MONOCLONAL ANTIBODIES TO Pgp-1/CD44 BLOCK LYMPHO-HEMOPOIESIS IN LONG-TERM BONE MARROW CULTURES

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Discrete microenvironments within the bone marrow are thought to regulate the production of eight types of blood cells through direct cell contact-mediated interactions and elaboration of cytokines. Stromal cells representing at least one component of the microenvironment for myeloid and lymphoid cell formation are now available as cloned cell lines that can be manipulated and studied in long-term cultures (1-3). It is clear that stromal cells can make a number of regulatory cytokines including macrophage CSF (M-CSF),[1] granulocyte/macrophage CSF (GM-CSF), IL-6, and IL-7 (3). However, little is known about how close physical proximity is maintained between stromal cells and the precursor cells they influence. Stromal cells can express several known adhesion molecules in culture, including N-CAM and N-cadherin, and lymphoid and myeloid progenitors bear LFA-1 and MEL-14 molecules (4-7). However, there is as yet no evidence that these have functional significance.

Serological identity has recently been found for a group of widely distributed cell surface glycoproteins that have been implicated in lymphocyte homing, as well as interaction between cells and the extracellular matrix (8-10). Originally known as Pgp-1/Ly-24 in the mouse and classified as CD44 in humans, these molecules have also been extensively studied as markers of T lymphocyte subsets and their precursors (8, 9, 11, 12). We prepared a panel of mAbs that recognize a cloned murine stromal cell line and selected four that might be of particular interest with an adhesion assay. These were then found to completely block production of lymphoid and myeloid cells when added to long-term bone marrow cultures (LTBMC). Sequential immunoprecipitation and other comparisons indicate that these new antibodies recognize epitopes on Pgp-1/CD44. Moreover, a previously described antibody to Pgp-1 (13) is also inhibitory, through a mechanism that does not involve direct toxicity to progenitor cells. These findings suggest that components of the Pgp-1/CD44 complex may be critically important for cell recognition functions necessary for lympho-hemopoiesis within bone marrow.

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[1] *Abbreviations used in this paper:* G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/macrophage CSF; LTBMC, long-term bone marrow cultures; M-CSF, macrophage CSF.

Materials and Methods

Animals. BALB/c, SCID mice and LOU/MN rats were bred in our Animal Resources Center. BALB/c mice were used for LTBMC, immunofluorescence, and colony-forming cell assays at 6–10 wk of age.

Antibodies and Cell Lines. The rat mAb IM 7.8.1 (anti-Pgp-1) was kindly provided by Dr. Ian S. Trowbridge (The Salk Institute, San Diego, CA) (13). The rat mAb M17/4.2 (anti-LFA-1) and MEL-14 (anti-peripheral lymph node homing receptor) were obtained via the American Type Culture Collection (ATCC, Rockville, MD). Rat mAb 14.8 against Ly-5_{220}/CD45R was established in this laboratory (14). KM 201, KM 703, and KMC 8 mAbs were produced by fusion of the hybridoma line Sp2/0 (15) with spleen cells from LOU/MN rats immunized with the bone marrow-derived stromal cell clone, BMS2 (16). BMS2 was established in our laboratory from (C57BL/6J × DBA/2J)F_1 female mice (16). BM-2 is an anti-2,4,6-TNP mAb-producing B cell hybridoma derived from fusion of P3X63Ag8U.1 myeloma cells and BALB/c spleen cells immunized with TNP-Ficoll, and a protocol was used to select for cells with marrow homing ability. That is, hybrids were injected intravenously and recovered from the bone marrow 24 h later. This B lineage cell line adhered well to the BMS2 stromal cell clone and was used for selecting the monoclonal antibodies that are detailed in this report. Screening and selection of four new hybridomas with similar specificity used the cell adhesion assay detailed below. All rat mAbs were semipurified from ascitic fluid of SCID mice by ABx column chromatography (J. T. Baker Inc., Philipsburg, NJ). Most batches had a single predominant band on SDS-PAGE analysis.

Cell Adhesion Assay. BM-2 cells were radiolabeled by incubation of 2×10^7 cells in 1 ml complete medium with 100 μCi of $Na_2\ ^{51}CrO_4$ for 1 h at 37°C and washed three times in complete medium. The labeled cells (2×10^5/well) were then added with or without antibodies to 24-well plates (Corning Glass Works, Corning, NY) that contained adherent BMS2 stromal cells plated (2×10^4/well) the day before. The plates were incubated for 1 h at 37°C, and unbound cells were removed by three cycles of washing in prewarmed complete medium with vigorous agitation on a Minishaker (Dynatech Laboratories, Inc., Alexandria, VA) for 30 s before each aspiration. Bound cells were solubilized with 0.1 N NaOH, 1% NP-40 (Sigma Chemical Co., St. Louis, MO) and the ^{51}Cr was counted with a gamma counter (Beckman Instruments, Fullerton, CA). Percentages of bound cells were determined by dividing cpm from bound cells by input cell-associated cpm, from which spontaneous release was subtracted and multiplied by 100%.

Long-Term Bone Marrow Cultures. LTBMC of B lineage cells were prepared according to Whitlock and Witte (Whitlock-White culture) (2). Briefly, pooled cells from femora and tibiae were placed into a 25-cm^2 flask ($5–6 \times 10^6$ cells; Corning Glass Works). The culture medium consisted of RPMI 1640 (Gibco Laboratories, Grand Island, NY), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 5×10^{-5} M 2-ME, and 5% FCS (Lot No. 1111794; HyClone Laboratories, Logan, UT). LTBMC of myeloid cells were initiated and maintained by the methods described by Dexter et al. (Dexter culture) (1). Briefly, $10–13 \times 10^6$ bone marrow cells were cultured in 25-cm^2 flasks. The culture medium consisted of α-MEM (Gibco Laboratories; without ribonucleosides and deoxyribonucleosides), penicillin, streptomycin, 10^{-7} M hydrocortisone sodium salt, and 20% horse sera (Gibco). These flasks were incubated in 5% CO_2 at 33°C. In both kinds of LTBMC, half of the medium (3 ml) was replaced with fresh medium weekly. The antibodies (5 μg/ml) were added at the beginning of culture and at every feeding.

Colony-forming Assays. The GM progenitor (CFU-c) assay was performed as in our previous studies (17). Proliferation was stimulated with 10 times concentrated WEHI-3 or L cell-conditioned medium (LCCM). Bone marrow cells (5×10^4) were plated in 1-ml dishes (Miles Laboratories Research, Elkhart, IN). A cloning assay for pre-B cells was used as recently described (18) with 500 U/ml of murine rIL-7 (donated by Dr. A. Namen, Immunex Corp., Seattle, WA).

Immunofluorescence Analyses. Cells from several lymphoid organs or harvested from cultures were incubated for 20 min on ice with tissue culture supernatants containing antibodies in RPMI 1640 supplemented with 5% FCS and 0.1% sodium azide. This was followed by two washes and incubation with FITC-conjugated mouse anti-rat κ (MAR 18.5) for an addi-

tional 20 min. Propidium iodide (Sigma Chemical Co.) was added during the second incubation to detect dead cells. Labeled cells were then analyzed on a EPICS V flow cytometer (Coulter Electronics Inc., Hialeah, FL).

Cell Surface Labeling and Immunoprecipitation. Cells were surface-labeled by an *N*-hydroxysuccinimidobiotin (NHS-biotin) procedure (19). Briefly, after three washes in HBSS, cells were suspended in HBSS (pH 8.0 adjusted by 0.1 M sodium bicarbonate/carbonate, pH 8.7) at a concentration of 3×10^7/ml. NHS-biotin (Pierce Chemical Co., Rockford, IL) dissolved in DMSO (50 mg/ml) was added to cell suspensions in such that the final concentration of NHS-biotin was 2 mg/ml. After a 40-min incubation on ice with occasional shaking, cells were washed three times in chilled RPMI 1640. The cell lysates were prepared by detergent lysis at a concentration of 5×10^7/ml for 15 min on ice in a lysis buffer containing 10 mM Tris-HCl, 0.15 M NaCl, 1% NP-40, 50 mM Iodoacetamide (Sigma Chemical Co.), 1 mM PMSF (Sigma Chemical Co.), 5 mM EDTA, and 0.1% sodium azide, pH 7.5. After centrifugation, the lysates were precleared three times with 50 μl of Sepharose 4B conjugated with goat anti-rat IgG (Zymed Laboratories, San Francisco, CA) that had been incubated with either normal rat IgG, IM7.8.1, or KM201. The precleared lysates were added to 30 μl of antibody-conjugated goat anti-rat IgG-Sepharose 4B. The mixtures were rotated for 60 min at 4°C and then washed three times with a buffer containing 10 mM Tris-HCl, 0.6 M NaCl, pH 7.5. This was followed by three washes with a 10 mM Tris-HCl buffer, pH 8.8, containing 0.6 M NaCl, 0.5% SDS, and 0.05% NP-40. The absorbed proteins were released by boiling for 5 min in a sample buffer containing 0.125 M Tris-HCl, 2% SDS, 10% glycerol, and 5% 2-ME, pH 6.8. SDS-PAGE was carried out on Laemmli 10% polyacrylamide gels.

Blotting and Developing. After electrophoresis, the gels were equilibrated for 30 min in transfer buffer (0.025 M Tris, 0.192 M glycine, and 20% methanol), and proteins were electrophoretically transferred to Immobilon P Transfer Membranes (Millipore Corp., Bedford, MA) by blotting overnight at 20 mV in a Trans-Blot Cell (Bio-Rad Laboratories, Richmond, CA). Transfer membranes were then soaked for 60 min in PBS containing 5% BSA, 3% gelatin, 0.05% Tween 20, and 0.2% thimerosal (Sigma Chemical Co.), followed by a 60-min incubation in PBS containing 0.1% avidin-horseradish peroxidase (Bio-Rad Laboratories), 1% BSA, and 0.05% Tween 20. After extensive washing in PBS with 0.05% Tween 20, membranes were developed in PBS containing 20% methanol 0.6 mg/ml 4-chloro-1-Naphthol (Sigma Chemical Co.), and 0.003% hydrogen peroxide.

Results

Production and Selection of mAbs. A panel of mAbs was prepared to a bone marrow-derived stromal cell clone (16). A screening procedure was then used to obtain reagents that might detect antigens associated with cell recognition or adhesion functions. Cells from the BM-2 anti-TNP-producing hybridoma line readily adhered to BMS2 stromal cells and were easily labeled with ^{51}Cr. A more complete description of BM-2 cells and their derivation will be submitted elsewhere, but this line was originally selected on the basis of its ability to home to the bone marrow in vivo (Miyake, K., unpublished observations). Of ~250 hybrids that recognized the immunizing stromal cell line in an initial screen by ELISA, only four inhibited in the adhesion assay as demonstrated for one of them (KM 201) in Fig. 1. KMC 8 detects an antigen that is also represented in high density on BM-2 and BMS2 cells, but had no effect in the adhesion assay. It is shown as a representative negative control antibody. Similarly, mAb 14.8 did not prevent binding of BM-2 cells to stromal cells (data not shown). An anti-Pgp-1 antibody (IM 7.8.1) inhibited in the adhesion assay, but less effectively than the new KM 201 mAb (data not shown).

Tissue Distribution and Similarity to Pgp-1. Immunofluorescence analyses with the new mAbs revealed a relatively high density of antigen on the immunizing stromal cell clone, as well as on the B lineage hybridoma used for the adhesion assay (Fig.

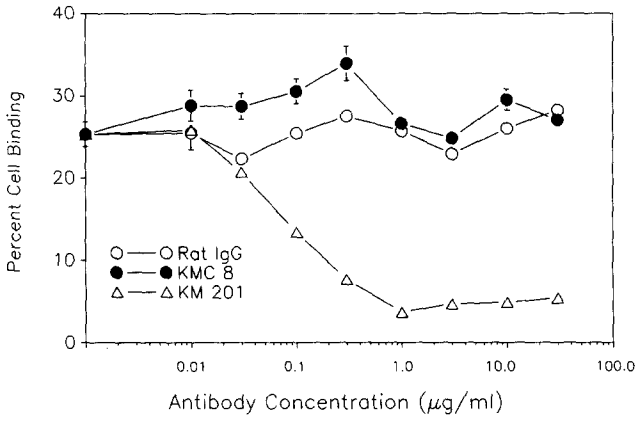

FIGURE 1. Inhibition of B lineage cell adhesion to a cloned stromal cell line by mAbs. Various concentrations of antibodies were inoculated together with ^{51}Cr-labeled BM-2 cells on monolayers of a cloned stromal cell line (BMS2) in 24-well culture plates. KMC 8 is representative of hundreds of mAbs that recognized the BMS2 stromal cell line, but did not inhibit in this adhesion assay. The results were calculated as detailed in the Materials and Methods and presented as means ± SE of triplicate wells. Total input cpm in this experiment were 43,598/well.

2). Nearly all of the nonadherent myeloid cells in Dexter-type, long-term cultures were brightly stained, and lower but significant staining was observed on most of the lymphoid cells harvested from Whitlock-Witte cultures (Fig. 2). With a more sensitive second antibody, all of the lymphocytes had above background amounts of this antigen (data not shown). Staining of normal bone marrow cells routinely revealed high and low density populations and this pattern was strikingly similar to published descriptions of Pgp-1 antibodies (13). Indeed, direct comparison of the

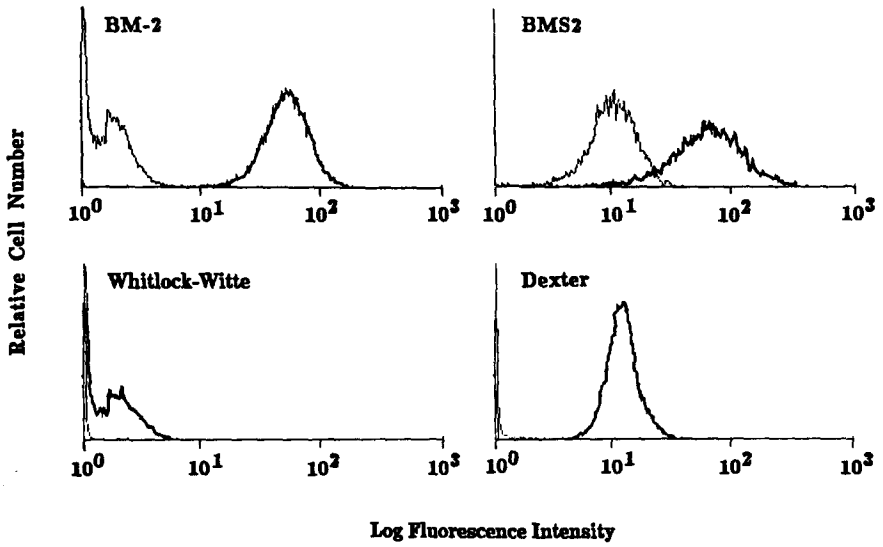

FIGURE 2. Recognition of B lineage cells, stromal cells, and nonadherent cells in LTBMC by mAb KM 201. Cells were stained and analyzed by flow cytometry as described in the Materials and Methods and the faint tracings represent staining when the primary antibody was omitted. BMS2 cells were detached from culture dishes before analysis and typically exhibit a relatively high level of background fluorescence.

mAb IM 7.8.1 to Pgp-1 with our new reagents revealed no significant differences by flow cytometry with a variety of cell types (Figure 3).

Biochemical Identification of Pgp-1 Antigen. Formal proof that our new antibodies detect epitopes on the Pgp-1 molecule was obtained by sequential immunoprecipitation. Identical size molecules were always precipitated from lysates of biotinylated BM2 cells with IM 7.8.1 and our antibodies (Fig. 4 and data not shown). With reduced samples run on 7.5% SDS-acrylamide gels, one major band corresponding to 80–95 kD was routinely observed, and a higher molecular mass species (~200 kD) was detectable in some experiments. Both of these species have been described with antibodies to Pgp-1 and the larger one is thought to be associated with chondroitin sulfate (20). On nonreducing gels, migration of the smaller species ranged from 68 to 88 kD (not shown). The presence of an intramolecular disulfide bond may account for this mobility difference as suggested by studies of human Pgp-1 (21). Preclearing of the lysates with antibody-coated beads revealed complete identity between the molecules recognized by IM 7.8.1 and our KM 201 antibodies (Fig. 4).

Inhibition of Lympho-hemopoiesis by Antibodies to Pgp-1. LTBMC provide a means to test the functional significance of cells and molecules derived from bone marrow. The original Dexter culture system permits extended propagation of neutrophil granulocytes, macrophages, and multipotential stem cells (1). B lineage lymphocytes selectively grow under the modified conditions developed by Whitlock and Witte (2). In three independent experiments, inclusion of mAbs to Pgp-1 completely prevented the production of nonadherent cells in both types of cultures (Figs. 5 and 6). Marked

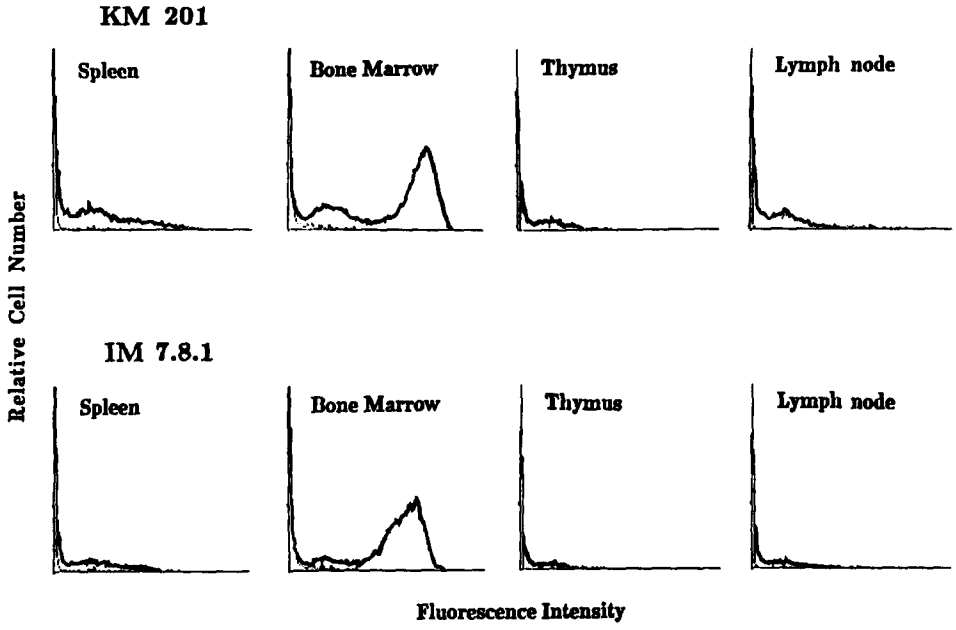

FIGURE 3. Tissue representation of antigens detected by mAbs KM201 and anti-Pgp-1. In this comparison, various freshly isolated tissues were stained with KM 201, or a previously described antibody to Pgp-1/CD44 (IM 7.8.1), and analyzed by flow cytometry.

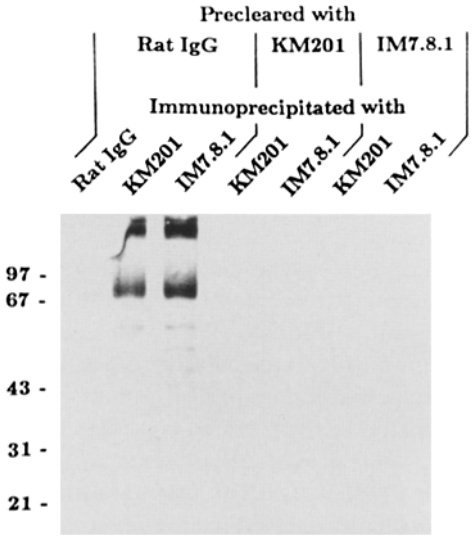

FIGURE 4. Sequential immunoprecipitation of surface membrane glycoproteins with KM 201 and anti-Pgp-1 antibodies. BM-2 hybridoma cells were biotinylated with NHS-biotin and extracts were prepared. These were precleared with beads bearing normal rat IgG and then beads coated with normal rat IgG (lane *1*), KM 201 (lane *2*), or anti-Pgp-1 (IM 7.8.1, lane *3*). Some samples were precleared with KM 201, and immunoprecipitated with KM 201 (lane *4*) or IM 7.8.1 (lane *5*). Others were precleared with IM 7.8.1 and immunoprecipitated with KM 201 (lane *6*), or IM 7.8.1 (lane *7*). Specifically bound molecules were resolved by electrophoresis on 10% SDS-polyacrylamide gels under reducing conditions and revealed by exposure to an avidin-peroxidase conjugate and development.

agglutination of Dexter cultured cells occurred with a mAb to the MEL-14 homing receptor and expansion of nonadherent cell numbers was apparently delayed by addition of M 17/4.2 antibody to the LFA-1 adhesion molecule (22, 23). However, Dexter cultures containing MEL-14 and LFA-1 antibodies were eventually productive. Likewise, these antibodies had no effect when added to Whitlock-Witte cultures (Fig. 6 and data not shown). Similarly, an antibody (14.8) to the Ly-5_{220}/CD45R common leukocyte antigen (14) did not influence growth of cells in either type of long-term culture (Figure 6 and data not shown). Suppressive IM 7.8.1 and nonsuppressive 14.8 antibodies were of the same isotype (IgG2b). Another new antibody to Pgp-1 (KM 703) was also suppressive (data not shown) and it is of the same IgG2a subclass as MEL-14 and M 17/4.2 (LFA-1) antibodies, which were without effect. The KM 201 antibody shown here is an IgG1. Therefore, inhibition of growth in long-term cultures is not dependent on a particular Ig isotype. Antibodies to various Pgp-1 epitopes could differ with respect to the efficiency with which they block lympho-

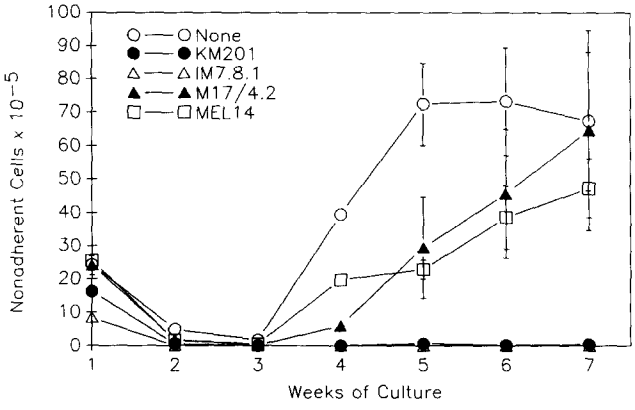

FIGURE 5. Inhibition of myelopoiesis in long-term cultures by addition of antibodies to Pgp-1/CD44. Bone marrow cells (10–12 × 10^6 cells/flask) were inoculated with the indicated semipurified antibodies (5 μg/ml) and maintained in culture under Dexter conditions. Nonadherent cells recovered in the medium removed at weekly feedings were counted and used to calculate the total number of nonadherent cells per flask. The data are presented as means ± SE for five replicate cultures.

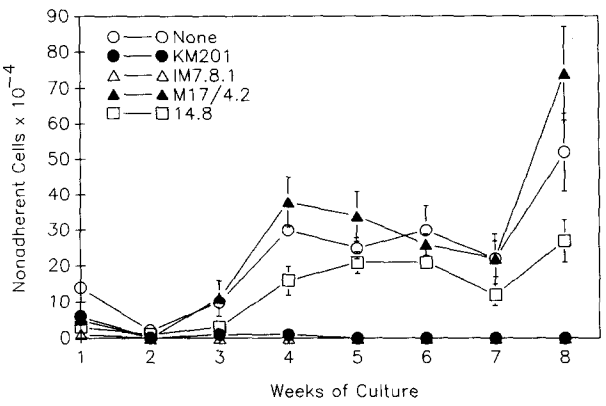

FIGURE 6. Inhibition of lymphopoiesis in long-term cultures by addition of antibodies to Pgp-1/CD44. Bone marrow cells (6–8 × 10^6 cells/flask) were inoculated and maintained with the indicated antibodies (5 μg/ml) under Whitlock-Witte conditions. The data represent means ± SE cells in six replicate flasks.

hemopoiesis. Greater than 1 μg/ml was sufficient to achieve suppression with some of the reagents, but a fraction of the cultures were productive with other antibodies at this lower concentration (data not shown). In most experiments, 5 μg of antibody/ml was added to insure saturating concentrations and this was completely suppressive with all of the anti-Pgp-1 antibodies that were tested.

Effects on Adherent Layer Formation and Progenitor Proliferation. Although lymphohemopoiesis was totally prevented by antibodies to Pgp-1, the adherent layers of LTBMC were not markedly influenced in terms of morphology (Fig. 7). Treated cultures contained fat cells and macrophages, as well as adherent cells with the appearance of stromal cells. The possibility that the antibodies might interfere with replication of myeloid and lymphoid progenitor cells was investigated with semisolid agar cloning procedures. Up to five times the concentration of antibodies used in long-term cultures had no influence on proliferation induced by IL-7 (pre-B cells) or two sources of CSF (myeloid progenitors) (Table I). These findings indicate that the antibodies to Pgp-1 are not directly toxic to hemopoietic cells or to cells that comprise the microenvironment required for their growth.

Discussion

A variety of cell surface glycoproteins may facilitate close interactions between hemopoietic progenitors and the microenvironment that regulates their proliferation and differentiation. Previous studies have implicated Pgp-1/CD44 in adhesion and cell recognition functions in peripheral lymphoid tissues. We now provide evidence with long-term cultures and mAbs that this molecule may also be critical to the development of cells in two hemopoietic lineages. Addition of mAbs to long-term cultures may provide an important experimental approach for testing the functional significance of other cell surface glycoproteins.

Pgp-1 was first identified as a major polymorphic surface glycoprotein on cultured murine fibroblasts and peritoneal exudate cells (24, 25). Later studies revealed that it is present on hemopoietic cells, and particularly abundant on myeloid cells and activated lymphocytes (13, 26). The human equivalent (CD44) was identified and shown to carry epitopes previously designated as p85, T lymphocyte-granulocyte-brain antigen, human medullary thymocyte antigen, and an erythrocyte an-

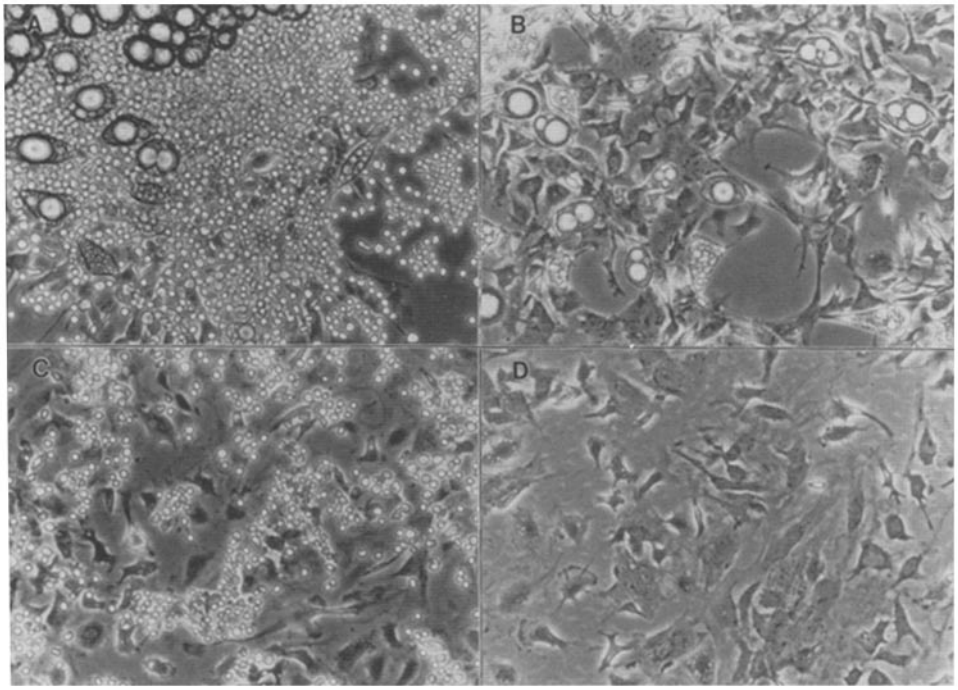

FIGURE 7. Effect of mAbs to Pgp-1/CD44 on the morphology of LTBMC. Phase contrast photomicrographs were prepared of Dexter (*A* and *B*) and Whitlock-Witte (*C* and *D*) cultures after 7–8 wk of continuous antibody treatment (*B* and *D*). Control cultures received medium alone (*A* and *C*). Original magnifications, ×250.

tigen (9, 27). Pgp-1/CD44 has been extensively used as a marker to distinguish precursors, subsets and memory cells in the T lymphocyte lineage (12, 26, 28).

More recently, identity between Pgp-1/CD44 and the Hermes class of molecule in humans was appreciated (8). The latter is thought to be involved in lymphocyte

TABLE I

Antibodies to Pgp-1 Do Not Influence Factor-dependent Colony Formation by Bone Marrow Cells

	No. of colonies/10^5 cells elicited by		
Antibodies	IL-7	WEHI-3	LCCM
None	405 ± 32	203 ± 6	192 ± 20
KM 201	379 ± 57	195 ± 13	213 ± 10
IM 7.8.1	371 ± 20	210 ± 16	189 ± 11
M17/4.2(*a*LFA-1)	410 ± 34	213 ± 16	194 ± 5
MEL14	407 ± 31	210 ± 7	186 ± 9
14.8	359 ± 25	192 ± 18	187 ± 16

Antibodies were added to three different colony assays at final concentrations of 25 μg/ml. Colonies were counted on day 6 (IL-7) or day 7 ((WEHI-3, LCCM). The number of colonies without stimulation was always <2, irrespective of addition of antibodies. Values are presented as means ± standard error for quadruplicate cultures.

recognition of high endothelial venules and the resulting effects on lymphocyte migration (29). There are also indications that Pgp-1 might participate in recognition of extracellular matrix components (10, 30, 31), and it is thought to be a transmembrane protein intimately associated with the cytoskeleton (30, 31). Molecular cloning of Pgp-1 has now been achieved and this revealed structural homology between one of its NH_2-terminal domains and a proteoglycan link protein (21, 32). Although the mAb MEL-14 raised to murine cells recognizes human Pgp-1/CD44, the murine gene is unrelated (8, 21, 32–34). This is consistent with the finding that MEL-14 did not inhibit murine long-term cultures. Perhaps this molecule is more important for interactions between cells in peripheral lymphoid tissues than those that occur within bone marrow.

Attention can now be focused on mechanisms through which antibodies to Pgp-1/CD44 inhibit long-term cultures. Direct toxicity to hemopoietic progenitors seems unlikely because much higher concentrations of antibodies had no effect on IL-7-dependent and CSF-dependent cloning assays done with semisolid agar. It remains possible that cell-mediated cytotoxicity with passively acquired antibodies is important for elimination of critical cell types in the long-term cultures. However, marked agglutination of myeloid cells in culture by MEL-14 and LFA-1 antibodies did not prevent emergence of hemopoietic cells. The presence of LFA-1 has been documented on a variety of hemopoietic precursors (6). Moreover, these two antibodies are of the same IgG2a isotype as KM 703, which is completely suppressive. Additional studies involving fragmented or immobilized antibodies may be informative in this regard. Similarly, while the adherent layers of treated cultures were not grossly affected, more subtle effects on the microenvironment can now be explored with cloned cell lines and different treatment protocols. One previous study revealed no influence of Pgp-1 antibodies on fibroblast growth or morphology (30). Stromal cells elaborate a number of cytokines (3) and effects of antibody treatment on this parameter should also be investigated.

It is probable that adhesive interactions are important for lympho-hemopoiesis in LTBMC. Indeed, preferential associations between precursors and stromal cells have been documented in previous studies (35, 36). Our new mAbs to Pgp-1 were selected on the basis of their ability to inhibit adhesion of a B lineage hybridoma cell to a cloned stromal cell line and flow cytometric analyses revealed that the antigen is expressed in relatively high density on both cell types. However, additional studies are required to determine if inhibition of close cellular interactions by anti-Pgp-1/CD44 antibodies accounts for their effects in long-term cultures. The resulting information may be informative about vital functions mediated by the Pgp-1 molecule.

Summary

A new panel of mAbs was prepared to a stromal cell line known to support lymphocytes in Whitlock-Witte type long-term bone marrow cultures. These antibodies were then screened with a cell adhesion assay and four were selected that inhibited the binding of B lineage cells to stromal cell monolayers. Immunofluorescent and biochemical analyses revealed that these new antibodies detected epitopes of the previously described Pgp-1/CD44 antigen complex. Addition of Pgp-1/CD44 antibodies to Dexter-type long-term bone marrow cultures completely prevented emergence of myeloid cells and they also blocked lymphocyte growth in Whitlock-Witte type

cultures. mAbs MEL-14, LFA-1, and CD45R did not inhibit under the same conditions and there was no apparent relationship to Ig isotype. Adherent layers in treated cultures were not unusual in terms of morphology and the antibodies did not affect factor-dependent replication of lymphoid or myeloid progenitor cells. Therefore, the mechanism of inhibition may not involve direct toxicity to precursors or microenvironmental elements. Previous studies in humans and mice have implicated Pgp-1/CD44-related glycoproteins in the migration of peripheral lymphoid cells, as well as interactions of cells with the extracellular matrix. These findings suggest that they may also be critical for formation of lymphoid and myeloid cells within bone marrow.

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