Hyaluronate Can Function as a Cell Adhesion Molecule and CD44 Participates in Hyaluronate Recognition

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Summary

A cell adhesion model was previously used to select a series of monoclonal antibodies (mAbs), which were subsequently found to recognize CD44/Pgp-1. Interest in these reagents increased with the finding that they totally inhibited production of lymphoid or myeloid cells in long-term bone marrow cultures. Further investigation has now revealed that hyaluronate is a potential ligand for CD44 and that hyaluronate recognition accounts for the adhesion between B lineage hybridoma and stromal cells. The hybridoma cells adhered to hyaluronate-coated plastic wells as well as to monolayers of stromal cells. The adhesion in both cases was inhibited by treatment with hyaluronidases, and did not require divalent cations. Addition of exogenous hyaluronate also diminished binding of lymphoid cells to stromal cells. One of several mAbs to Pgp-1/CD44 was particularly effective at blocking these interactions. Since hyaluronate and Pgp-1/CD44 were present on both cell types, experiments were done to determine the cellular location of interacting molecules required for the adhesion process. Treatment of lymphoid cells with an anti-Pgp-1/CD44 antibody was more inhibitory than antibody treatment of the stromal cells. Conversely, hyaluronidase treatment of stromal cells reduced subsequent binding more than treatment of the lymphoid cells. Adhesive interactions that involve hyaluronate and CD44 could contribute to a number of cell recognition processes, including ones required for normal lympho-hemopoiesis.

The avid binding of a B lineage hybridoma to a cloned stromal cell line recently permitted the selection of a new panel of mAbs based on their ability to inhibit this adhesion (1). Several of these reagents also blocked lympho-hemopoiesis when included in long-term bone marrow cultures, suggesting that the molecule(s) they recognize has considerable functional importance. Immunoprecipitation and other analyses revealed that these antibodies identify epitopes on the Pgp-1/CD44 glycoprotein (1). Molecular cloning was recently achieved for human and murine Pgp-1/CD44 (2–5). Of particular interest was the finding that the NH_2-terminal domain of this protein is homologous to link protein and the core protein of large proteoglycans, both of which bind to a glycosaminoglycan, hyaluronate. In addition, this domain is highly conserved between humans and mice (4), suggesting that hyaluronate might be a ligand for Pgp-1/CD44. Hyaluronate is widely distributed in extracellular matrices and has been implicated in a number of biological phenomena, including cell–cell adhesion, cell migration, embryonic development, and pathogenesis (6–8). A receptor for hyaluronate has also been described that is similar to Pgp-1/CD44 with respect to size, cellular representation, and interaction with the cytoskeleton (7, 9–16). We now report that adhesive interactions between one lymphoid cell line and certain adherent cell clones are totally dependent on hyaluronate and Pgp-1/CD44. This mechanism of cell recognition could have general importance because both molecules are widely distributed on hemopoietic and other tissues.

Materials and Methods

Cells and Cell Cultures. The BMS2 stromal cell line was established from bone marrow and has been extensively studied for its ability to support growth of stromal cell–dependent lymphocyte clones (17–20). Other adherent cell clones were isolated from either bone marrow (BMS1) or spleen (SNS1, SS1) and share some properties with BMS2 (17). BALB/3T3 and NIH/3T3 cells were obtained through the American Type Culture Collection (ATCC, Rockville, MD). BM-2 is a B cell hybridoma producing anti-TNP mAb. All of these lines were maintained in RPMI 1640 medium supplemented with 10% FCS, 50 μM 2-ME and antibiotics.

Antibodies. The rat mAb IM 7.8.1 (anti-Pgp-1) (21) was kindly provided by Dr. Ian S. Trowbridge (The Salk Institute, San Diego, CA). Rat mAbs were prepared in our laboratory using the bone marrow–derived stromal cell clone BMS2 as an immunogen (1).

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The KM 201 and KM 703 mAbs detect different epitopes on murine Pgp-1/CD44, and KMC 8.8 recognizes an unrelated antigen (Miyake, K., unpublished observations). All mAbs were semipurified from ascitic fluids of SCID mice by ABx column chromatography (J.T. Baker Inc., Philipsburg, NJ).

Reagents and Hyaluronidase Treatment. Glycosaminoglycans were obtained from the following commercial sources: hyaluronate, Sigma Chemical Co., catalog no. H-4015 (St. Louis, MO); chondroitin sulfate A (CalBiochem-Behring Corp., La Jolla, CA); chondroitin sulfate C and heparan sulfate (Seikagaku Kogyo Co., Tokyo, Japan); heparin (Organon Inc., W. Orange, NJ). For coating the tissue culture dishes, these glycosaminoglycans were dissolved in PBS (5 mg/ml), except heparin, which was used without dilution (20,000 U/ml). Testicular hyaluronidase was obtained from CalBiochem-Behring Corp. and Streptomyces hyaluronidase was purchased from ICN Immunobiologicals (Lisle, IL). These were dissolved in HBSS and used at 5,000 U/ml (testicular) and 20 TRU/ml (Streptomyces) final concentrations. The biotinylated proteoglycan probe (b-PG) for staining hyaluronate was purified from cartilage proteoglycan using the methods of Green et al. (10). BM-2 cells (5×10^6/ml) were suspended in 10% FCS RPMI 1640 with hyaluronidase for 1 hr at 37°C. Adherent cells were treated in the culture wells (2×10^4/well) with the same concentration of hyaluronidase as for BM-2 cells. When treated cells were subsequently stained with b-PG, no residual hyaluronate could be detected, indicating that the conditions for enzymatic reaction were appropriate (see below).

Detection of Membrane-bound Hyaluronate. Flow cytometry was used to evaluate expression of hyaluronate on cell surfaces. BM-2 or BMS2 cells (5×10^6/ml) were suspended in 10% FCS RPMI 1640 and aliquots were treated with 5,000 U/ml of testicular hyaluronidase for 1 h at 37°C. The suspensions were then incubated for 20 min on ice with the b-PG probe at a concentration of 1 μg/ml in RPMI 1640 containing 1% BSA and 0.1% sodium azide (10). After thorough washing, the cells were incubated with FITC-conjugated avidin (Zymed Laboratories, San Francisco, CA) for an additional 20 min. Propidium iodide (Sigma Chemical Co.) was added during the second incubation to exclude dead cells. Labeled cells were then analyzed on an EPICS V flow cytometer (Coulter Electronics Inc., Hialeah, FL).

Cell Adhesion Assay. B cell hybridoma BM-2 cells were radiolabeled by incubating 4×10^7 cells in 1 ml complete medium with 100 μCi of $Na_2[^{51}Cr]O_4$ for 1 h at 37°C and then washing three times in complete medium. The labeled cells (2×10^5/well) were added to 24-well plates (Corning Glass Works, Corning, NY). For direct adhesion to glycosaminoglycans, the plates were precoated with 5 mg/ml of the substances dissolved in PBS the day before (22), and then washed three times with PBS immediately before the addition of BM-2 cells. For BM-2 adhesion to adherent cells, adherent cells were plated (2×10^4/well) in complete medium and allowed to grow overnight before the binding assay.

For assessment of divalent cation requirements, HBSS was used supplemented with 10% FCS that had been dialyzed against PBS without divalent cations. Cells were washed in this medium three times immediately before the adhesion assay and all possible combinations of 0, 0.1, and 1.0 mM final concentrations of Ca^{2+} and Mg^{2+} added at the same time as labeled BM-2 cells. 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 lysed with 0.1 N NaOH, 1% NP-40 (Sigma Chemical Co.) and the ^{51}Cr counted with a gamma counter (Beckman Instruments Inc., Fullerton, CA). Percentages of bound cells were determined by the formula: percent bound = [(cpm from bound cells)/(input cell associated cpm − spontaneously released cpm)] × 100.

Results

Adhesion of B Lineage Hybridoma Cells to Hyaluronate. Hyaluronate can be coated on plastic dishes (22) and we exploited this to determine that lymphocytes may directly recognize and adhere to this glycosaminoglycan. Of several substances that were tested, binding was only significant in wells coated with hyaluronate (Table 1). Attachment involved up to 96% of the lymphocytes and was appreciable within 15 min of their addition to the culture plates. This binding was completely abolished when the coated plates were pretreated with Streptomyces hyaluronidase (data not shown). These results suggested that hyaluronate might participate in adhesion between two cell types such as stromal cells and lymphocytes. To determine if either of these cells bear hyaluronate, we used a biotinylated probe obtained from cartilage proteoglycan (b-PG) which has been previously shown to bind to hyaluronate with high affinity and specificity (10). Indeed, flow cytometry revealed measurable quantities of hyaluronate on the BM-2 cells, as well as on a cloned stromal cell line (Fig. 1). The specificity of binding was confirmed by hyaluronidase treatment.

Similarities between Binding of Lymphocytes to Stromal Cells and Hyaluronate. We previously selected mAbs on the basis of their ability to inhibit recognition of cloned stromal cells by the BM-2 lineage hybridoma (1). Immunochemical and other analyses revealed that these antibodies detect epitopes on the Pgp-1/CD44 glycoprotein. We therefore tested the ability of the same antibodies to block adhesion of lymphocytes to hyaluronate coated culture wells (Table 2). Binding was virtually abolished by the KM 201 antibody and slight, but consistent inhibition was observed with two other antibodies that also recognize Pgp-1/CD44 (KM 703 and IM

Table 1. *Adhesion of B Lineage Hybridoma Cells to Glycosaminoglycans*

Coated with:	Bound cpm	Percent cells bound
None	425 ± 68	1.5 ± 0.2
Hyaluronate	16,840 ± 677	63.4 ± 2.5
Chondroitin Sulfate A	576 ± 103	2.2 ± 0.4
Chondroitin Sulfate C	304 ± 12	1.1 ± 0.1
Heparan Sulfate	240 ± 21	0.9 ± 0.1
Heparin	350 ± 28	1.3 ± 0.1

The wells of plastic plates were coated with glycosaminoglycans and radiolabeled BM-2 cells added. The results were presented as mean values ± SE for quadruplicate wells. Similar results were obtained in two independent experiments. Prior treatment of hyaluronate-coated wells with Streptomyces hyaluronidase completely abolished the binding of BM-2 cells in this assay (data not shown).

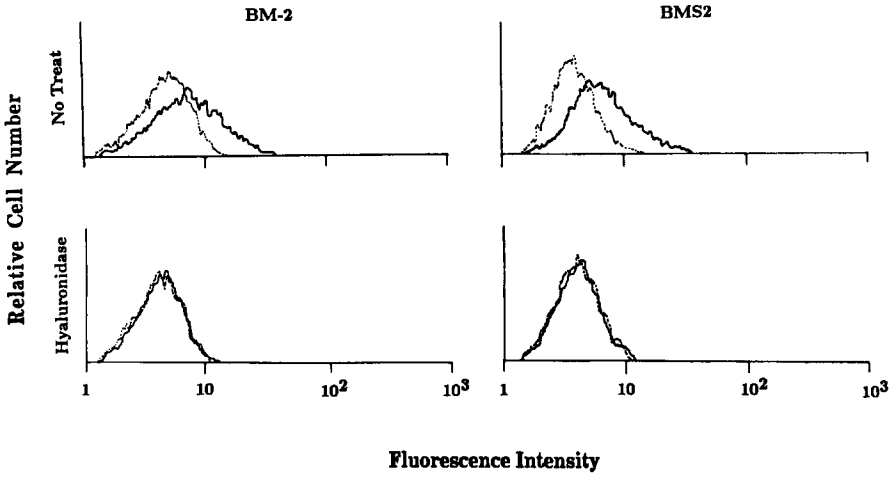

Figure 1. Demonstration of hyaluronate expression on stromal cells and hybridoma cells by flow cytometry. Cells (5×10^6/ml) were incubated with or without testicular hyaluronidase for 1 h at 37°C in complete medium, and then stained with a biotinylated proteoglycan probe as previously described (10). After washing three times, the cells were visualized with FITC-labeled avidin. Control profiles (*dotted line*) show the background fluorescence of cells that were stained with the second reagent only.

7.8.1). It should be noted that KM 201 was also the most effective of our anti Pgp-1/CD44 antibodies in blocking adhesion of lymphocytes to stromal cells (reference 1, and see Table 6 below). A pool of purified normal rat IgG, or an antibody that recognizes a different cell surface glycoprotein (KMC 8.8), had no effect. Adhesion of the BM-2 hybridoma cell line to stromal cells or hyaluronate-coated dishes was not remarkably dependent on divalent cations. Addition of 0.1 or 1.0 mM concentrations of Ca^{2+} and/or Mg^{2+} to the assay did not influence the numbers of cells that bound. Prewashing of cells and dishes with 5 mM EDTA also had no effect on the subsequent binding to hyaluronate-coated dishes (data not shown).

Hyaluronate Is an Important Mediator of B Cell Hybridoma Adhesion to Stromal Cells. When added to the medium in relatively high concentrations, hyaluronate inhibited the adhesion of hybridoma cells to stromal cells (Fig. 2). Similar amounts of another glycosaminoglycan, chondroitin sulfate A, had no effect. This result is consistent with the inability of lymphocytes to bind to chondroitin sulfate–treated dishes (Table 1) and suggests the selectivity of binding to hyaluronate. Since the inhibition of cell–cell adhesion by exogenous hyaluronate was only partial, other ligands of CD44 might be involved. Also, it was not clear from these results whether stromal cell associated, or lymphocyte membrane–associated hyaluronate was important for this interaction. To clarify these issues, the two cell types were treated separately with hyaluronidase before the binding assay (Table 3). Treatment of either the adherent stromal cell layer alone, or both the lymphocytes and stromal cells with enzyme prevented binding. Either bovine testicular hyaluronidase (Exp. 1), or Streptomyces hyalurolyticus (Exp. 2) derived enzymes were effective. Hyaluronidase treatment had no effect on the density of CD44 on lymphocytes (results not shown). Furthermore, neither enzyme caused significant cell death, as assessed by chromium release from labeled BM-2 cells or inspection of adherent layer cells by phase-contrast microscopy (data not shown). There was a small, but reproducible reduction in the extent of binding when only the hybridoma cells were enzyme treated. These

Table 2. *Antibodies Block Adhesion of Hybridoma Cells to Hyaluronate*

mAb (5 μg/ml)	Bound cpm	Percent cells bound
None	23,170 ± 980	45.3 ± 1.9
Rat IgG	23,088 ± 608	45.2 ± 1.2
KMC 8.8	22,350 ± 1021	43.7 ± 2.0
KM 201	441 ± 165	0.8 ± 0.3
KM 703	15,160 ± 3605	29.7 ± 7.1
IM 7.8.1	17,844 ± 3184	34.9 ± 6.2

Hyaluronic acid (5 mg/ml) was coated on the bottom of plastic wells, and radiolabeled BM-2 was added with the purified antibodies. The values were presented as the means ± SE for quadruplicate wells, and the result was confirmed for reproducibility. Adhesion of BM-2 cells to uncoated wells averaged 405 ± 90.

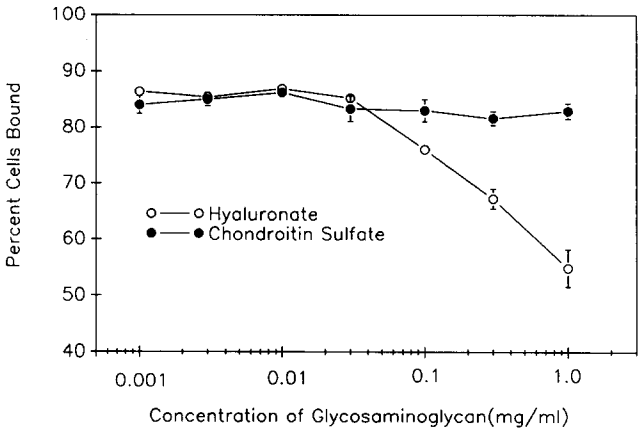

Figure 2. Inhibition of cell adhesion by addition of exogenous glycosaminoglycans. BM-2 cells were added to monolayers of BMS2 along with the indicated amounts of hyaluronate or chondroitin sulfate A. The results are presented as mean values ± SE for triplicate determinations.

Table 3. *Hyaluronidase Treatment Affects Interaction of Hybridoma and Stromal Cells*

Exp.	Hyaluronidase treatment BM-2	Hyaluronidase treatment BMS2	Bound cPM	Percent cells bound
1	–	–	34,815 ± 437	84.9 ± 1.1
	+	–	20,719 ± 1385	54.7 ± 3.7
	–	+	473 ± 47	1.2 ± 0.1
	+	+	528 ± 48	1.4 ± 0.1
2	–	–	38,718 ± 376	89.1 ± 0.1
	+	–	30,090 ± 402	62.9 ± 0.1
	–	+	1,400 ± 133	3.2 ± 0.1
	+	+	2,081 ± 40	4.3 ± 0.1

Radiolabeled BM-2 (5 × 10^6/ml) and BMS2 (2 × 10^4/well) cells were treated with testicular hyaluronidase (5,000 U/ml) in Exp.1 or Streptomyces hyaluronidase (20 TRU/ml) in Exp.2 for 1 h at 37°C, and then used in the adhesion assay after washing three times in complete medium. Treatment with these enzymes did not contribute to spontaneous chromium release (data not shown).

results strongly suggested that hyaluronate is a functional ligand for recognition between these two cell types and that hyaluronate associated with stromal cell membranes is particularly important. The receptor for hyaluronate might be Pgp-1/CD44, which is well represented on the hybridoma cells (1). Consistent with this interpretation, pretreatment of hybridoma cells with the KM 201 mAb was consistently more effective than pretreatment of stromal cells (Table 4). The inhibition was even higher when both cell types were antibody treated, or when the antibody was continually present in the culture medium.

Participation of Hyaluronate in Recognition Between Other Cell Types. Both the cell surface glycoprotein Pgp-1/CD44 and its putative ligand hyaluronate are widely distributed in various organs, and multiple adhesion mechanisms involving lymphocytes have previously been described (6, 7, 14–16). The particular combination of BM-2 hybridoma and BMS2 stromal cells might not be representative of binding between cells taken from other tissues. Therefore, we used a panel of adherent spleen-, embryo-, or bone marrow–derived stromal cells to determine the relative importance of this particular cell adhesion mechanism. A substantial fraction of the BM-2 cells bound to each of these types of adherent cells (Table 5). However, hyaluronidase treatment revealed marked differences in the degree to which hyaluronate was involved. The combination of BM-2 and BMS2 cells was most inhibited, whereas the interaction between BM-2 cells and another marrow-derived stromal cell clone, BMS1, was only slightly affected. As might be predicted, the adhesion to BMS1 was also relatively resistant to treatment with the KM 201 antibody (Table 6). In other preliminary experiments, the binding

Table 4. *Pretreatment with Antibodies to Pgp-1/CD44 Affects Interaction of Hybridoma and Stromal Cells*

KM 201 pretreatment BM-2	KM 201 pretreatment BMS2	Bound cpm	Percent cells bound	Percent inhibition
–	–	23,120 ± 856	51.6 ± 2.1	–
+	–	9,586 ± 338	23.2 ± 0.8	55.0
–	+	17,505 ± 1229	42.4 ± 3.0	17.8
+	+	6,195 ± 202	15.0 ± 0.5	70.9
Continuous	Addition	2,457 ± 89	5.9 ± 0.2	88.6

BM-2 hybridoma cells or adherent layers of BMS2 stromal cells were treated separately with purified KM 201 antibodies (10 μg/ml) in complete medium for 30 min at 4°C and then washed three times before use in the adhesion assay. The results are means ± SE of quadruplicate determinations. This experiment is representative of three independent experiments.

of pre-B lymphoma cells to stromal cells was less avid than observed with the BM-2 hybridoma (K.M., unpublished observations).

These results suggest that multiple mechanisms can potentially contribute to the binding strength between lymphoid cells and fibroblast-like cells. One of these involves cell surface hyaluronate and Pgp-1/CD44.

Discussion

The direct interaction between a glycosaminoglycan, hyaluronate, and the cell surface glycoprotein, Pgp-1/CD44, accounts for most, if not all, of the binding between a B

Table 5. *Differential Effects of Hyaluronidase Treatment on Other Adherent Cell Lines*

Adherent cell	Percent cells bound: No treatment	Percent cells bound: Hyaluronidase	Percent inhibition
BMS1	79.1 ± 1.1	66.0 ± 3.1	16.6
BMS2	81.5 ± 1.0	7.1 ± 0.1	91.3
SS1	80.7 ± 1.3	50.0 ± 0.5	38.0
SNS1	59.4 ± 1.0	38.3 ± 2.2	35.5
NIH/3T3	85.6 ± 1.3	17.5 ± 0.7	80.0
BALB/3T3	80.4 ± 1.6	10.4 ± 0.8	87.1

Radiolabeled BM-2 (5 × 10^6/ml) and adherent cell lines (2 × 10^4/well) were treated with testicular hyaluronidase (5,000 U/ml) for 1 h at 37°C in complete medium. These cells were then used in the adhesion assay after washing three times. The results are presented as mean values ± SE for quadruplicate determinations.

Table 6. *Contribution of Pgp-1/CD44 to Binding of Hybridoma Cells to Other Adherent Cell Lines*

Adherent cell	Percent cells bound with the following antibodies:				
	None	Rat IgG	KM 201	IM 7.8.1	KM 703
BMS1	51.4 ± 1.5	50.3 ± 1.7	27.0 ± 0.3	42.9 ± 1.2	52.0 ± 2.4
BMS2	35.2 ± 0.7	31.4 ± 0.3	7.7 ± 0.5	22.1 ± 0.6	23.4 ± 1.3
SS1	40.9 ± 1.9	36.9 ± 2.8	12.1 ± 0.8	27.9 ± 1.8	31.9 ± 0.3
SNS1	40.8 ± 2.6	38.4 ± 1.7	12.7 ± 0.6	22.0 ± 1.0	29.1 ± 1.8
NIH/3T3	34.5 ± 0.7	32.1 ± 1.1	9.7 ± 0.8	21.4 ± 1.2	25.2 ± 2.1
BALB/3T3	51.1 ± 1.1	50.5 ± 2.1	12.0 ± 0.3	31.5 ± 1.0	37.4 ± 1.1

Adherent cell lines were placed in 24-well plates (2×10^4/well) 1 d before assay. Radiolabeled BM-2 were added with the indicated antibodies (10 μg/ml) and washed out after 30 min incubation at 37°C. Results are presented as mean values ± SE for quadruplicate determinations.

lineage hybridoma and a bone marrow–derived stromal cell line. The hybridoma quickly attached to hyaluronate-coated plastic dishes and this binding was abolished by a mAb to Pgp-1/CD44. Furthermore, the adhesion of hybridoma to cloned stromal cells was blocked by hyaluronidase treatment, exogenous hyaluronate, or the mAb. Finally, the binding mediated by these two cell-associated molecules was independent of divalent cations, as has been previously described for a hyaluronate receptor (23). These findings suggest one possible mechanism for cell–cell recognition.

The Pgp-1/CD44 molecule has been independently detected in multiple species and in association with a variety of biological phenomena (see reference 16 for a review). CD44 was originally described as a membrane glycoprotein on human T cells (24) and the murine homologue (Pgp-1) first derived from a study of membrane glycoproteins on 3T3 cells (25). Subsequent studies revealed a broad tissue distribution and some size heterogeneity of this molecule (14–16). In mice, Pgp-1/CD44 expression has been correlated with maturation and function of T lymphocytes (26). A subset of antibodies to human CD44 block interaction of lymphocytes with high endothelial venules (27), and at least some of these glycoproteins act as receptors for extracellular matrix proteins (28). Of particular relevance to this study was the recent finding that Pgp-1/CD44 has an NH_2-terminal domain with structural features of link protein and the core proteins of large proteoglycans, both of which can bind to hyaluronate (22).

Treatment with anti-Pgp-1/CD44 mAbs or hyaluronidase, as well as addition of exogenous hyaluronate, inhibited binding between BM-2 hybridoma and BMS2 stromal cells. Thus, both Pgp-1 and hyaluronate seem to participate in the adhesion phenomenon. Both cell types used in our adhesion model express hyaluronate, as well as Pgp-1/CD44 (reference 1 and Fig. 1). However, separate antibody treatment of BM-2 cells was more effective than treatment of BMS2 cells in reducing binding in the adhesion assay (Table 4). The reciprocal result was consistently obtained by separate hyaluronidase treatment of these two cell types. That is, enzyme treatment of BMS2 cells influenced binding more dramatically than treatment of BM-2 cells (Table 3). However, immunochemical studies of Pgp-1/CD44 extracted from the two cell types did not reveal obvious structural differences (reference 1 and Miyake, K., unpublished observations).

A simple interpretation of our findings is that Pgp-1/CD44 on our B lineage hybridoma cells recognizes and directly binds to hyaluronate on the BMS2 stromal cell line. Binding of BM-2 cells to hyaluronate-coated plates is consistent with this notion, as are recent findings that certain other Pgp-1/CD44-bearing cells adhere to such dishes, bind fluorescein-labeled hyaluronate, or are agglutinated by it. All of these interactions were inhibitable by anti-Pgp-1/CD44 antibodies (29). Furthermore, the previously described hyaluronate receptor is similar in size, divalent cation independence, and distribution to Pgp-1/CD44 (6, 7, 9–15, 23), suggesting that the two are either closely related or identical. Indeed, one mAb to Pgp-1/CD44, KM 201, completely blocked binding of radiolabeled hyaluronate to the hyaluronate receptor on SV-3T3 cells (Underhill, C.B., unpublished observations).

It is important to stress that other interpretations of these findings are possible. For example, Pgp-1/CD44$^+$ cells might interact with Pgp-1/CD44$^+$ cells via a low affinity, homotypic interaction that is stabilized by hyaluronate. Another, more complex explanation would be that the link protein–like domain of Pgp-1/CD44 stabilizes interactions between hyaluronate and other glycosaminoglycans. Chondroitin sulfate is covalently attached to some isoforms of Pgp-1/CD44 (30). However, we found no evidence for participation of chondroitin sulfate in our adhesion assay (Table 1 and Fig. 2). Furthermore, the binding was completely inhibited by Streptomyces hyaluronidase, which is thought to be highly specific for hyaluronate (31). Our findings also do not exclude the possibility that in some circumstances hyaluronate and Pgp-1/CD44 might interact with each other on the same cell surface.

In addition to hyaluronate, there are probably other ligands for Pgp-1/CD44, including an endothelial cell glycoprotein and certain forms of collagen (14, 28). Other well studied adhesion molecules are known to have multiple ligands

(32–34). Moreover, a recent study revealed that some Pgp-1/CD44-bearing hemopoietic cells do not bind to hyaluronate (29). It is as yet unclear if different transcription products and/or postsynthetic modifications will correspond to different binding specificities of this molecule. Alternatively, competition between ligands and differences in their availability might govern the outcome of interactions with Pgp-1/CD44. We and others have prepared panels of mAbs to this glycoprotein and the unique epitopes they recognize may be instructive in this regard. The KM 201 antibody was a potent inhibitor in the cell adhesion experiments described in this report (Table 2 and reference 1), and might preferentially interact with a putative hyaluronate binding domain of Pgp-1/CD44.

Mechanisms responsible for holding the various types of hemopoietic cells in close association with the inductive microenvironment are potentially complex and a number of candidate molecules have been identified (35–39). Our results show that adhesion of the B cell hybridoma was not limited to bone marrow–derived stromal cell clones but also occurred with adherent cell lines derived from spleen or embryos (Table 6). Interactions with some of these cells were relatively resistant to Pgp-1/CD44 antibody or hyaluronidase treatment, suggesting that additional adhesion mechanisms are involved. Also, it has previously been found that some of the lymphoid cells detach from the adherent layers of long-term bone marrow cultures after treatment with chelating agents or phosphatidylinositol-specific phospholipase C (40 and Kincade, P.W., unpublished observations). Neither of these results are consistent with Pgp-1/CD44–hyaluronate-mediated adhesion. Thus, while adhesion mediated by these two molecules might be very important to hemopoiesis and other functions, it is likely that other mechanisms contribute to the specificity and degree of binding required for development and cell migration. It is important to determine the molecular basis of these critical cell recognition processes and determine how each is used and modulated in discrete sites.

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