Highly Restricted Expression of a Stromal Cell Determinant in Mouse Bone Marrow In Vivo

By Karen Jacobsen,* Kensuke Miyake,‡ Paul W. Kincade,‡ and Dennis G. Osmond*

From the *Department of Anatomy, McGill University, Montreal H3A 2B2, Canada; and the ‡Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104

Summary

B lymphocyte precursor cells in mouse bone marrow develop in close association with stromal cells which provide essential growth signals. To identify molecules that may normally play a role in this interaction we have examined the in vivo binding of a new monoclonal antibody (mAb) (KMI6) that recognizes a determinant on a bone marrow stromal cell line (BMS2) in vitro. Flow cytometric and radioautographic evaluations revealed that the antigen recognized by KMI6 is represented on the surface of an extremely small number of cells in bone marrow cell suspensions from adult mice. An apparent molecular mass of 110 kD was obtained by surface labeling of a stromal cell clone and immunoprecipitation. Purified mAb KMI6 labeled with ^{125}I was then given intravenously to young C3H/HeJ mice. Unbound mAb was washed out by cardiac perfusion and femoral bone marrow was examined by light and electron microscope radioautography. KMI6 labeling was heavy on the plasma membrane of many stromal cells, especially those located towards the outer subosteal region. The KMI6-labeled stromal cells were usually associated with cells of lymphoid morphology which they often completely surrounded. The labeling was restricted to areas of stromal cell plasma membranes in contact with lymphoid cells. The lymphoid cells themselves, as well as macrophages and other hemopoietic cells, failed to bind mAb KMI6 significantly. Stromal cells in bone marrow depleted of hemopoietic cells by γ-irradiation (9,5 Gy) bound mAb KMI6 at reduced intensity. The results demonstrate that the KMI6 determinant, a 110-kD protein, is expressed on bone marrow stromal cells in vivo. Its restriction to areas of interaction with lymphoid cells suggests a role in forming microenvironmental niches of B lymphopoiesis. The surface membrane of individual stromal cells may thus be functionally polarized towards interacting B cell precursors and other hemopoietic cells.

B cells are continuously generated in the bone marrow under the influence of local microenvironmental controls (1, 2). Both in long-term bone marrow cultures and in vivo, precursor B lymphocytes develop in close association with certain stromal cells (2–4). Actual adhesion to the stromal cells and access to stromal cell products are needed for early steps in B lymphocyte differentiation and proliferation (2, 5–8). Clones of individual stromal cells capable of supporting B cell development have been defined (5–14). Stromal cells and their products are equally important, however, in supporting other hemopoietic lineages (15). The molecular interactions between individual stromal cells and associated precursor cells in vivo are thus central to the functional organization of stromal cells and their role in regulating B lymphopoiesis and other aspects of hemopoiesis.

Adhesion molecules so far shown to play a role in B lymphopoiesis in vitro include CD44, hyaluronate, very late antigen 4[1] (VLA-4) and vascular cell adhesion molecule 1 (VCAM-1) (16–19). These products appear to be functionally important because antibodies to the individual molecules can prevent lymphopoiesis when added to bone marrow cultures (16, 19). The effects are not restricted to B cell genesis, however, as myelopoiesis is also being impaired when the antibodies are added to appropriate cultures (16, 19).

A new mAb (mAb KMI6) has now been raised against a B cell-supportive stromal cell clone, BMS2, as a potential marker of B lineage interaction. The mAb was selected for its strong binding to BMS2 stromal cells, and lack of hematopoietic cell labeling. It has yet to be shown, however, that

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[1] *Abbreviations used in this paper:* EM, electron microscopy; LM, light microscopy; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

the KMI6 cell surface determinant is constitutively expressed in vivo. Some other surface molecules on stromal cells are expressed only under culture conditions (2).

The present work has been designed to determine the expression of the KMI6 determinant and its precise location at the stromal–precursor cell interface under normal circumstances in vivo. Radiolabeled purified mAb KMI6 has been administered intravenously to mice, and its binding sites in the bone marrow visualized by electron microscopy and high resolution radioautography (3, 4). The findings reveal a constitutive, in vivo expression of KMI6 determinant, apparently restricted to certain areas of stromal cell surface membranes at the interface with undifferentiated lymphoid cells.

Materials and Methods

Antibody Isolation and Immunoprecipitation of Determinants Recognized by mAb KMI6. The BMS2 stromal cell clone was established from the bone marrow of (C57BL/6 × DBA/2)F_1 mice and has been extensively characterized (12, 15). These cells were used repeatedly to immunize LOU/MN rats before fusion of their spleen cells with the murine Sp2/0 hybridoma. Drug-resistant hybrids were selected for antibody production by an ELISA assay with fixed BMS2 cells. Positive wells were additionally tested for reactivity with hemopoietic cells by flow cytometry. Antibody produced by the cloned KMI6 hybridoma stained the BMS2 cells, but not suspensions prepared from spleen, thymus, lymph nodes, or bone marrow. Ascites was then prepared in immunodeficient SCID mice and the antibody purified by ABx column chromatography (J.T. Baker; Phillipsburg, NJ).

BMS2 cells were surface labeled with N-hydroxy-sulfosuccinimidobiotin (Pierce Chemical Co., Rockford, IL) before extraction with NP-40 (Sigma Chemical Co., St. Louis, MO) exactly as described (19). The labeled material was then precleared by repeated incubation with sepharose beads coated with goat anti–rat IgG antibodies. Immunoprecipitations were then performed with similar beads loaded with either normal rat IgG, or purified KMI6 antibody. Recovered protein was eluted by boiling in sample buffer and run on 10% SDS-PAGE. After transfer to Immobilon P membranes (Millipore Corp., Bedford, MA) immunoprecipitates were revealed by treatment with an avidin–horseradish peroxidase conjugate, followed by 4-chloro-1-napthol and hydrogen peroxide (Sigma Chemical Co.).

Mice. Male C3H/HeJ mice, 3–4-wk-old, were purchased from the Jackson Laboratory (Bar Harbor, ME). All mice were housed in sterile microisolator cages (Laboratory Products Inc., Maywood, NJ). Food and water were provided ad libitum.

Irradiation. Mice were placed in a Gamma-Cell irradiator (^{132}Cs) chamber and received a single dose of 9.5 Gy γ-irradiation (1.37 Gy/min).

Radioiodination of Affinity-purified Rat IgG. mAb KMI6 (rat IgG 2a) was used at a concentration of 2.5 mg/ml. To radiolabel tyrosine residues, 150 μg aliquots of mAb were coupled to carrier-free Na^{125}I (1.5 mCi; sp act 1.5 × 10^7 μci/μg) by a modified chloramine-T method. The final concentration of mAb was ∼50 μg/ml in PBS (pH 7.2).

Bone Marrow Cell Sampling and In Vitro Binding of ^{125}I-mAb KMI6. Mice were killed by cervical dislocation and femurs removed. Bone marrow cell suspensions were prepared by flushing the femoral shafts with 10% FCS in Eagle's MEM (Gibco Laboratories, Grand Island, NY). Bone marrow cell suspensions were

counted with an electronic particle counter (Coulter Electronics, Hialeah, FL) and the cellularity adjusted to 40 × 10^6 cells/ml before 100 μl aliquots were incubated with 100 μl of ^{125}I-KMI6 for 30 min on ice. The cells were then centrifuged through 6% BSA in PBS at 1,100 rpm for 8 min. The supernatant was aspirated and the pellet washed four times by centrifugation in PBS (pH 7.4). Cell pellets were suspended in 6% BSA/PBS and smeared onto gelatin-coated glass slides, fixed in absolute methanol, and dipped in Kodak d52 radioautographic emulsion (Kodak Canada, Toronto, ON) (3, 4).

In Vivo Immunolabeling with Radioiodinated mAb KMI6. Groups of six control and six irradiated mice were anaesthetized with 1.6% chloral hydrate in sterile saline intraperitoneally (0.25 ml/gm body weight). The external jugular vein was exposed and 100 μl of ^{125}I-mAb was injected directly into the blood stream. 3 min later, the blood was cleared from the circulation by whole-body cardiac perfusion. A syringe pump (model 355; Sage Instruments, White Plains, NY) was used to deliver (3 ml/min) cold Ringer's solution to wash out unbound antibody and blood followed by a cacodylate-buffered solution of 2.5% glutaraldehyde and 2.0% acrolein (pH 7.2) to fix the tissues in situ.

Tissue Processing and Radioautography. Femoral shafts of perfusion-fixed mice were removed and placed in fixative at 4°C for 12 h before decalcification in EDTA (10% wt/vol, pH 7.2) at 4°C for 5–7 d. Decalcified femurs were postfixed in potassium ferrocyanide-reduced osmium tetroxide, dehydrated in acetone, infiltrated and embedded in Epon 812 (J.B.EM, St. Laurent, QC) as described (3, 4). Mid-diaphyseal transverse sections of bone marrow were cut for light microscopy (LM) and electron microscopy (EM). LM sections were stained with iron hematoxylin and iron alum before they were dipped in Kodak NTB2 emulsion (Kodak Canada, Toronto, ON) and exposed for 1–4 wk. EM sections were placed on celloidin-treated glass slides, coated with a monolayer of Ilford L4 emulsion (Ilford, Essex, UK), and exposed for 8–24 wk. Sections were stained in aqueous uranyl acetate and lead citrate. Specimens were scanned and photographed on an electron microscope (model 400; Philips, Eindhoven, the Netherlands).

Morphometric Analysis of mAb KMI6 Binding in Bone Marrow Sections. Photomicrographs of labeled stromal cell processes were traced at a constant magnification of 14,500 and the information was stored using the MOP-Videoplan image analysis program (Carl Zeiss of Canada Ltd., Don Mills, ON). The perimeters of lymphoid cells associated with labeled stromal cell processes were traced, and measurements of their maximum diameters at x- and y- axes were derived. The MOP Videoplan was also used to measure the distance between the center of individual radioautographic grains and hemopoietic cell plasma membranes.

Analysis of mAb KMI6 Labeling in Mouse Bone Marrow Suspensions. Subsequent to in vitro exposure to ^{125}I-mAb, three bone marrow smears from each mouse were analyzed after 1, 7, and 21 d of radioautographic exposure. By standard hematologic criteria, cells were categorized as lymphoid, erythroid, granulocytic, or other, and the number of radioautographic grains associated with each cell was counted.

Results

mAb KMI6, a new antibody raised against the BMS2 stromal cell clone, bound strongly to the cultured stromal cells, but by flow cytometry was found to label an extremely small number of cells in suspensions of bone marrow from adult mice. Radioautographic evaluation of bone marrow cells
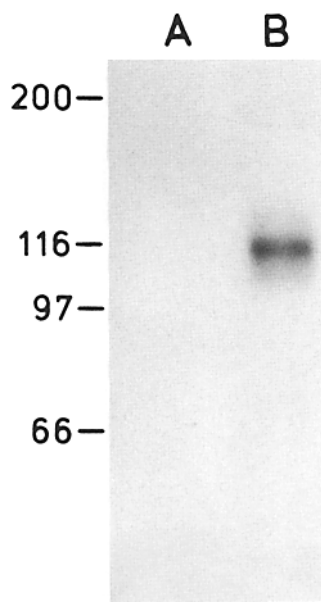
Figure 1. The KMI6 mAb detects an ~110-kD protein on BMS2 stromal cells. Biotin-labeled cells were extracted and immunoprecipitated with normal rat IgG (*A*) or KMI6 antibody (*B*). The positions of size standards are indicated.

from young mice exposed to ^{125}I-mAb KMI6 also showed only near-threshold labeling of a few bone marrow cells (1.8–2.5%), mainly of lymphoid morphology. Immunoprecipitation and PAGE of the new stromal cell determinant defined by mAb KMI6 resolved a single band with an apparent molecular mass of ~110-kD (Fig. 1). The mobility of the immunoprecipitated species was identical under reducing and nonreducing conditions (not shown).

mAb KMI6 Binding to Bone Marrow Stromal Cells In Vivo. After administering ^{125}I-mAb KMI6 intravenously to young mice, well-marked binding of the mAb was detected in sections of femoral bone marrow. Radioautographic grains, indicative of KMI6 binding, were revealed by LM, forming linear arrangements between and around the hemopoietic cells (Fig. 2 *a*). This labeling was particularly evident in outer regions of the bone marrow towards the surrounding bone. Low power EM demonstrated the presence of high intensities of KMI6 labeling localized to certain extravascular cellular elements, background grains being rarely seen (Fig. 2 *b*). Endothelial cells and components of the walls of venous sinusoids and arterioles were devoid of KMI6 labeling (Fig. 2 *b*).

KMI6 binding often appeared to be located around the perimeter of hemopoietic cells which exhibited an undifferentiated lymphoid morphology (Fig. 2). High magnification revealed, however, that these lymphoid cells were actually surrounded by thin cytoplasmic extensions from stromal reticular cells (Fig. 3, *a–d*). There was intimate contact, but no specialized junctions, between the adjacent cell membranes. The majority of the radioautographic grains were localized over the stromal cell processes, suggesting that these, rather than the lymphoid cell plasma membrane, were the sites of KMI6 binding (Fig. 3, *a* and *b*).

Sheets of cytoplasm of mAb KMI6-labeled stromal cells, up to 1 μm in width, often completely encircled the associated lymphoid cell, and could be followed three-dimensionally around the cell in serial sections (Fig. 3, *a–d*). The lymphoid cell thus appeared to be virtually enclosed within a cup-like
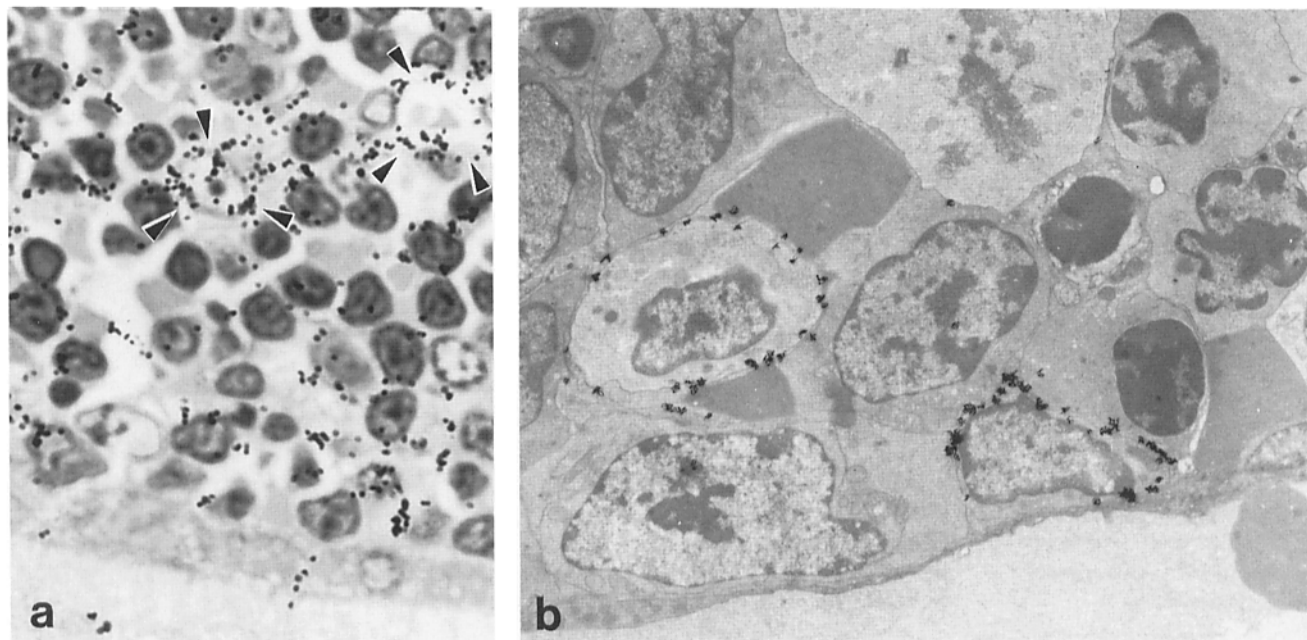

Figure 2. Light and electron microscope radioautography of mAb KMI6 binding in mouse femoral bone marrow. (*a*) Two hemopoietic cells (*arrowheads*) surrounded by radioautographic grains revealing binding of ^{125}I-mAb KMI6 in an area adjacent to the surrounding cortical bone (radioautographic exposure 7 d, ×3600). (*b*) Radioautographic grains associated with the plasma membranes of stromal reticular cell processes surrounding two hemopoietic cells (the underlying sinus endothelium does not bind the antibody). Exposure 10 wk, ×5,200.
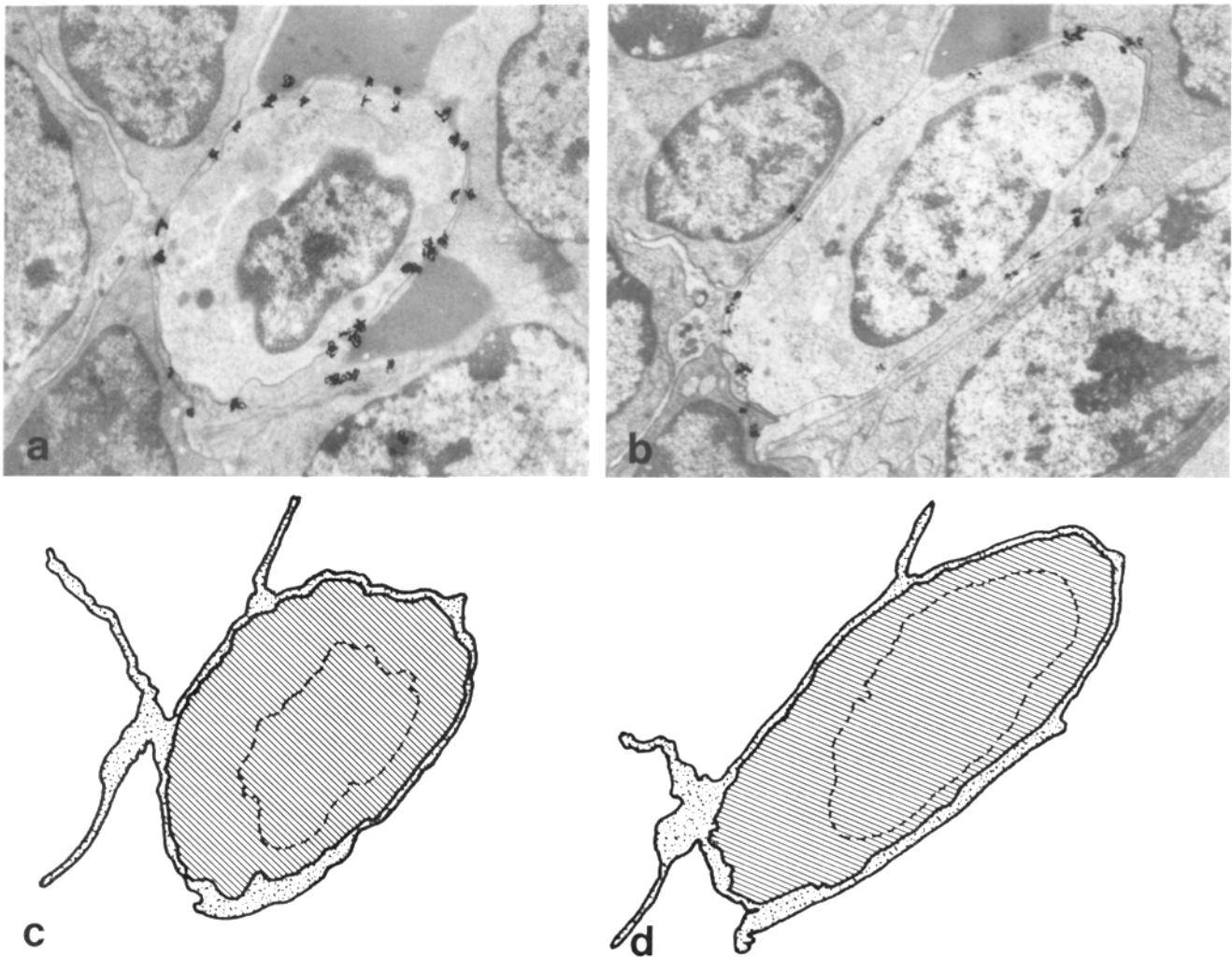
Figure 3. Two serial sections of a single lymphoid cell (*a* and *b*) (from Fig. 1 *B*) and accompanying drawings (*c* and *d*) illustrating extensive association with a stromal cell. Processes from the stromal cell surround the lymphoid cell in two planes of the section and express the KMI6 determinant on surfaces of contact between the two cells. Exposure 10 wk, ×7700.

extension of the stromal cell cytoplasm. Whereas this extension exhibited KMI6 binding, other processes of the same stromal cell, not enveloping the lymphoid cell, remained completely unlabeled by mAb KMI6 (Fig. 3, *a–d*). In some planes of section, stromal cell processes extended only partially around a lymphoid cell. In such cases, the KMI6 determinant was localized strictly to the area of plasma membrane contact between the two cells (Fig. 4). Other regions of the plasma membrane of both cells showed no mAb KMI6 binding. The expression of the KMI6 determinant thus appeared to be restricted to the stromal–lymphoid cell interface.

The intensity of KMI6 expression by various stromal cells within the same bone marrow sample varied widely, as revealed by the density of radioautographic grains per unit length of stromal cell profile (Fig. 5). Not all stromal cell processes associated with undifferentiated lymphoid cells were labeled by mAb KMI6 (data not shown).

The morphology of KMI6-expressing stromal cells and associated lymphoid cells is illustrated in Figs. 3–5. The stromal cell cytoplasm was electronlucent and contained a few profiles of rough endoplasmic reticulum, small vesicles and occasional mitochondria, but was devoid of lysosomes or phagosomes. Electron-dense granules resembling fat droplets were sometimes seen. The stromal cell nucleus, revealed occasionally in the plane of section, had prominent nucleoli and a moderately dispersed chromatin pattern. The associated lymphoid cells were large cells, having a mean diameter of ~7.5 μm, as measured in sections using the MOP-Videoplan and correcting for the random planes of section. The cytoplasm was relatively plentiful, the mean ratio of cytoplasmic to nuclear area in sections being 1.0:1.4. The cytoplasm contained 2–9 mitochondria per cell profile, a few cisternae of rough endoplasmic reticulum, many free ribosomes, a small Golgi complex of 3–4 saccules, and some electron-dense vesicles. The
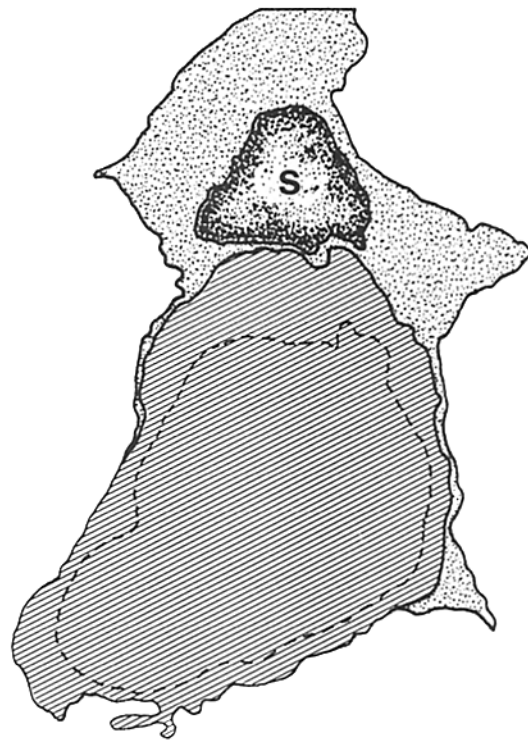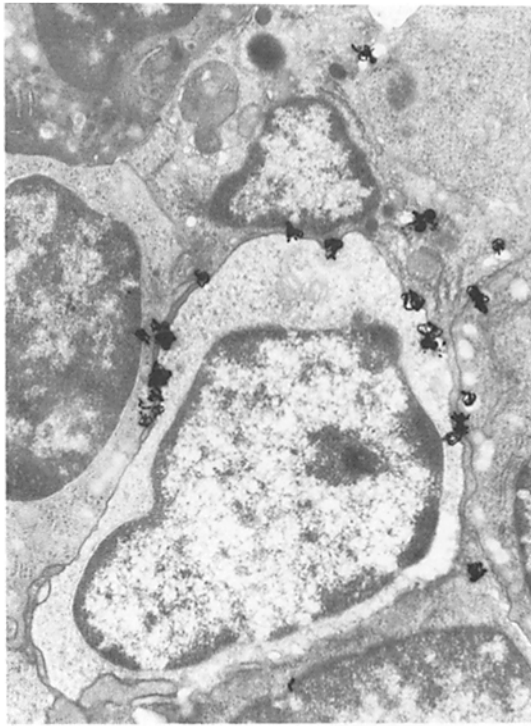
Figure 4. A stromal cell (S) sectioned through its nucleus, sends cytoplasmic processes around an undifferentiated lymphoid cell (see drawing). Radioautographic grains, indicating KMI6 binding, are restricted to regions where stromal cell processes contact the lymphoid cell. Exposure 10 wk, ×8,800.

lymphoid cell nucleus had a relatively dispersed euchromatin pattern with patchy heterochromatin around the nuclear envelope and prominent nucleoli. There appeared to be no consistent morphological differences between lymphoid cells associated with stromal cell processes of high and low KMI6 expression, respectively. Some cells associated with KMI6-bearing stromal cells were in mitosis (data not shown). Occasionally, small groups of lymphoid cells were encompassed in a network of KMI6+ stromal cell processes.

Resident macrophages in the bone marrow, whose complex cytoplasmic processes extended through the parenchyma, did not bind KMI6. These cells were clearly distinguishable from the stromal reticular cells by their abundance of phagosomes and other inclusion bodies, characteristic lysosomal network and voluminous cytoplasm.

No cells other than those of undifferentiated lymphoid morphology appeared to be associated with sites of KMI6 expression on stromal cells. Nonlymphoid cells, notably granulocytic cells, were often in contact with KMI6-binding stromal cells, but were not adjacent to the region of KMI6 labeling. Measurements of the distance between radioautographic grains and the nearest plasma membrane of lymphoid cells and granulocytic (myeloid) cells revealed that the KMI6 epitope was expressed highly on stromal elements immediately adjacent to lymphoid cells. Over 75% of all the grains were located within a distance of 0.2 μm from their center to the plasma membrane of a lymphoid cell (Fig. 6). Most granulocytes were further removed from KMI6 binding sites.

Effect of γ-Irradiation on mAb KMI6 Binding to Bone Marrow Stromal Cells In Vivo. The bone marrow 2 d after 9.5 Gy whole-body γ-irradiation was largely devoid of hemopoietic cells, but stromal reticular cells and their processes were prominent and more clearly delineated than in normal bone marrow (Fig. 7). The parenchyma, reduced to hypocellular columns between widely dilated sinusoids, contained stromal reticular cell bodies and cytoplasmic processes that formed concentric layers in the outer subosteal region (Fig. 7 b). Other cells in the parenchyma included adipocytes, which were often adjacent to sinusoids and were more abundant than usual, blast-like cells, megakaryocytes, erythrocytes, and small clusters of granulocytes.

mAb KMI6 binding was distinctly evident on the stromal cell processes throughout the irradiated bone marrow, in both outer and central areas (Fig. 7). Most radioautographic grains were localized directly over stromal cell profiles. Compared with nonirradiated bone marrow, the labeling was reduced in intensity and was not localized in patches. Sites of mAb KMI6 binding, were distributed at intervals evenly along the length of some, but not all, stromal cell processes (Fig. 7 b).

γ-irradiated bone marrow also showed mAb KMI6 labeling of the endothelium of venous sinusoids in low intensities, the media of arterioles external to the elastic lamina (Fig. 7 a) and, occasionally, canaliculi of some megakaryocytes.

Extramyeloid Binding of mAb KMI6. In addition to bone marrow stromal reticular cells, ^{125}I-mAb KMI6 bound to stromal cells in the red pulp of the spleen, most evidently
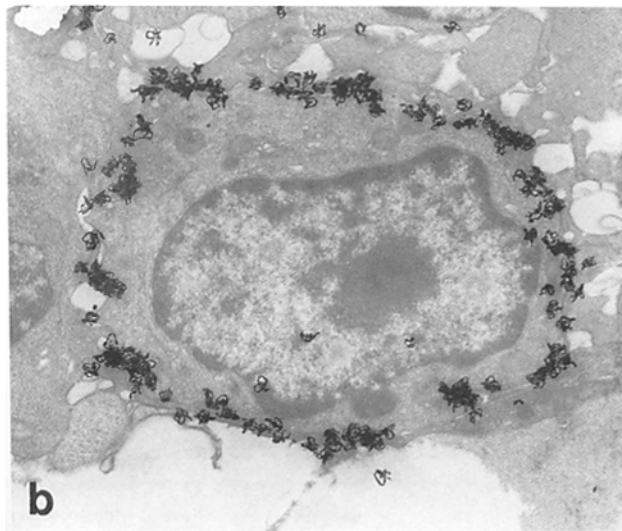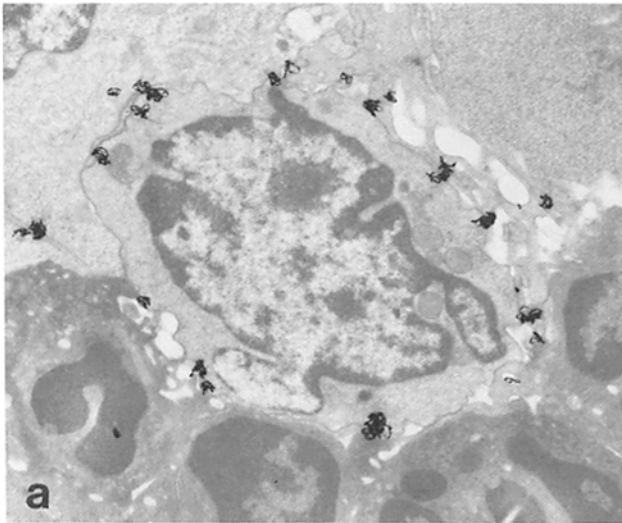
Figure 5. The density of the KMI6 determinant expression on stromal cells varies considerably. (a) A lymphoid cell with an undulating nuclear profile surrounded by stromal cell processes that show a low level of mAb KMI6 binding. (b) A lymphoid cell surrounded by a heavily labeled stromal cell. Exposure 10 wk, ×8,250.
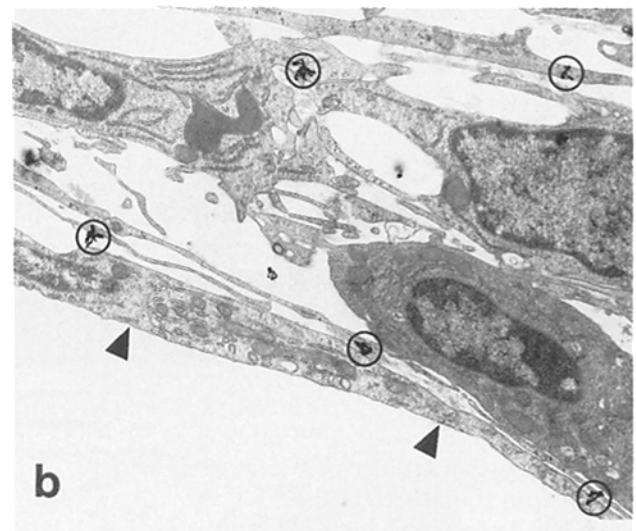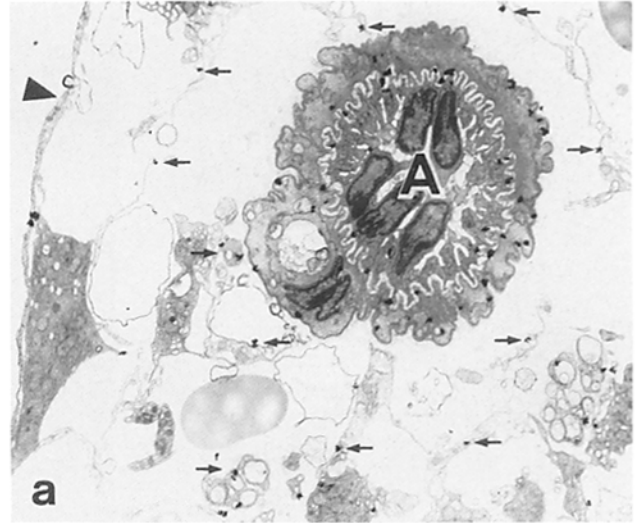
Figure 7. Binding of mAb KMI6 to radioresistant bone marrow stromal cells 2 d after whole-body γ-irradiation (9.5 Gy). (a) Central area of bone marrow showing sectioned stromal cell processes with overlying radioautographic grains (arrows). Arterioles (A) and endothelial cells (arrowheads) also show mAb KMI6 labeling (×3,500). (b) Peripheral area of bone marrow containing layers of stromal cell processes, showing low levels of KMI6 labeling (circles). Exposure 10 wk, ×11,000.
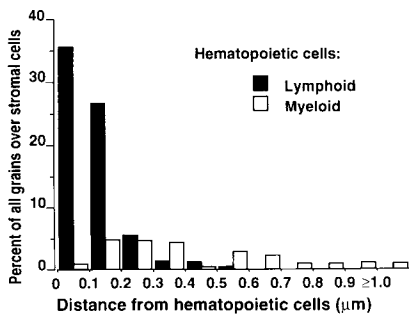

Figure 6. Distance between radioautographic grains and plasma membranes of lymphoid or myeloid cells.

after irradiation, and to microvilli on the sinusoidal surface of hepatocytes. Immunoperoxidase KMI6 staining of cryosections revealed KMI6 binding to a variety of tissue elements, including endothelial cells in the heart, kidney glomerular capsule, hair follicles and epidermis, reticular elements in extrafollicular regions of lymph nodes and thymus, and bone marrow megakayrocytes (data not shown). In 14-d fetal liver, erythroid cells, accounting for approximately one half of the total nucleated cells, showed KMI6 cell surface binding as detected by fluorescence labeling, flow cytometry, and cell sorting (data not shown).

Discussion

The present study demonstrates that a cell surface molecule expressed by a lymphocyte-supporting stromal cell clone in vitro is expressed by certain stromal reticular cells in the bone marrow under normal circumstances in vivo. The protein determinant recognized by mAb KMI6 appears to be the first example of a molecule whose expression on stromal cell processes is limited to restricted areas of the surface membrane, interfacing with cells of undifferentiated lymphoid morphology. The findings suggest that stromal cells and some of their products are polarized towards individual interacting hemopoietic cells in vivo, providing a possible basis for the multilineage function of bone marrow stromal cells.

The KMI6 cell surface protein is expressed only by stromal reticular cells in the bone marrow, not by hemopoietic cells. In vivo administration of radiolabeled mAb KMI6 gives radioautographic labeling localized to stromal cell processes both in normal bone marrow, when stromal cells are closely associated with hemopoietic cells, and after lethal γ-irradiation, when hemopoietic cells are virtually absent. Single cell suspensions of bone marrow, from which stromal cells have been eliminated by disruption, show virtually no mAb KMI6 binding by either flow cytometry or radioautography. The low level of radioautographic mAb KMI6 labeling shown by some lymphoid cells in vitro could represent the presence of fragments of adherent stromal cell membrane remaining after the breakdown of stromal cell–lymphoid cell associations. Not all reticular cells in the bone marrow express the KMI6 determinant, however, suggesting that this property defines a particular subset of stromal reticular cells.

Comparable heterogeneity is evident among cultured bone marrow stromal cells. Cloned stromal cell lines exhibit a variety of phenotypic and functional properties (2, 5–12). With respect to B cell genesis, some stromal cell clones support the development of the earliest progenitor B cells, others can sustain the proliferation of more differentiated IL-7–responsive pro-B and pre-B cells, whereas some are devoid of lymphocyte supporting activity (5, 10–14). In contrast with reticular cells, the resident macrophages of the bone marrow stroma, which do not bind lymphoid progenitor cells in long-term bone marrow culture (20), are entirely lacking in KMI6 surface protein in vivo.

KMI6 expression demarcates areas of intimate association between stromal cell processes and individual lymphoid progenitor cells. As seen by EM, depending upon the plane of section, this association takes the form of either a thin ring of KMI6-bearing stromal cell cytoplasm completely encircling the lymphoid cell (Figs. 2, 3, and 5) or extensions from the body of the stromal cell partially embracing the lymphoid cell (Fig. 4). These appearances suggest that three-dimensionally, the lymphoid cell is located within a deep goblet-like stromal cell extension whose inner surface displays KMI6 protein. Small clusters of lymphoid cells have been observed to be associated with a single stromal cell. In addition, however, nonlymphoid cells, notably granulocytic precursors, may be associated with other indentations of the stromal cell surface not expressing KMI6. This interpretation, shown schematically in Fig. 8, suggests the formation of microenvironmental "niches" for lymphoid cell development limited to restricted portions of the surface of stromal cells whose activities are directed specifically towards the membrane interface.

The concept of such a polarization of stromal cells, directing KMI6 protein expression towards lymphoid cells, finds parallels in associations between immune cells. In interacting with B lymphocytes to promote Ig synthesis, T helper lymphocytes show a polarization of cytoskeletal elements and cell organelles, as well as a vectorial direction of cytokine secretion towards the interface with the B cell (21). CTLs become polarized towards target cells before lysing them (22). The present findings suggest a mechanism whereby individual hemopoietic lineages can be selectively regulated by soluble factors in the bone marrow, despite the close packing of diverse hemopoietic cells in the extravascular parenchyma. Stromal cell factors released in small amounts at KMI6-expressing interfaces could stimulate the associated lymphoid cells without influencing nearby cells. In this way, more than one cell lineage could be independently regulated on the surface of an individual stromal cell.

Interlineage competition in the bone marrow has been a long-recognized phenomenon, but its cellular basis has been unclear. A reciprocal relationship between lymphocytopoiesis and granulocytopoiesis has been repeatedly observed in perturbations of steady state conditions (23–25). Individual stromal cells are multifunctional with respect to cytokine interactions. They can produce both CSFs and IL-7 and they display receptors for many cytokines (2, 14). Stimulation of pre-B cell proliferation by administered IL-7, however, is associated with decreased granulocytopoiesis (26) whereas, conversely, the stimulation of neutrophil production by administered G-CSF diminishes B lymphocytopoiesis (27, 28). These effects have been conjectured to reflect a competition for a common pool of stem cells, but it now seems more probable that the underlying mechanism involves a competition for stromal cell-related factors. The rapid proliferation of a stromal cell-associated precursor cell of a given lineage may induce an increased polarization and expansion of the respective lineage-directed area of stromal cell membrane at the expense of other associated lineages. The variation in intensity of expression of the KMI6 protein from one lymphoid
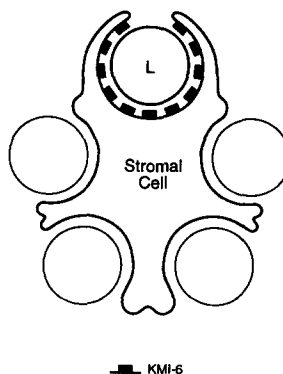

Figure 8. Schematic representation of the proposed localisation of KMI6 determinant at the interface between a bone marrow reticular stromal cell and a lymphoid progenitor cell (L).

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niche to another (Figs. 2 and 5) may reflect the differentiation state of the associated lymphoid cell and its capacity to induce stromal cell activities. In the absence of lymphoid cells in irradiated bone marrow, the expression of KMI6 protein by bone marrow stromal cells becomes diffuse and of low intensity. This seems unlikely to be the result of radiation-induced injury to the stromal cells because the expression of another surface molecule, VCAM-1, actually increases after identical irradiation treatment (Jacobsen K. and D.G. Osmond, unpublished data). Rather, the findings suggest that interaction with lymphoid progenitor cells may normally be instrumental in inducing the patchy expression of KMI6 protein in high intensities on polarized stromal cells. The ability of lymphoid interactions to influence stromal cell activities has been documented. A stromal cell clone, ST2, which does not constitutively produce IL-7, can be induced to transcribe RNA for IL-7 when cocultured with the IL-7–dependent B cell line, DW34 (29).

Further studies are indicated to identify positively the nature of the KMI6-associated lymphoid cells. These cells exhibit remarkably consistent appearances within the heterogeneous cell populations of the bone marrow. Their undifferentiated morphology would be generally consistent with that of primitive hemopoietic precursors, including multipotential stem cells (30). Their size and cytoplasmic volume appear in most cases to be larger than those of hemopoietic stem cells (30), however, and they would appear greatly to exceed the stem cell population in frequency (30–33). On the other hand, their morphology is entirely consistent with that of early B220⁺ precursor B cells, such as those identified in SCID mice (34). The region of the bone marrow in which KMI6 labeling is most evident, tending towards peripheral areas near the surrounding bone, is one in which early precursor B cells are concentrated (3, 4, 34, 35). Double marker studies combining KMI6 with B220 and μ chains will test the hypothesis suggested by the present work that the KMI6-associated lymphoid cells are mainly precursor cells of the B lymphocyte lineage.

Studies of the molecular nature of the 110-kD KMI6 antigen, as well as its functional significance are underway. The addition of mAb KMI6 to long-term cultures of B lymphocyte precursor cells on BMS2 stromal cells has had no effect on B cell genesis (19). This is not surprising since the KMI6 antibody was not selected on the basis of a functional assay. However, the molecule recognized by KMI6 antibody is an interesting candidate for a number of possible functions. Firstly, it could be a transmembrane growth or differentiation factor. A number of recently characterized cytokines, such as stem cell factor (MGF, kit ligand) have a membrane orientation. The KMI6 antigen is constitutively expressed on cloned stromal cell lines and is detectable as a cytoplasmic protein in sections of a variety of tissues.

Although human CD34 is of similar size, preliminary experiments suggest that the KMI6 antibody does not recognize the human homologue. Another candidate is CD10 (CALLA) and experiments are underway to see if KMI6 recognizes an endopeptidase like molecule. Still other possibilities are that it is a cell adhesion molecule of unknown function. If that is the case, the antibody is either directed to a functionally unimportant domain or has not been tested in an appropriate adhesion system. Molecular cloning would clarify these issues. Aside from demonstrating that stromal cells can be polarized to provide physical niches for interaction with hemopoietic progenitors, the present findings will stimulate structural studies of the KMI6 molecule.

Address correspondence to Dr. Dennis G. Osmond, Department of Anatomy, McGill University, 3540 University Street, Montreal, Quebec H3A 2B2, Canada.

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