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REVIEW

Cellular and Molecular Regulation of an Erythropoietic Inductive Microenvironment (EIM)

Masuo Obinata* **and Nobuaki Yanai**

Department of Cell Biology, Institute of Aging, Development and Cancer, Tohoku University, Seiryo-machi 4-1, Aoba-ku, Sendai, Japan

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I. Introduction to an erythropoietic inductive microenvironment (EIM)

Two hemogenic sites are present in mouse embryos before the onset of fetal liver hematopoiesis. While the yolk sac provides for immediate erythropoiesis, an intraembryonic region encompassing the dorsal aorta produces definitive hematopoietic stem cells. At early developmental stages this region, called the paraaortic splanchnopleura, produces multipotent progenitors. At the time of fetal liver colonisation, the paraaortic splanchnopleura further evolves into aorta, gonads and mesonephros (AGM) and contains progenitors capable of long term multilineage reconstitution (26). The majority of hematopoietic cells generated in the yolk sac are nucleated erythroid cellscalled primitive erythroid cells which express an embryonic type of hemoglobins. After their switch to fetal liver, the majority of hematopoietic cells are erythroid cells, but they are enucleated cells containing adult type hemoglobins (β-globin) in mice, whereas they contain fetal type (γ-globin) hemoglobin in human. In the adult mouse, bone marrow is the major hematopoietic organ where hematopoietic stem cells and their progenitors develop and myelopoiesis seems to dominate; spleen is another hematopoietic organ where the stem cells are predominantly committed to erythroid development (3).

Self-renewal and commitment of hematopoietic stem cells are believed to be regulated by the hematopoietic inductive microenvironment (HIM) created in these hematopoietic organs and the stem cells may migrate to and localize in appropriate microenvironments during ontogeny. Dexter (4) developed a culture for bone marrow hematopoietic cells on the adherent cells from bone marrow and showed the long-term maintenance of mature blood cells as well as progenitors and, therefore, stem cells in this culture. The adherent cells are called the stromal cells of the bone marrow and contain a mixture of fibroblasts, endothelial cells, and preadipocytes. In this *in vitro* culture, it is shown that the stromal cells may support maintenance of hematopoietic cells by cell-to-cell communication through secreting cytokines, producing extracellular matrices and direct cell-to-cell contact. Thus, the long-term bone marrow culture is the first clear experimental evidence of the HIM.

Similar to HIM, erythropoiesis may be regulated by a microenvironment suitable for proliferation and differentiation of the erythroid cells, and such erythropoietic organs may provide an erythropoietic inductive microenvironment (EIM). This review is focused on cellular and molecular evidence of the EIM based on our observations.

II. In vitro reconstruction of EIM with the stromal cell lines

A. EIM in spleen

In lethally irradiated mice receiving low doses of bone marrow cells intravenously, each type of hematopoiesis normally occurring in the bone marrow (erythroid, neutrophilic granulocyte, eosinophilic granulocyte, and megakaryocyte) may appear in the form of gross or microscopic discrete colonies in the spleen, but the erythroid colonies are predominant in this organ, whereas granulocyte colonies predominate in bone marrow. In addition, erythroid spleen colonies are found throughout the red pulp, whereas granulocyte colonies are found primarily adjacent to the capsule and traculae (white pulp) and, less frequently, in the center of atrophic lymphoid follicles, indicating a different distribution of erythropoiesis versus granulopoiesis within the spleen. A richer supply of HIM for erythropoiesis in spleen and the reverse in bone marrow suggested HIM may be function on a particular lineage of hemato-

* To whom correspondence should be addressed:
Tel: +81–22–717–8483, Fax: +81–22–717–8488
E-mail: mobinata@idac.tohoku.ac.jp

Abbreviations: AGM, aorta, gonads and mesonephros; HIM, hematopoietic inductive microenvironment; EIM, erythropoietic inductive microenvironment; Epo, erythropoietin; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late activation antigen-1; SCF, stem cell factor.

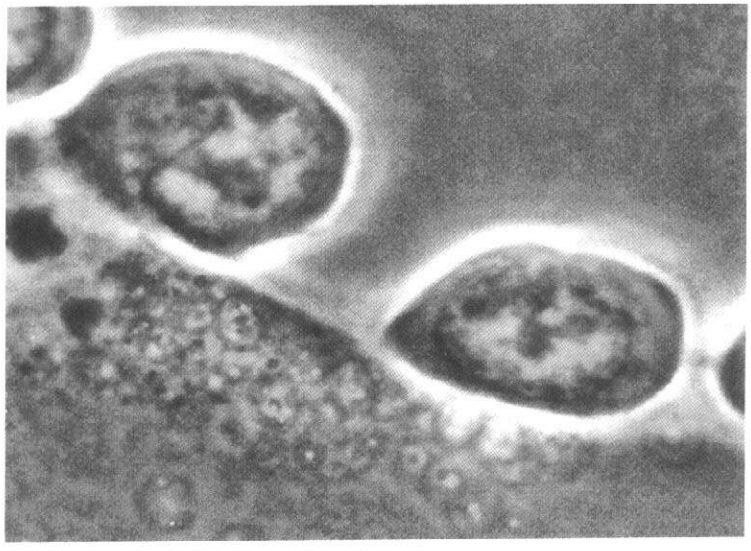

Fig. 1. Erythroid progenitor cells (top) adhere to the stromal cell layers (bottom). Proliferation and differentiation of erythroid progenitor cells are supported by stromal cells by direct cell-to-cell contact in the presence of Epo.

poietic cells and that the spleen may supply adequate HIM for erythropoiesis (3).

We postulated that there was an EIM for the HIM which is adequate for erythropoiesis and tried to find evidence of it at cellular levels. Since circumstantial evidence of the EIM has been found in the mouse spleen, we first established stromal cells of the spleen and examined the supporting ability of these cells for erythroid cells by coculturing the hematopoietic progenitor cells on the stromal cell layers (35, 36).

Most of the established stromal cell lines (MSS cell lines) from newborn spleen seemed to be of endothelial cell origin because they formed microcapillaries in the collagen matrix-culture (36). To create the EIM *in vitro*, the hematopoietic progenitor cells recovered from 13-day-old fetal livers, where the erythroid progenitors are abundant, were cultured on the MSS stromal cells in a semisolid medium containing erythropoietin (Epo). After 4 days of culture, large erythroid colonies of over 1,000 benzidine-positive erythroid cells developed from a single erythroid progenitor cell (35). This first experimental evidence for spleen EIM that stromal cells support erythropoiesis *in vitro* prompted us to examine further the studies made of its molecular and cellular regulatory mechanisms. Tsuchiyama *et al.* (32) recently established a stromal cell line, SPY3-2, from a three-dimensional spleen primary culture in collagen gel matrix. These cells were negative for preadipocytic and endothelial markers, but showed a fibroblastoid morphology. They supported erythropoiesis in the presence of Epo, similar to MSS cells.

B. EIM in fetal liver

Fetal liver is a major erythropoietic organ in the mouse and rapid expansion of the erythropoietic cell population is observed in mouse fetal liver during the 12 to 16 days of gestation. Thus, we established stromal cell lines from fetal livers (FLS lines) of 13-day gestation mouse fetus (19). They are epithelial-like cells in their morphology and supported the proliferation and differentiation of the erythroid progenitor cells from mouse fetal livers and bone marrow in a semisolid medium in the presence of Epo by forming large erythroid colonies. The erythroid progenitor cells responsible for FLS cells are CFU-E (colony forming unit-erythroid) as determined by dose-dependency of Epo and cell fractionation. When in close contact with the layer, the erythroid progenitor cells divided rapidly with an average generation time of 9.6 h for 10 cycles of division and completed maturation into enucleated erythrocytes. Thus, the EIM created by the FLS stromal cells *in vitro* is sufficient to explain the rapid expansion of an erythropoietic cell population in the fetal liver of mice.

Subsequently, Ohneda and Bautch (17) reported that the two established endothelial cell lines allowed for the proliferation and differentiation of erythroid and monocyte-macrophage precursors. Erythropoiesis was dependent on the addition of Epo. Epo- and stroma-dependent erythroid colony formation requires close contact between progenitor cells and the endothelial cells, whereas monocyte-macrophage colonies formation does not, suggesting that different molecular mechanisms are used by endothelial cells to support erythroid development and myeloid development in the

mouse fetal liver.

Tsai *et al.* (30) established a diploid fibroblastoid cell strain, termed "ST-1," from a long-term liquid culture of human fetal liver cells. ST-1 cells are nonphagocytic, negative for nonspecific esterase and factor VIII-related antigen but positive for fibronectin and type I collagen. The ST-1 cells stimulated the development of erythroid bursts, mixed granulocyte-macrophage colonies, pure granulocyte colonies, and pure macrophage colonies, suggesting that the fetal liver stromal cells may support erythropoiesis in human. Li and Congote (14) reported a bovine fetal-liver stromal cell line that supports erythroid colony formation in the presence of Epo. The cells are cytokeratin-negative and vimentin-positive, indicating a mesenchymal origin, and have phagocytic activity and show endothelial-like morphology. They showed that presence of physiological concentrations of insulin-like growth factor II and unidentified factors escreted from the stromal cells enhanced stroma-supported erythropoiesis in fetal liver. These results indicate a contribution by stromal cells for EIM in fetal livers in a variety of animals.

C. EIM in bone marrow

A novel cover slip-transfer culture system to study the functional roles of stromal cells in erythropoiesis was developed by Tsai *et al.* (30). Human bone marrow stromal cell colonies were allowed to develop on small glass cover slips in liquid medium. The cover slips along with human bone marrow stromal cell colonies and progenitors attached to them were cultured in a culture dish by overlaying methylcellulose medium in the presence of Epo. Large erythroid bursts, comprising multiple subcolonies, were developed on the stromal cells. Harigaya and Handa (8) established five clonal human bone marrow stromal cell lines from the adherent cell populations in long-term liquid cultures after transfection with the recombinant plasmid pSV3gpt and they demonstrated that eraly erythroid stem cell (BFUe)-derived colonies and mixed colonies with erythroids were increased in the presence of the conditioned medium from these cell lines and Epo. These results indicated that stromal cell-associated erythropoiesis occurs in bone marrow.

Because bone marrow HIM may be more complex than those of fetal liver and spleen and different types of stromal cells may contribute to HIM, we attempted to immortalize a variety of stromal cell lines by using temperature-sensitive SV40 large T-antigen gene transgenic mouse (12, 21, 22). Thirty-three bone marrow stromal cells including preadipocytes, endothelial cells, and fibroblasts were established and their selective stimulatory abilities to support large colony formation of lineage-specific hematopoietic progenitor cells (erythroid, monocyte/macrophage, granulocyte, and monocyte-granulocyte) were examined. On myeloid progenitors, the stromal cells showed lineage-restricted stimulatory activity and a reciprocal relationship was observed between granulocyte formation and macrophage formation, but these activities were not dependent on the amount of produced colony-stimulating factors (11). Interestingly, a majority of the established stromal cells (27 among 33 clones) showed erythropoietic stimulatory activity in the presence of Epo, although bone marrow HIM was expected to be more appropriate for granulopoiesis than for erythropoiesis. These results indicated that each stromal cell in the bone marrow may provide a preferable microenvironment for a rapid expansion of the lineage-restricted progenitor cells by the induced signals.

D. EIM in other hematopoietic organs

The demonstration of the erythropoietic supporting ability of the stromal cells established from fetal liver, spleen and bone marrow provided clear evidence of EIM in these hematopoietic organs.

Yolk sac is the erythropoietic organ in the embryo. In the wall of the yolk sac, angiogenetic clusters are derived to develop blood vessels by the mesenchyme condensation and the centers of the clustered cells form the blood cells. Although stromal type cells have not been established from the yolk sac, the endothelial cells may function as stromal cells and constitute EIM, because cell-to-cell interaction is expected to be important in the generation of erythropoietic cells in the yolk sac blood island.

AGM was shown to contain hematopoietic stem cells capable of long-term multilineage reconstitution (26), thus HIM may be involved. Stromal cells were established from AGM and their supporting activity for stem cells was reported (18). It is interesting to know whether they have potential for EIM, although erythropoiesis was not observed in AGM.

III. Molecules involved in EIM

Similar to HIM, the EIM in which the stromal cells may support maintenance of hematopoietic cells may require cell-to-cell communication through secreting cytokines, producing extracellular matrices and direct cell-to-cell contact. Formation of the large erythroid colonies requires Epo as a cytokine, but these colonies did not develop when transwell filters were used between the stroma and hematopoietic cells, or when conditioned medium was used in place of stromal cells (17, 35). Thus, direct cell-to-cell communication may be necessary and we reviewed the molecules required for it in EIM.

A. Very late activation antigen-4 (VLA-4) integrin and its ligands

Cell adhesion molecules may be the most likely candidate molecules involved in development of the erythroid progenitor cells in EIM. We reported the role of adhesion molecules in erythropoiesis using blocking antibodies in the stroma-dependent erythropoiesis (38). The development of the erythroid cells on stroma cells was inhibited by anti-very late activation antigen-4 (VLA-4 integrin) antibody, but not by anti-VLA-5 antibody, although the erythroid cells express both VLA-4 and VLA-5. Whereas high levels of expression of vascular cell adhesion molecule-1 (VCAM-1) and fibronectin, ligands for VLA-4, were detected in the stroma cells, the adhesion and development of the erythroid progenitor cells were partly inhibited by the blocking antibody against VCAM-1. VLA-5 and fibronectin could mediate adhesion of the erythroid progenitor cells to the stromal cells, but the adhesion itself may not be sufficient for the stroma-supported erythropoiesis. The stromal cells may support erythroid development by adhesion through a new ligand molecule(s) for VLA-4 in addition to VCAM-1, and such collaborative interaction may provide adequate signaling for the erythroid progenitor cells in the erythropoietic microenvironment.

Papayannopoulou and Brice (23) isolated selected populations of cells from human fetal liver after immunoadherence to anti-β2 integrin (CD18) coated plates to study the expression of integrins at the erythroid progenitor level. β2 Cells which did not adhere to CD18 were mostly later erythroid progenitors, while CD18 adherent cells had a blast-like cell morphology and a higher proliferative potential and diversity. CD18-adherent cells express αL (CD11a) chain, but not αM (CD11b) chain. CD11a is present in all types of progenitors, but is selectively lost at later stages of erythroid differentiation/maturation. By contrast, CD11b appears to be virtually absent from all progenitors but it has an enhanced expression during granulomonocytic differentiation/maturation. These data indicate stage-specific expression of integrin during erythroid differentiation.

The role of VLA-4 in hematopoiesis *in vivo* was demonstrated by in utero treatment of mice with an anti-VLA-4 monoclonal antibody (10). Although all hematopoietic cells in fetal liver expressed VLA-4, the treatment specifically induced anemia. It had no effect on the development of nonerythroid lineage cells, including lymphoids and myeloids. In the treated liver almost no erythroblast was detected, whereas erythroid progenitors, which give rise to erythroid colonies *in vitro*, were present. These results indicate that VLA-4 plays a critical role in erythropoiesis, while it is not critical in lymphopoiesis *in vivo*. This is contrast with the *in vitro* observation that antibodies against VLA-4 or VCAM-1 partially inhibited stroma-supported erythropoiesis while they completely suppressed growth of stroma-dependent B-lymphoid cells (16) and the cobblestone formation of the sorted stem cells committed to lymphoid cells (22).

B. SMAP-1 (stromal membrane-associated protein)

To identify cell surface molecules involved in providing erythropoietic support of the stromal cells, monoclonal antibodies against cell surface molecules of mouse spleen stromal cell line, MSS31, were produced. One monoclonal antibody, 11D, specifically stained the red pulp of spleen in which erythropoiesis dominates, suggesting the involvement of erythropoiesis (27, 37). 11D antibody recognized the 90 Kd glycoprotein whose molecular weight was shifted from 90 Kd to 66 Kd by treatment with N-glycanase. The gene for the 11D-recognized protein was cloned by immunoscreening. It coded a new protein without an entire homology with the known proteins and was called SMAP (stromal membrane-associated protein)-1 (31). SMAP-1 is a type II membrane protein with 3 N-glycosylation sites and contains KE (lysine and glutamine) rich sequences that shared homology with human microtubule associated proteins (MAP1A and MAP1B) and yeast centromere binding protein (CBF5p). Since KKD/EX repeat domain of MAP1B and CBF5p was shown to be responsible for the interaction with microtubules, SMAP-1 may function through the protein-protein interaction.

To know the function of the SMAP-1 of stromal cells in erythropoiesis, the smap-1 cDNA was transfected in the antisense orientation into MSS62 cells, and the effect of its reduced levels on the stroma-supported erythropoiesis examined *in vitro*. Four antisense cDNA transfectants that significantly reduced expression of SMAP-1 suppressed the large erythroid colony formation, indicating the stimulatory function of SMAP-1 on EIM.

Expression of SMAP-1 in erythropoietic organs was shown to be well correlated with EIM in the hematopoietic organs. In yolk sac, SMAP-1 was detected in the blood vessel endothelial cells, but not in the blood cells. Blood vessel formation was first seen in the wall of the yolk sac where the undifferentiated mesenchyme condenses to form angiogenetic clusters, the centers of these clusters form the blood cells, and the outsides of the clusters develop into blood vessel endothelial cells. Cell-to-cell interaction is expected to be important in generation of erythropoietic cells in the blood island. In the fetal liver, expression of smap-1 mRNA started to accumulate on day 11.5, increased up to day 14.5 and then decreased, thus levels of SMAP-1 were well correlated with the erythropoietic activity of the fetal liver.

The number of stromal cells expressing SMAP-1 was decreased depending on the expansion of hepatic parenchymal cells in the fetal liver on day 18. In the spleen, the expression was restricted in the red-pulp and its expressed areas were increased depending on the induction of erythropoiesis by hemolysis. Combining the result of the functional importance of SMAP-1 in the stroma-supported erythropoiesis *in vitro*, SMAP-1 may be a key molecule to induce EIM in the hematopoietic organs.

In addition to the importance of SMAP-1 in EIM, its restricted expression during embryonic development is intriguing. The regions of SMAP-1 expression were restricted to the limb bud, the cardial wall, the yolk sac, and the liver in the mouse embryos. Expression of SMAP-1 in the limb bud is quite interesting because it was detected only in the apical ectodermal ridge (AER), whose inductive role upon the underlying mesenchyme has been extensively studied. In the cardial wall, its expression was detected in both muscle cells and endothelial cells. The presumptive heart cells from a double-walled tube consisting of an inner endocardium and an outer epimyocardium and fusion of the endocardial tubes occurs to form a single pumping chamber following movement. The restricted expression of SMAP-1 in these embryonic organs suggests that it may function in the inductive process of tissue formation during development.

C. IAP (integrin-assocciated protein)/CD47

MAb100.1, a monoclonal antibody, was selected by inhibition of the stroma-supported erythropoiesis. FACS analysis using MAb100.1 indicated that the protein recognized by MAb100.1 was localized on the surface of MSS62. The gene encoding the protein recognized by MAb100.1 was isolated by expression cloning. cDNA library made from MSS62 stromal cells was transfected into COS7 cells and the transfected cells expressing surface molecules recognized by MAb100.1 were collected by the antibody coupled magnetic beads method. The cDNAs recovered after 4 rounds of procedures were sequenced and 4 independent clones contained the same ORF identical to CD47/IAP (integrin-associated protein) (6). Comparison of nucleotide sequences of different cDNA clones with mouse, rat and human cDNAs showed differentially spliced forms. IAP was first identified by its association with $\alpha V\beta 3$ integrin and is a 50-k-Da membrane protein with an amino-terminal immunoglobulin domain and a carboxyl-terminal 5 membrane-spaning region; it is expressed on a variety of cell types including hematopoietic cells and stromal cells. Ig variable domain (IgV) of IAP was shown to be necessary for adhesion of the cells to vitronectin. IAP appears to be involved in signal transduction by $\alpha V\beta 3$ and perhaps other integrins in phagocytosis, oxidant-bursts stimulation of neutrophilds and monocytes. Its expression in erythrocytes suggested its role in erythropoiesis or erythrocyte function. IAP was also shown to be a receptor for the C-terminal domain of thrombospondin, which is a family of proteins implicated in regulation of the motility, proliferation, and differentiation of many cell types. Because IAP acts both as a component of $\beta 3$ integrin signalling and as a receptor for the ECM protein thrombospondin, it would be ideally suited to integrate signals from several matrix proteins to inform the erythroid progenitors, although more work is necessary to understand its molecular function in EIM.

D. c-Kit and Kit-ligand (stem cell factor=SCF)

Mutations of the receptor tyrosine kinase c-Kit coded by the murine dominant-white spotting locus (W) or its ligand stem cell factor (SCF), which is encoded as a soluble and membrane-associated protein by the Steel gene in mice, lead to deficiencies of germ cells, melanocytes, and hematopoiesis, including the erythroid lineage; thus the role of c-Kit in erythropoiesis was examined by antibodies against c-Kit in the fetal liver stromal cell-dependent large colony formation of the erythroid progenitors (20). The antibody inhibited the colony formation of the progenitors and in more detailed analysis, it inhibited only proliferation but not differentiation of the progenitor cells. The inhibition was effective only at the early phase (within 6 hours after Epo addition) before the cells started to proliferate induced by Epo. During this early phase, Epo down-regulated c-Kit gene expression. These results suggest a mechanism of combined action of c-Kit with Epo on the lineage-restricted erythroid progenitor cells, and therefore SCF, a c-Kit ligand expressed in the stromal cells is a key molecule for EIM.

Kapur *et al.* (13) showed genetic evidence for the effective signaling through the interaction of membrane-restricted SCF and c-Kit in erythropoiesis. They used genetic methods to study the role of membrane or soluble presentation of SCF in hematopoiesis. Bone marrow-derived stromal cells expressing only a membrane-restricted (MR) isoform of SCF induced an elevated and sustained tyrosine phosphorylation of both c-Kit and Epo receptor (Epo-R) and significantly greater proliferation of an erythrocytic progenitor cell line compared with stromal cells expressing soluble SCF. Transgene expression of MR-SCF in Steel-dickie (Sld) mutants resulted in a significant improvement in the production of red blood cells. In contrast, overexpression of the full-length soluble form of SCF transgene had no effect on red blood cell production but corrected the myeloid progenitor cell deficiency seen in these mutants. These data provide the first evidence of differential functions of SCF isoforms *in vivo* and sug-

gest an abnormal signaling mechanism as the cause of the severe anemia seen in mutants of the Sl gene.

Wu *et al.* (34) reported further that functional interaction of Epo and c-Kit is essential for erythroid colony formation. They showed that fetal liver erythroid progenitors from Epo-R (-/-) knockout mice, infected *in vitro* with a retrovirus expressing the wild-type Epo-R, required addition of both Epo and SCF to form CFU-E colonies. Thus, a functional interaction between c-Kit and the Epo-R is essential for the function of CFU-E progenitors. In contrast, CFU-E colony formation *in vitro* by normal fetal liver progenitors requires only Epo; the essential interaction between activated c-Kit and the Epo-R must have occurred *in vivo* before or at the CFU-E progenitor stage.

Socolovsky *et al.* (28, 29) reported that the prolactin receptor rescues EpoR (-/-) erythroid progenitors and replaces EpoR in a synergistic interaction with c-Kit. The addition of SCF potentiates the ability of the prolactin receptor to support differentiation of both EpoR (-/-) and wild-type CFU-e progenitors. Therefore, there is no requirement for an EpoR-unique signal in erythroid differentiation and EpoR signaling has no instructive role in red blood cell differentiation, nor is an EpoR-unique signal required in the synergistic interaction between c-Kit and EpoR. Interestingly, Bellone *et al.* (1) reported that development of burst-forming unit-erythroid (BFU-E) colonies from $CD34^+$ hematopoietic progenitors cultured on a bone marrow stroma cell layer was significantly reduced in the presence of an anti-human prolactin antibody, and suggested a role of exogenous prolactin at an early step of *in vitro* erythroid differentiation in a bone marrow stroma environment. Wessely *et al.* (33) also reported cooperation of c-Kit with Epo-R as a novel way to induce erythroid progenitor self renewal.

Quesniaux *et al.* (25) reported combined action of SCF with Interleukin-11 (IL-11), a pleiotropic cytokine originally isolated from a primate bone marrow stromal cell line on multiple phases of erythropoiesis *in vitro*. In the presence of SCF, IL-11 has profound stimulatory effects on the erythroid precursors representing various stages of erythroid differentiation including BFU-E and CFU-E and highly proliferative erythroid progenitors.

Administration of cytokines brings about redistribution of stem/progenitor cells from bone marrow to peripheral blood. This cytokine-induced mobilization encompasses the view that cytokines disrupt, directly or indirectly, cytoadhesive interactions of stem/progenitor cells with their bone marrow stroma regulated by HIM. Craddock *et al.* (2) found that anti-VLA4/VCAM-1 treatment combined with either G-CSF or SCF treatment leads to significant enhancement of mobilization efficiency, and cooperative signaling through the c-Kit-SCF pathway was reported by Papayannopoulou (24) for the anti-VLA4-induced mobilization. Mobilization by anti-VLA4 does not depend on functional receptors for G-CSF, IL-7, or IL-3, but the functional c-Kit receptor is required because W/Wv mice responded minimally, whereas Steel-Dickie (Sl/Sld) responded normally. Neither Wv nor Sl/Sld mice responded to anti-VCAM-1 treatment. The defective response to anti-VCAM-1 in W/Wv mice was corrected after their transplantation with +/+ cells. After anti-VLA4 treatment, c-Kit expression was down-modulated in normal bone marrow cells. Thus, anti-VLA4/VCAM-1-induced mobilization likely requires signaling for stimulation of cell migration and this cooperative signaling involves the c-Kit-SCF pathway. This provides a novel example of integrin/cytokine crosstalk in stroma-stem/progenitor cell interaction which may be involved in the stroma-supported erythropoiesis.

To identify the regulatory factors for EIM, MSS62 cells were transfected with v-src oncogene (39), because the latter's effect on the HIM was suggested. Transfectants with high v-Src activity showed reduction in erythropoietic stimulatory activity. A decrease in cell-surface VCAM-1 and SCF mRNA was accompanied by high v-Src activity. These results suggest that v-Src interferes with the erythropoietic stimulatory activity of the stromal cells through repression of VCAM-1 and SCF between stromal and erythroid cells, and suggested the possible role of integrin/cytokine crosstalk in EIM.

E. TIMP/EPA (erythroid potentiating activity)

Hayakawa *et al.* (9) demonstrated that tissue inhibitor of metalloproteinases (TIMP) produced by human bone marrow stromal cell line KM-102 has erythroid-potentiating activity (EPA) which stimulates the proliferation of erythroid progenitor cells. They proposed a scheme for the bifunctional role of TIMP/EPA in a hematopoietic microenvironment; maintenance of the integrity of bone marrow matrix and the proliferation of erythroid progenitor cells proceeding on the matrix. Docherty (5) reported sequence identity of human TIMP to EPA. TIMP-1/EPA was induced in connective tissues by IL-6 (15) and by Newcastle Disease virus (NDV) in mouse and hamster cell lines (7), thus TIMP/EPA may be one of the inducible regulatory factors of EIM.

F. TC-PTP (T cell protein tyrosine phosphatase)

You-Ten *et al.* (40) reported impaired bone marrow microenvironment in T cell protein tyrosine phosphatase-deficient mice. All homozygous mutant mice with the T cell protein tyrosine phosphatase (TC-PTP), the most abundant mammalian tyrosine phosphatases in

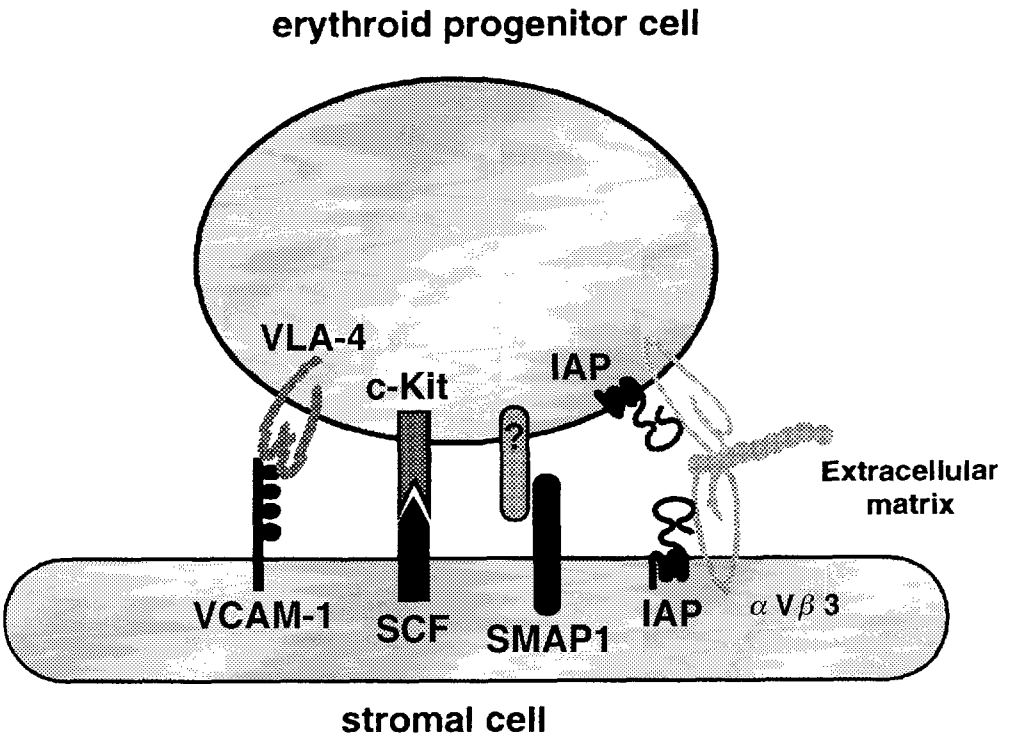

Fig. 2. Molecules required for the cell-to-cell interaction between erythroid progenitors and stromal cells, identified by our studies.

hematopoietic cells, died by 3–5 wks of age, displaying runting, splenomegaly, and lymphadenopathy. Homozygous mice exhibited specific defects in bone marrow (BM), B cell lymphopoiesis and erythropoiesis, as well as impaired T and B cell functions. BM transplantation experiments showed that hematopoietic failure in TC-PTP -/- animals was not due to a stem cell defect, but rather to a stromal cell deficiency. This study demonstrates that TC-PTP plays a significant role in both hematopoiesis and immune function.

IV. EIM and HIM

HIM has been most extensively studied for its regulation of self-renewal and commitment of the hematopoietic stem cells by the stromal cells. However, complete maintenance of the long-term repopulating stem cells *in vitro* was not possible either by coculture with the stromal cells or in the presence of mixtures of known cytokines, thus more studies are necessary.

The microenvironments of the hematopoietic progenitor cells have not been studied because most progenitors are derived from the stem cells and expansion of the committed progenitors could be regulated by various cytokines, although HIM may have been originally defined as the microenvironment for the stem cells and their progenitor cells.

In this review, we have discussed cellular and molecular analysis of the microenvironment required for erythropoiesis (EIM); *in vitro* reconstruction of EIM with the established stromal cells and the molecules required for EIM. Further studies will identify other functional molecules.

In addition to EIM, HIMs may act for other lineages of hematopoietic progenitor cells, in fact, we observed that the bone marrow stromal cells selectively stimulate rapid expansion of myeloid progenitors (12), thus suggesting a GIM (granulopoietic inductive microenvironment) in bone marrow. Cellular analysis of GIM showed that the stromal cells support the myeloid progenitor cells through direct cell-to-cell contact and their support does not require cytokines such as G-CSF, GM-CSF or M-CSF (11). Involvement of stromal cells on lymphopoiesis is well known and has been extensively studied at cellular and molecular levels (16).

HIM for hematopoietic stem cells should therefore be called the stem cell inductive microenvironment (SIM) and the previously stated HIM may consist of SIM, LIM, GIM and EIM; these individual HIM may contribute in different ways to the regulation of constitutive hematopoiesis in various hematopoietic organs.

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