhesive interaction between these cells is a biphasic process, which involves an early phase of attachment mediated by late antigen-4 (VLA-4) expressed on lymphoblastic cells and vascular cell adhesion molecule–1 (VCAM-1) exposed on stromal cells. Initial attachment is followed by a late adhesive phase, not yet characterized, which seems to involve novel adhesive partners. However, data indicate that the late heterotypic adhesion between lymphoblastic and marrow stromal cells is mediated neither by certain members of the immunoglobulin, integrin, or selectin families nor by CD44. It is assumed that the early phase has a role in the homing of lymphoblastic precursors to the marrow, whereas the late phase may contribute to a firm adhesion or a stabilization process for retention of cells within the marrow space. A similar concept for the participation of a vast array of cytoadhesive molecules in progenitor-stroma interactions involving molecules that permit both homing and retention has already been proposed (2, 6, 14).

A surface glycoprotein, a product of the MIC-2 gene, which is involved in antigen-independent adhesion pathways in T cell subsets (15), has recently been described in hematopoietic progenitor cells (16). The expression of this protein is high in $CD34^+$ progenitor cells and decreases progressively as cells differentiate along the granulocytic maturation pathway. Although its adhesion role in hemopoiesis has not been established, it is speculated that the MIC-2 product may be a potential candidate for an adhesion molecule and may function in cell-cell or cell-matrix signaling.

Stem Cell Factor and the c-*kit* Receptor. Stem cell factor (SCF), a multipotent growth factor exists in either a membrane-bound or a soluble, secreted form (17). Upon binding of SCF to its receptor, the c-*kit* (CD117) protooncogene (18), a cascade of signal transduction occurs which leads to a proliferative response (19). In bone marrow, while hematopoietic progenitor cells express the c-*kit* receptor in a differentiation-dependent fashion (20), stromal cells such as fibroblasts (21, 22) and endothelial cells (23) express the bound form of SCF. Data from several authors suggest that the binding of the membrane-bound form of SCF to its receptor may represent an additional adhesive event in hematopoiesis (21, 24, 25). Moreover, the adhesive interaction between c-*kit* receptor (in megakaryocytes) and bound SCF (in marrow fibroblasts) seems to be a prerequisite for the expression of the proliferative effect of SCF (21). It remains to be established whether, in other cells expressing the c-*kit* receptor or co-expressing both the c-*kit* receptor and the bound form of SCF, adhesive interactions or autocrine loops, *via* c-*kit*/SCF, are required for proliferation (20, 26, 27). The involvement of c-*kit* receptor and its ligand in the regulation of adhesive interactions in hematopoiesis is further suggested by the observation that cytokines like interleukin-3 (IL-3), granulocyte-macrophage colony–stimulating factor (GM-CSF), interleukin-4 (IL-4), transforming growth factor–β (TGF-β) and tumor necrosis factor–α (TNF-α), down-regulate the expression of c-*kit* receptor in several SCF-responsive progenitor cells (28–30).

CD34 Antigen. Recent studies have assigned to the CD34 antigen an adhesive function for interactions between progenitors and various types of stromal cells. The CD34 antigen is a monomeric glycoprotein with an apparent Mr of 110 kDa, containing heavily glycosylated regions, either N- and/or O-linked glycan types at a proportion that is an attribute of the cell type expressing the antigen (31). CD34 is strongly expressed in the most primitive hematopoietic progenitor cells and is progressively lost as these differentiate (31). Other cells, like human marrow fibroblasts, endothelial, and certain neoplastic cells, also express CD34 (32–34). In turn, ligands for CD34 have been suggested to be present in stromal cells (35, 36).

Several lines of evidence suggest that CD34 may be involved in adhesive interactions between progenitor cells and stroma. Direct evidence for such a role has originated from studies in which CD34 was transfected into CD34-nonexpressing murine T cells. Compared with the nontransfected, CD34-expressing cells exhibited stronger cytoadherence to human marrow stromal cells (37). Additional evidence for the involvement of CD34 in adhesive events has resulted from studies showing that, depending on the differentiation stage of the progenitor cells, the occupancy of the CD34 by monoclonal antibodies (MoAb) against certain epitopes of CD34, results in signal transduction and in the formation of cell aggregates, via β_2 integrin–associated cytoadhesive pathways (38). Since most immature $CD34^+$ progenitors lack β_2 expression (39), occupancy of CD34 by its putative ligand in stroma may result in β_2 integrin expression. This in turn allows the formation of heterotypic cell clusters by interaction with β_2 integrin–counterreceptor–expressing cells in the bone marrow microenvironment. Furthermore, it has been demonstrated that CD34 in endothelial cells, after selective glycosylation, may act as an

adhesive ligand for the lectin domain of L-selectin expressed in lymphocytes and thus modulates leukocyte trafficking (36).

These findings, together with a finely tuned control of CD34 expression (40, 41), support the speculation that CD34 may have an important role in defining whether progenitor cells remain in a proper stromal environment for hematopoiesis to occur.

CD44 and Hyaluronan Binding. CD44 is a transmembrane molecule (42) which mediates the binding of several cells to hyaluronan, a major glycosaminoglycan component of the extracellular matrix. Considering the abundance of hyaluronan in human bone marrow, it has been suggested that CD44/hyaluronan interaction may mediate adhesive processes between hematopoietic progenitors and marrow stroma (43, 44). This interaction underlies not only cell attachment, but also migration and matrix remodeling (45). Alternative splicing of the CD44 gene, which is developmentally regulated (46), generates isoforms to which distinct functions have been ascribed. It has been demonstrated that the main CD44 isoform expressed on resting hemopoietic cells is the CD44H variant which fully retains the property to bind hyaluronan (47). The binding of CD44 to hyaluronan is a highly regulated process; however the nature of this regulation is a matter of controversy. Data have shown that this is either mediated by a phosphorylation-independent (45) or by a protein kinase (PK) C-or A-dependent process'(44). In the latter case, phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) was used to investigate the involvement of PKC phosphorylation in CD44 regulation. Under these conditions, hyaluronan binding was enhanced, but CD44 reactivity against anti-CD44 MoAb was partially decreased, suggesting that another protein, distinct from CD44, was activated by the PKC-dependent process. Whether the activated protein is intercellular adhesion molecule–1 (ICAM-1), which also binds hyaluronan in a cytokine-dependent fashion (48), has not been established. In this context, the observations that certain isoforms of CD44 can bind growth factors (49) and that interleukin-5 (IL-5) modulates CD44 expression (50) highlight a new and potentially important role for CD44 in the control of cell-matrix interactions.

Anti-Adhesive Mechanisms. The identification of novel extracellular molecules involved in repulsive or inhibitory interactions, as a result of studies by neurobiologists, has opened an exciting area of research for improved understanding of the regulation of cell adhesion and/or migration. Several extracellular matrix proteins, like tenascin, thrombospondin, laminin, mucin, and proteoglycans, have been postulated to display anti-adhesive properties (51). Although various of these molecules are produced by progenitor and stromal cells (7, 32, 52, 53), it is not known, so far, whether, besides their well-established role in hemopoiesis, they also display anti-adhesive properties. This seems to be the case for perlecan, a component of the heparan sulfate proteoglycan family isolated from bone marrow stroma. It has been shown that hematopoietic cells are repelled from attaching to perlecan-coated dishes, the anti-adhesive moiety being located in the core protein of the proteoglycan (54). Further evidences for the anti-adhesive effect of perlecan are given by the observation that collagen VI, a marrow stroma component (55) with strong cytoadhesive properties, while combined with perlecan loses its capacity to bind progenitor cells (56). The mechanisms involved in the expression of the anti-adhesive properties of extracellular matrix molecules has not yet been elucidated, but preliminary evidence suggests that this may be related to steric hindrance at the cell surface rather than to a transmembrane signal transduction event (51). In the case of the hematopoietic system, such an effect may regulate the proper compartmentalization, and therefore the proliferation, of precursors within the marrow microenvironment.

The existence of anti-adhesive molecules invites reconsideration of the current concepts thought to be important in cell-cell and cell-matrix interactions. Thus, cytoadhesive processes and their physiological significance should be considered as the algebraic sum of the intervening adhesive and anti-adhesive components, rather than the consequences of adhesion or nonadhesion processes *per se*, as is currently accepted. Perhaps the availability of disintegrins, a novel family of integrin inhibitory proteins (57), as well as new monoclonal antibodies to dissect the anti-adhesive regions of adhesive molecules, may help to elucidate the functional equilibrium between adhesive and anti-adhesive processes.

Modulation by Cytokines of the Expression and Function of Adhesive Receptors and Ligands in Progenitor and Stromal Cells

Cytokines display a multiplicity of activities in hematopoietic cells both at the level of early multilineage and in late committed or mature progenitors. Although individual factors may show preferential activity in one compartment, they typically manifest a spectrum of effects crossing the boundaries between early and late action and between specific lineages (58). Optimal proliferation of early, noncommitted precursors requires multiple cytokines and in addition the full expression of their avidity to tightly adhere to the marrow stroma. The adhesiveness of early progenitors contrasts with that of the more mature progeny which tend to be either less adherent or nonadherent. Therefore, upon binding to stroma early progenitors become more prone to receive the regulatory signals associated with the microenvironmental niche (59, 60). Thus,

it seems that specific growth factors and adhesion molecules are intimately involved (61) in directing early events and/or in providing permissive conditions for the proliferation and differentiation of progenitor cells (62). However, recent findings, which will now be briefly discussed, have demonstrated that cytokines modulate the expression or function of cytoadhesive molecules both in progenitor and in stromal cells. This adds another set of primary actions to those elicited by cytokines in hematopoiesis (63).

Effect of Cytokines on the Expression/Function of Cytoadhesive Molecules in Progenitor Cells. *Effects of G-CSF and GM-CSF.* It has been observed that after administration of G-CSF or GM-CSF to patients or normal donors, large numbers of hemopoietic progenitors are released from their reservoirs into the peripheral blood, which can be collected by leukapheresis. The mobilized product includes $CD34^+/CD33^-$ cells, an immunophenotype marking early progenitor cells (64, 65). These studies have prompted conjectures about the mechanisms involved in the growth factor–mediated release of marrow progenitor cells. It has been speculated that, after exposure to G-CSF or GM-CSF, adhesion molecules are shed from the surface of marrow resident primitive multilineage cells, therefore allowing them to enter the circulation (66).

An insight into the adhesive molecules that can be affected by the treatment with growth factors was obtained by experiments in which primates were injected with saturating amounts of anti β_1 or β_2 integrin antibodies. Under these conditions, anti-β_1, but not anti-β_2 antibodies, induced the mobilization of all classes of hematopoietic progenitors cells. Furthermore, antibody treatment after a course of G-CSF additively increased circulating progenitors (67). These data suggest that β_1 integrins which are expressed in progenitor cells (6, 14, 68, 69, 70) are involved in the trafficking of progenitor cells. Leavesley *et al.* (71) provide evidence that steady-state and G-CSF-mobilized progenitor cells manifest different levels of expression of adhesive molecules. While cells from both sources express similar levels of the platelet/endothelial cell adhesion molecule–1 (PECAM-1), those of VLA-4 and lymphocyte function–associated antigen–1 (LFA-1) were lower in mobilized than in steady-state progenitor cells. In spite of this, the adhesiveness of $CD34^+$ cells from both sources to VCAM-1–transfected CHO cells was similar. This observation agrees with the concept that changes in the expression of integrins on the cell surface does not always correlate with their adhesive function, which may shift from a low- to a high-affinity state (72). The observation that VLA-4 function in cells from both sources was modulated by the co-expressed adhesion receptor PECAM-1 (71) strengthens the notion that interplay between several adhesive receptors (73) regulates the activation of adhesive molecules. Thus, it seems that after G-CSF administration, progenitor cells express not only lower levels of certain adhesive molecules but a modified adhesive phenotype which may affect their location within the bone marrow.

Effect of SCF. The effectiveness of SCF to mobilize progenitor cells from the marrow (74), has prompted studies to investigate the associated mechanisms and to establish whether adhesion molecules are involved. Evidence indicates that SCF may affect stem cell redistribution (75) in a process that seems to involve β_1 integrins (76). In the latter study, using a SCF-responsive myeloid cell line (MO7E), it was observed that SCF modulates adhesive function in a dose- and time-dependent manner, *via* integrins $\alpha_4\beta_1$ and $\alpha_5\beta_1$. Thus, MO7E cells showed increased adhesiveness to endothelial cells or VCAM-1 transfected cells shortly (30 min) after SCF treatment. This effect, however, was transient, and after longer exposures (up to 24 hr) to SCF a time-dependent decrease in integrin-mediated adherence occurred. This bimodal adhesive effect of SCF was not paralleled by changes in the density of integrin receptors on the cell surface as determined by flow cytometry. Thus, it seems that upon binding of SCF to its receptor, which implies receptor internalization (25), the function of a subpopulation of β_1 integrin receptors is modulated by uncoupling or disengagement of its adhesive activity. The latter could be mediated by the tyrosine kinase activity of the stem cell factor receptor (24). It has to be established whether these *in vitro* results may explain the *in vivo* SCF-induced mobilization of progenitor cells (77). If this proves to be the case, the SCF-induced transient increase in adhesion may result in progenitor cell redistribution, which, followed by the period of decreased adhesion, permits progenitor migration and their final release into the circulation. Bimodal adhesive effects produced by cytokines *in vivo* are not without precedent. It has been documented that the transient leukopenia observed after GM-CSF administration is due in part to a rapid rise in neutrophil adhesion-promoting glycoproteins, which cause neutrophil margination in the pulmonary vasculature. The recovery in circulating neutrophil and monocyte numbers coincided with the release of these cells from the lungs, mediated by adhesive processes not well identified (78, 79).

Effect of α-Interferon. α-Interferon (IFN-α), which frequently normalizes peripheral blood counts in chronic myelogenous leukemic (CML) patients, seems to be involved in the regulation of the adhesive properties of hemopoietic progenitor cells. In CML, marrow progenitors, unlike normal progenitors, fail to adhere *in vitro* to normal stromal layers, fibronectin, or its proteolytic fragments. This defect probably re-

sults in the increased numbers of circulating progenitors seen in CML (80, 81). Incubation of CML-purified marrow progenitors with IFN-α induces a dose-dependent increase in their adhesion to normal stroma. This effect, which is partly mediated by β_1 integrins, does not depend on the cell density of $\alpha_4\beta_1$ or $\alpha_5\beta_1$ integrins. Rather, it depends on the restoration of β_1 integrin function (10, 82), probably mediated by early signals after IFN-α treatment, like the production of macrophage-inflammatory protein–1α (MIP-α) or diacylglycerol release and/or protein kinase C activation (83, 84). It has to be established whether in CML β_1 integrin presents alterations in its ligand binding site or in certain cytoplasmic domains. This is the case in the alternatively spliced variant (β_{1c}) of integrin β_1, which has already been detected in hematopoietic cell lines (85) and may be important, not only in the transmission of distinct adhesive signals, but also in the control of cell growth (86). In this respect it is worth mentioning that various cytokines (IFN-α, TGF-β, and TNF) acting through their receptor families are involved, like the β_{1c} variant, in the inhibition of cell growth (84, 87).

Effect of IL-3. IL-3, a multilineage growth factor, affects proliferation, maturation, survival, and function of a variety of primitive, committed, and mature hematopoietic cells. Animal studies have shown that short-term *ex vivo* preincubation of marrow cells with IL-3, either decreases (88) or increases the grafting efficiencies of transplanted marrow cells (89, 90). In the latter case it has been postulated that increased grafting may be related to the upmodulation of homing receptors by IL-3 (91). These results suggest that IL-3 may also have a role in the regulation of the adhesive properties of progenitor cells. This concept was strengthened by the observation that IL-3–depleted progenitors, compared with control cells, exhibited a rapid decrement in their attachment to fibronectin. This effect was reverted by the readdition of IL-3, but not GM-CSF, IL-6, or TGF-β (92). Further studies demonstrated that the adhesion of hematopoietic progenitors to fibronectin is modulated by IL-3 through a decrease in the expression of the integrin and not by a change in its affinity for its substrate (70). Moreover, the IL-3–dependent modulation of cytoadhesion was not related to other late IL-3 effects like those involved in cell viability and in growth/survival (92, 93).

In addition to the effect of IL-3 in modulating $\alpha_5\beta_1$ integrin expression, we have recently reported that the synthesis of proteoglycans by progenitor cell lines (94, 95) is also modulated by IL-3. In these studies, proteoglycan synthesis by multipotent but not by bipotent murine progenitor cells, was increased after exposure to IL-3. This does not involve changes in charge density, hydrodynamic size, nature of the glycosaminoglycan chains or membrane stability of the proteoglycan. IL-3–treated cells, compared with untreated cells, exhibited increased adhesiveness to the 40-kDa fibronectin fragment containing the heparin-binding region (96).

Effects of Cytokines on the Expression/Function of Cytoadhesive Molecules in Stromal Cells and the Associated Extracellular Matrix. It has not been clearly established whether growth factors may affect the adhesive properties of marrow stroma. This concept implies that after cytokine stimulation, either endogenous or exogenous, the architecture and function of cellular elements of the bone marrow microenvironment, or the nature of the adjacent extracellular matrix, may be changed, thus affecting the extent or nature of its interaction with progenitor cells.

Effects on the cellular structure of marrow stroma. As already mentioned, current clinical practice to mobilize stem cells involves the use of growth factors, like G-CSF, GM-CSF, SCF, and/or IL-3. Although data favor the concept of "shedding" of adhesive molecules on the side of the progenitor cells, several lines of evidence have shown that cytokines also modify or disrupt the steady-state architecture of marrow stroma (97).

It has been reported that cytokine administration *in vivo* after high-dose chemotherapy results in profound morphological and immunohistochemical changes in marrow stromal cells. Thus, marrow specimens from patients treated with IL-3 or GM-CSF, compared with control marrow, exhibit an increased vascular network with a high proportion of endothelial cells expressing CD34. The concentration of other marrow stromal cells, like macrophages and fibroblasts, is also increased (98). *In vitro* studies using human umbilical vein endothelial cells (HUVEC) have shown that G-CSF or GM-CSF induces in a dose dependent fashion cell proliferation and migration, the latter effect in relation with changes in cell shape and cytoskeletal organization (99). Whether HUVEC and marrow endothelial cells respond to growth factor in the same way is not known, but certainly the availability of methods to obtain marrow endothelial cells (100) will allow the exploration of the mechanisms involved in their regulation by growth factors. Similarly, growth factors affect the proliferation and properties of marrow stromal cells. SCF and IL-6, both produced by the stroma, preferentially induce the differentiation and proliferation, respectively of marrow stromal cells (101). Fibroblast precursors from human marrow when cultured in the presence of GM-CSF increased their rate of proliferation and gave rise to larger colonies (CFU-F) than control cells (102). In the same vein, we have observed that the plating efficiency of human bone marrow fibroblasts is decreased after exposure to G-CSF or GM-CSF, probably by an effect

on cytoskeletal arrangement (Fernández M, unpublished data).

Effects on the expression of cytoadhesive molecules. Several reports have documented the involvement of cytokines in the expression or function of adhesion molecules in marrow stromal cells. Human marrow fibroblastic cells establish heterotypic interactions with progenitor cells by means of the VCAM-1/VLA-4 adhesive system (103). The expression of VCAM-1 in marrow stromal cells is upregulated by IL-4 (104) in a mode that does not involve the cascade of events activated by other cytokines, as it occurs in other cells (105). Moreover, marrow stromal cells preincubated with IL-4 exhibit an enhanced capacity for binding $CD34^+$ progenitor cells (106). Evidence suggests that the effect of IL-4 on VCAM-1 expression, and the concomitant change in stromal cell adhesiveness, may be related to an increase in the half-life of VCAM-1 mRNA (106, 107).

Bone marrow endothelial cells are likely to play an important role in the homing of hematopoietic progenitor cells (108). The recent fruitful results of the isolation and cultivation of marrow endothelial cells (100, 109) will permit studies to map adhesion receptors and their regulation by endogenous or exogenous cytokines. Among this line, a recent study has shown that the expression of several adhesive molecules (ICAM-1, VCAM-1, E-selectin, and PECAM-1) on marrow endothelial cells is regulated by TNF-α (109). The scarce information available on adhesive molecules and their regulation in marrow endothelial cells contrasts with the ample data demonstrating cytokine modulation of adhesive molecules in human umbilical vein endothelial cells (HUVEC) (33, 110–114). However, based on the knowledge that marked phenotypic and functional differences exist between sinusoidal endothelium from different anatomical sites (115), any attempt to extrapolate information from HUVEC to marrow endothelial cells should be done with caution.

As previously discussed, CD34 antigen seems to play a role as an adhesive molecule. In this context, not only progenitor but also stromal cells (32, 33) express the antigen. It has been observed that freshly isolated endothelial cells, which are $CD34^+$, become $CD34^-$ after cultivation, probably as consequence of a proliferation-dependent downregulation process. The expression of CD34 is also downregulated by cytokines, such as interleukin-1β (IL-1β), IFN-γ, and TNF-α, under conditions where these cytokines upregulate adhesion molecules, like ELAM-1 and ICAM-1 (33). Taken together, these data suggest that antigen CD34 in stromal cells may have a role in regulating progenitor-stroma cell adhesion.

Effects on the production of extracellular matrix molecules. Several cytokines affect the capacity of marrow stromal cells to produce matrix molecules. Thus, it has been reported that the deposition in marrow stroma of reticulin and collagen III is selectively increased by IL-3, but not by GM-CSF (98, 116). TGF-β enhances the synthesis of collagen and fibronectin by marrow stromal cells as well (117). In nonhematopoietic cells, TGF-β and EGF induce tenascin-C production (118), which acts as a cytoadhesive (anti-adhesive?) molecule in the bone marrow microenvironment (52).

In endothelial cells, TNF induces a decrease in the rate of synthesis of proteoglycans as well as in the level of mRNA for the core protein of the proteoglycans, perlecan and biglycan (119). Although it is not known whether in marrow endothelial cells TNF produces similar effects, the above-mentioned studies are interesting in view of the anti-adhesive properties of perlecan (54) and in light of the observation that proteoglycans are involved in the capture and presentation of growth factors within the marrow microenvironment (120). Further evidence for the regulation of matrix molecule production by growth factors is provided by the observation that the expression of mRNA for collagen VI as well as the synthesis of collagen VI by marrow stromal cells is regulated by G-CSF (Fernández M, unpublished results). Since the genes for collagen VI are constitutively expressed by marrow stromal cells (55, 56), changes in the level of expression of this protein may alter proper progenitor-stromal interactions by hampering the organization of the extracellular matrix (121).

Finally, steroid hormones are also involved in the regulation of the synthesis of matrix components by stromal cells. Marrow fibroblasts, after exposure to hydrocortisone, increase their steady-state level of stem cell factor mRNA, an effect that involves coordinated increases in transcripts encoding soluble and membrane-bound proteins (22). Furthermore, treatment of marrow stromal cells with methyl prednisolone results in a decreased synthesis of hyaluronic acid and in the production of structurally modified proteoglycans. These changes in turn modify the capacity of the stroma to bind and stimulate the formation of progenitor colonies (122).

Perspectives

Adhesive interactions play a central role as determinants of hematopoietic progenitor cell location within the marrow microenvironment and in their migration, proliferation, survival, and differentiation. An important clue deducted from the overview of data presented here points to the fact that adhesive interactions taking place in the bone marrow are under the regulation of cytokines, among other factors. Thus, the reductionist concept of adhesion molecules as cell surface components facilitating only cell contacts and of cytokines as soluble effectors with a broad range of

biological activities does not seem to be valid anymore (123). Incidentally, adhesion molecules and cytokines are now known to exist also as soluble and bound species, respectively. On the other hand, it has been reported that molecules related to extracellular matrix components regulate cytokine expression (124).

Besides the evident effect of cytokines on the expression and function of adhesive molecules, the data reviewed here suggest the idea that in the bone marrow microenvironment there exists a sort of interdependence with overlap and similarity between the expression of adhesion molecules and cytokines. This notion is strengthened by the "classical" observation of a physical association between cytokines and matrix molecules (i.e., proteoglycans) and by the "new" evidence that cytokine-receptor pairs (i.e., c-*kit* receptor and bound-SCF) can mediate or consolidate adhesion. The above idea is not new, since the information evolving from immune and inflammatory responses has shown that the coordinated expression of cytokines and adhesion molecules is essential for the development of the sequence of molecular events involved (61, 125).

Although considerable amounts of work have been performed to elucidate the nature and regulation of the adhesive processes in the marrow microenvironment, there remain gaps in our understanding of the mechanisms involved in the cytoadhesive properties of progenitor and stromal cells. Briefly, we feel that research should be carried out to clarify, among other issues, whether growth factors are involved in the regulation of the pattern of glycosylation or sulfation of cytoadhesive molecules/ligands (36, 122), the production of soluble adhesive molecules (126, 127), the interplay between the expression and function of adhesive and anti-adhesive molecules (56), the expression of "key" molecules for the organization of extracellular matrix (55, 128), and, finally, the expression and overexpression of regulatory genes and their impact in cell adhesion (129).

It is likely that a better understanding of the nature of cytoadhesive molecules and the modulation of their expression and function by growth factors or other signals will help to define an "adhesive phenotype" for progenitor cells at each stage of their differentiation. Such a concept will not only improve our knowledge of steady-state hematopoiesis but will undoubtedly shed light on our understanding of hematological diseases. The clinical application of this information will certainly focus new research on the development and selection of appropriate prognostic and therapeutic strategies. In this respect, recent data shows that the adhesive phenotype of cells from diseased marrow differs from that of the normal, steady-state marrow (130–133).

In addition, since growth factors are intensively used for the mobilization of progenitor cells from marrow to peripheral blood, more research should be performed to understand whether prolonged exposure to high doses of cytokines also entails the blocking of attachment of newly generated progenitors, redistribution/circulation of progenitors attached to other tissues (75, 134) or the activation of secondary effects, such as endogenous cytokine release (135, 136).

The helpful comments of Oscar Brunser, MD, are gratefully acknowledged.

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