

Identification of a 107-kD Glycoprotein that Mediates Adhesion between Stromal Cells and Hematolymphoid Cells

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Summary

The mechanism of cell complex formation between lymphocytes and stromal cells was investigated. We found that lymphoid lines of both T and B lineages could form cell complexes with stromal cells from the thymus as well as bone marrow but not with macrophages or typical fibroblast lines. Formation of these cell complexes is temperature dependent and requires the presence of Mg^{2+} , active cellular metabolism, and microfilament assembly of cytoskeleton. We raised an antiserum against a thymic stromal cell clone (BATE-2) in rats and found that, after absorption, this serum could effectively block cell complex formation between lymphocytes and stromal cells from both thymus and bone marrow. An efficient blocking was obtained only when the antiserum was added at the initial stage of cell interaction. From the blocking experiments and the SDS-PAGE analysis of immunoprecipitated materials from the stromal cell surface, we identified a unique 107-kD glycoprotein on the stromal cells as a molecule for mediating stromal cell-lymphocyte interaction. This is further supported by the findings that an antiserum raised in hamsters against the excised gel band corresponding to 107 kD, which specifically immunoprecipitated the 107-kD molecule, effectively blocked the lymphocyte-stromal cell interaction. The possible function of this molecule in hematolymphoid development is discussed.

The growth and differentiation of myeloerythroid and lymphoid cells from stem cells is strictly regulated by the microenvironmental elements of their generative organs. Long-term bone marrow cultures (LTBMC)¹, first described by Dexter et al. (1), result in the establishment of the major elements of hematopoiesis: the bone marrow stromal microenvironments and the hematopoietic cells that respond to them. Whitlock and Witte (2) developed a modified LTBMC that supports the differentiation of lymphoid progenitors into pre-B and B cells. The microenvironmental stroma of both types of LTBMC appears to be made up of fibroblastoid and endothelial cells (1-6). Recently, functional stromal cell lines have also been established from the thymus, and these stromal cells can support at least a part of the developmental pathway of immature thymocytes (7). Further, stromal cell clones es-

tablished from the spleen have been shown to support the differentiation of erythroid precursors to mature erythrocytes in the presence of erythropoietin (8). Taken together, these results suggest that a given pathway of hematopoietic differentiation must be regulated by distinctive types of stromal cells.

The regulation of hematopoiesis might be controlled by cell-to-cell and/or cell-to-extracellular matrix (ECM) adhesion (3-6, 9, 10), and by the elaboration of various cytokines (11). When hematolymphoid precursor cells are cocultured with stromal cells, some of the cells attach and actively crawl underneath the stromal cell surface, resulting in the formation of multicellular complexes between precursors and stromal cells. This phenomenon has been described in the thymic stromal cell-lymphocyte interactions and termed as pseudoemperipoiesis (12). Although the mechanisms and functional significance of this interaction have not been clarified, this kind of cell interaction seems to be an important step for stem cells and hematopoietic precursors to differentiate to a committed lineage, since this multicellular complex has gener-

¹ Abbreviations used in this paper: CAM, cellular adhesion molecule; ECM, extracellular matrix; GM-CSF, granulocyte/macrophage colony-stimulating factor; LTBMC, long-term bone marrow culture.

ally been observed in various hematopoietic and lymphopoietic systems *in vitro* (3, 6). Cell adhesion molecules might be involved in this cellular interaction, and several cell adhesion molecules are known to be expressed on stromal cells and hematopoietic precursors (13–15). Recently, an antibody to a murine cell surface glycoprotein, CD44, also known as Pgp-1, was found to block the production of lymphoid and myeloid cells when added to some types of LTBM (16), suggesting that this molecule plays an important role for inducing hematopoiesis. It is not clear, however, how the antibody exerts its inhibitory activity on the cell interaction, because Pgp-1 glycoprotein is expressed on both lymphoid/myeloid precursors and stromal cells (16). Heparan sulfate, a component of ECM proteoglycans is expressed on bone marrow stromal cells (3); this molecule has been suggested to bind soluble factors, such as IL-3 and granulocyte/macrophage colony-stimulating factor (GM-CSF) (9, 10), providing at least one function of ECM molecules in hematopoiesis.

Stromal cells have been shown to produce a number of cytokines, including macrophage CSF (M-CSF), GM-CSF, IL-6, and IL-7 (17–19). These factors support the growth of factor-dependent cell lines without stromal cells *in vitro*. However, in *in vivo* conditions, even if these factors are produced, they are likely to be rapidly removed and/or diluted out by blood and/or lymph circulation. Thus, short-range interactions (cell contact) between stromal cells and hematopoietic precursors may be necessary for the specific regulation of hematopoiesis to occur. In the present study, we demonstrate that lymphoid tumor cell lines can make multicellular complexes with stromal cells, and use this model to analyze the characteristics of this cell complex formation. By the use of polyclonal antisera raised against these stromal cells and absorbed to monospecificity, we identified a large cell surface molecule consisting of 107-kD glycoprotein subunits as a mediator of such an adhesive event. The possible relationship and/or identity of this molecule with known stromal/endothelial adhesion molecule is discussed.

Materials and Methods

Cell Lines. The cloned thymic stromal cell lines (BATE-2, BATE-5, BATE-10, and BATE-12) were established from the cultures of day 14 fetal thymus fragments of C57BL/Ka mouse (S. Heimfeld, manuscript in preparation). The stromal cell line TSt-thymo was established from the thymus of preleukemic B10.thy-1.1 mouse irradiated with split doses of X-ray (Watanabe et al., manuscript in preparation). The stromal cell line LDF-1 was established from mesenteric lymph nodes (B. Holzmann, unpublished results). Bone marrow stromal cell lines AC-6 (4) and ST2 (5) were established from LTBM. These stromal cell lines were established by using Whitlock-Witte-type culture conditions (2) and cloned by limiting dilution. Fibroblast lines (NIH-3T3, LTK⁻, and Cos-7) and monocyte/macrophage lineage lines (J774.1 and RAW-264.7) were obtained from the American Type Culture Collection (Rockville, MD). T cell lines (TK-1, TK-5, VL-3, RPL-1, AKR-179, 5C2, 16C1, and C6VL.B) and pre-B cell lines (70Z/3, DW8, SCID7 and DW34) were also used (5, 20). Cell lines were maintained in tissue culture using RPMI 1640 (Irvine Scientific, Santa Ana, CA) with 5% FCS.

Measurement of Cell Complex Formation. For measuring the for-

mation of cell complexes between lymphocytes and stromal cells, 10⁴ stromal cells in 100 μ l of RPMI 1640 containing 5% FCS (5% FCS-RPMI) were precultured overnight in a microtiter plate (3596; Costar, Cambridge, MA). After washing with warm RPMI 1640, the wells were overlaid with 100 μ l of 2% FCS-RPMI containing 3×10^5 indicator lymphocytes, which were radiolabeled with Na₂⁵¹CrO₄ (⁵¹Cr, New England Nuclear, Boston, MA) as described (21). Cells were cultured for 0.5–8 h at 37°C in 5% CO₂ in air. At the end of incubation time, each well was flushed with 200 μ l of warm RPMI 1640 with a multichannel pipet, and flicked down vigorously to remove the washing medium. This procedure was repeated several times and the wells were microscopically checked for the absence of lymphocyte on the stromal cells or plastic surface. Cells remaining in the wells were then lysed by adding 200 μ l of lysing buffer consisting of 1% Triton X-100 in 0.1 M NaOH solution, and the radioactivity of ⁵¹Cr was counted in a gamma counter. Spontaneous release of ⁵¹Cr from the labeled lymphocytes (within the range of 5.0–10% of total input cpm) was counted and subtracted from the counts in all the assay cultures. Triplicate cultures were set up for each experimental group and the arithmetic means plus or minus standard errors were calculated.

When the effects of antisera on the cell complex formation were tested, the monolayer of stromal cells was pretreated with 100 μ l of appropriate dilutions of antiserum for 30 min at room temperature before the addition of indicator lymphocytes. In the experiments to investigate the time course of inhibition, antiserum was added to the stromal cell monolayer at the same time or various times after the addition of indicator lymphocytes.

Production of Antiserum. For raising antiserum against BATE-2 cells, Fisher rats were immunized subcutaneously with BATE-2 stromal cells together with CFA (Difco Laboratories, Detroit, MI), and then boosted four times with intact BATE-2 cells intraperitoneally every 2 wk. Serum samples were collected individually and checked for the reactivity against BATE-2 cells by FACS (a registered trademark of Becton Dickinson & Co.) analysis. The final immunization was performed by injecting BATE-2 cells with IFA, and serum was collected 3 wk later. The serum was heat inactivated and extensively absorbed with RPL-1 cells, normal thymocytes, and erythrocytes before use.

For the production of a monospecific antiserum against cell surface 107-kD molecules, TSt-thymo stromal cells ($\sim 10^9$) were lysed with 5 ml of lysing buffer consisting of 1.0% Triton X-100, 0.15 M NaCl, 2 mM PMSF (Sigma Chemical Co., St. Louis, MO), and 10 U/ml of aprotinin (Sigma Chemical Co.) in 10 mM Tris-HCl buffer (pH 8.0) for 1 h on ice. The lysate was spun down at 15,000 g for 15 min, and mixed 1:1 with 4% SDS sample buffer in the presence of 2-ME (2%). One-fourth of the extract was loaded onto a 2-mm-thick SDS-polyacrylamide gel, and the gel was run for 6 h at 40 mA. The gel was stained with 0.01% Coomassie brilliant blue and fixed in 2% glutaraldehyde solution for 1 h. After destaining overnight, the gel band corresponding to 107 kD was excised and homogenized with CFA. The mixture was then injected subcutaneously into Syrian hamsters. Subsequently, they were injected intraperitoneally three times with the excised band without adjuvant every 2 wk. The serum was collected 2 wk after the final boost and heat inactivated before use.

Radioiodination of Stromal Cells and Extraction. Monolayer cultures of stromal cells in five 10-cm tissue culture dishes were scraped with rubber policeman, washed three times with HBSS (Irvine Scientific), and then radiolabeled with 1 mCi of Na¹²⁵I (New England Nuclear) by using the glucose oxidase-coupled lactoperoxidase method as described by Pink and Ziegler (22). Cells were then lysed with 50 mM Tris-HCl buffer (pH 7.4) containing 1.0% Triton

X-100, 0.15 M NaCl, 2 mM PMSF, and 10 U/ml of aprotinin for 1 h at 4°C. The lysate was centrifuged at 15,000 *g* for 15 min and precleared with an equal volume of Pansorbin cells (Calbiochem-Behring Corp., San Diego, CA) and subjected to immunoprecipitation.

Immunoprecipitation and SDS-PAGE Analysis. Immunoprecipitation of radiolabeled cell surface molecules with protein A-coupled Sepharose CL-4B beads (Sigma Chemical Co.) was performed as described (20). Briefly, protein A-Sepharose beads (10 μ l) were incubated with sufficient amount of rabbit anti-rat or anti-hamster IgG antiserum (Pel-Freeze Biologicals, Rogers, AR) for 2 h at room temperature. After washing the beads with PBS, immune rat or hamster antiserum was added to the beads and incubated for 2 h at room temperature, and the beads were blocked with 1% BSA in PBS for 1 h. The beads were then incubated with ¹²⁵I-labeled precleared lysate (10⁷ cpm) for 5–6 h at 4°C, and extensively washed with 1.0% Triton X-100 in 10 mM Tris-HCl buffer, pH 8.0. Samples for SDS-PAGE were prepared by boiling 2 min in the presence of 2% SDS with (reducing conditions) or without (nonreducing conditions) 1% 2-ME, and analyzed on 10% polyacrylamide gels. Molecular weights of proteins were estimated by comparison to the molecular weight standards (Bio-Rad Laboratories, Richmond, CA).

Endo-glycosidase Digestion. Endo-glycosidase F digestion of immunoprecipitated materials was done as described (23). After the immunoprecipitation, protein A beads were washed four times with lysing buffer and suspended in the elution buffer containing 1% SDS, 1% 2-ME, and 1% NP-40 in 10 mM Tris (pH 6.8). The samples were boiled for 2 min, and the supernatant-containing immunoprecipitated materials were then digested with varying units of endo- β -*N*-glycosidase F (Endo F; New England Nuclear) for 2 h at 37°C in the reaction buffer consisting of 1% NP-40, 1% 2-ME, and 56 mM EDTA in 0.1 M phosphate buffer (pH 6.1). Digested samples were boiled and loaded onto the 10% polyacrylamide gel for the analysis in SDS-PAGE.

Results

Adhesion between Stromal Cells and Hematolymphoid Lines Can Be Measured In Vitro. The formation of multicellular complexes (pseudo-emperipoiesis) between various hematolymphoid lines and stromal cell lines was compared in Fig. 1. In Fig. 1 *a*, the formation of complex between lymphoid cell lines and the thymic stromal cell clone BATE-2 are shown. Whereas lymphoid lines showed various levels of activities, T cell lines RPL-1 and TK-1 (a cloned cell line from preleukemic thymus of irradiated C57BL/Ka mouse and a spontaneous thymic lymphoma of AKR mouse, respectively) and a B cell line, DW8 (derived from Whitlock-Witte-type LT BMC), showed the highest activity of binding to stroma (with no nonspecific binding to the plastic surface), thus presenting useful indicator cells for measuring the activity of cell complex formation. VL-3 and 70Z/3 lines bound to wells with or without stromal cells, while the cell lines (5C2 and C6VL.B) did not bind to the stromal cells. It is notable that cell lines of both T and B lineages showed the binding to thymic stromal cells.

Using RPL-1 cells, we compared the binding of this line to various stromal cell lines, fibroblasts, and macrophage lines. The results are shown in Fig. 1 *b*. High levels of binding were observed with thymic stromal cell lines (BATE-2, -5,

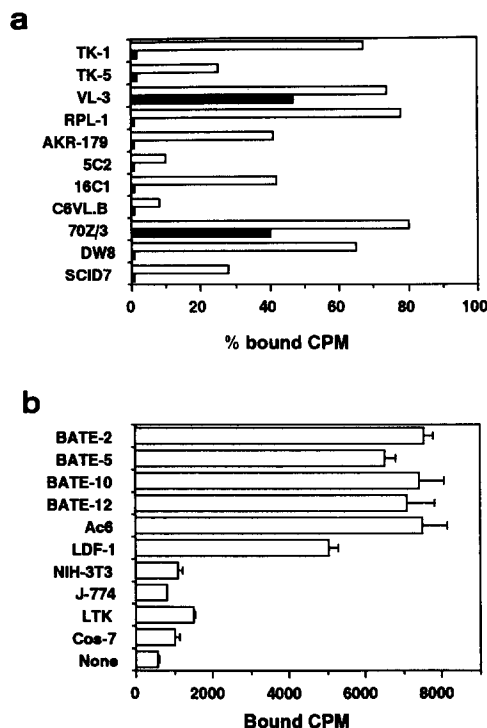


Figure 1. Cell complex formation in various combinations of lymphoid and stromal cell lines. (a) After an overnight preculture of BATE-2 cells (10⁴) in a microplate, 3 × 10⁵ cells of ⁵¹Cr-labeled T and B lymphoid cell lines (15,000–25,000 cpm) were added to the monolayer of BATE-2 cells (open columns) or to the control wells without BATE-2 cells (closed columns), and incubated at 37°C for 1 h. The microplate was extensively washed with warm RPMI 1640, and cells remaining in the wells were then lysed in 1% Triton X-100 in 0.1 M NaOH solution. Bound counts per minute was calculated and presented as percentages of total input counts per minute of lymphocytes. (b) Stromal cell lines of various origins, fibroblasts, and a macrophage cell line (10⁴) were precultured in a microplate, ⁵¹Cr-labeled RPL-1 cells (3 × 10⁵, 15,000 cpm) were cocultured for 1 h, and the radioactivities were counted after the extensive washings.

-10, and -12) as well as with stromal cell lines derived from bone marrow (AC-6) and mesenteric lymph node (LDF-1), whereas the binding activities to typical fibroblast lines (NIH-3T3 and LTK⁻), a macrophage line (J-774.1), and a monkey fibroblast line (Cos-7) were very low.

In Fig. 2, we characterized several functions of cell complex formation using RPL-1 cells and BATE-2 stromal cells. The binding was detectable by 0.5 h and reached maximum level at 4 h after the initiation of assay (Fig. 2 *a*). This activity is temperature dependent, in that an optimal response can be seen at 37°C (Fig. 2 *b*). We also analyzed whether this interaction depends on energy metabolism and a functional cytoskeleton. As shown in Fig. 2 *c*, this cell interaction was markedly inhibited by the addition of sodium azide plus 2-deoxy-D-glucose, or cytochalasin B, demonstrating the dependence on both energy and a functional cytoskeleton. Since it has been shown in many cell adhesion systems that the presence of divalent cations are required for binding of cells to extracellular matrices (24) or for homotypic and heterotypic cell conjugate formation (25), we next investigated

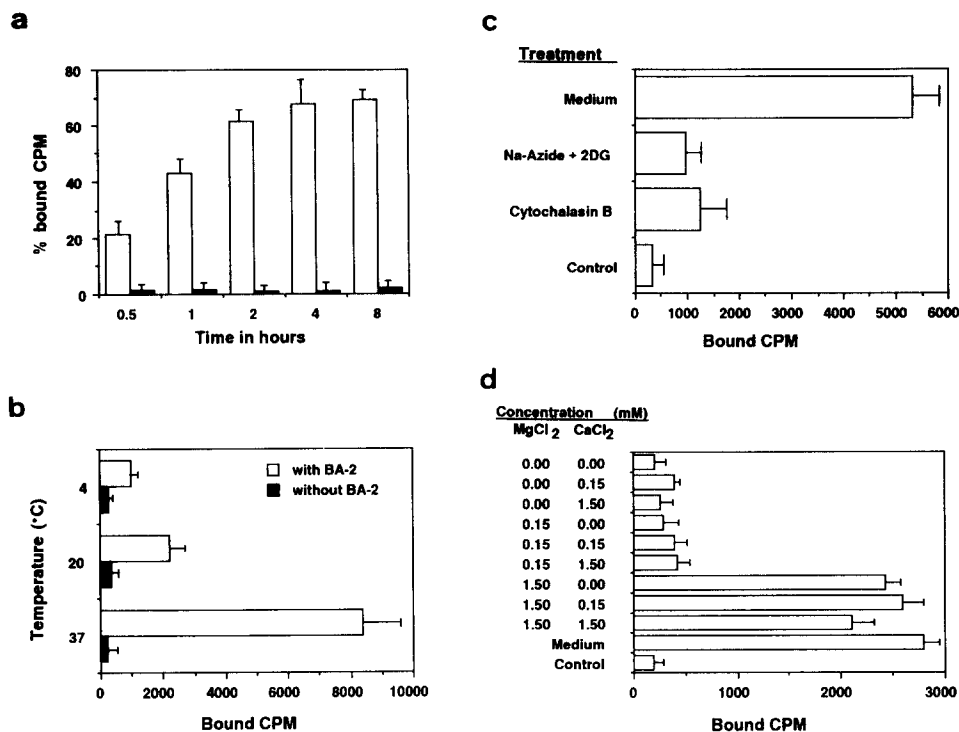


Figure 2. Characterization of cell complex formation between RPL-1 and BATE-2 cells. (a) Time course of cell complex formation. ⁵¹Cr-labeled RPL-1 cells were added to the wells precultured with BATE-2 cells (*open columns*) or to wells without BATE-2 cells (*closed columns*), and the cell complex formation activity was measured on 0.5, 1, 2, 4, and 8 h later. (b) Temperature dependence. ⁵¹Cr-labeled RPL-1 cells were cocultured with BATE-2 stromal cell monolayer for 1 h at 4, 20, or 37°C, and the bound counts per minute was calculated as the percentages of input counts per minute. (c) Requirement for energy metabolism and cytoskeleton function. Assay cultures of complex formation between RPL-1 and BATE-2 were set up in the presence of 10 mM sodium azide plus 50 mM 2-dideoxy-D-glucose, 20 μM cytochalasin B, and medium alone, as a control. ⁵¹Cr activities remaining in the wells were measured after 1 h of incubation. (d) Requirement for Mg²⁺ cation. After an overnight preculture of BATE-2 stromal cells, the monolayers were washed three times with 200 μl of Ca²⁺ and Mg²⁺-free HBSS, and ⁵¹Cr-labeled RPL-1

cells (3×10^5) were coincubated with stromal cell monolayers in the presence of various concentrations of MgCl₂ and CaCl₂ in Ca²⁺ and Mg²⁺-free HBSS with 5% dialyzed FCS, and the cell complex formation activity was determined 1 h later.

whether divalent cations are required for the cell complex formation in our system. As shown in Fig. 2 d, the absence of divalent cations resulted in no measurable cell complex formation. The activity was recovered when physiological concentrations of Mg²⁺ were added to the culture, while the presence of Ca²⁺ is not necessary for this cell complex formation.

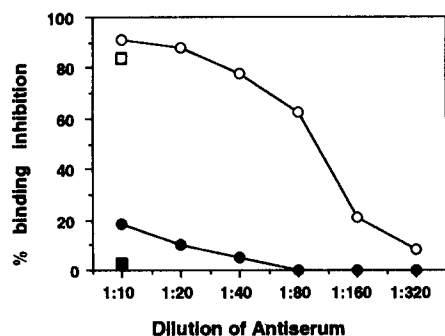


Figure 3. Inhibition of cell complex formation by rat anti-BATE-2 serum. 10^4 BATE-2 and Ac-6 stromal cells were precultured overnight in a microplate, and 50 μl of various dilutions of rat antiserum against BATE-2 stromal cells (after the extensive absorption with lymphoid cells and erythrocytes) was added to the stromal cell monolayers. ⁵¹Cr-labeled RPL-1 cells (3×10^5) were added immediately thereafter, and the cell complex formation was determined 1 h after the incubation. Rat anti-BATE-2 serum was added to BATE-2 monolayers (○) or to Ac-6 monolayers (□). Normal rat serum was added to BATE-2 monolayers (●) or to Ac-6 monolayers (■).

An Antibody to Thymic Stromal Cells Blocks Adhesive Interaction between Lymphocytes and Stromal Cells. Although we have tested the effect of various mAbs on the cell complex formation between lymphocytes and stromal cells, so far, none of antibodies (including anti-LFA-1, anti-CD2, anti-Pgp-1, anti-VLA4, and anti-CD3) were able to block the cell interaction (data not shown). From these results, it is considered that: (a) a new cell surface molecule, undefined so far, may mediate this kind of cell interaction; (b) several kinds of molecules may be involved in this phenomenon; or (c) multiple binding sites (epitopes) of a single molecule may be involved in this interaction. Thus, we tried to make polyclonal antiserum against stromal cells that can block lymphocyte-stromal cell interaction. Fischer rats were hyper-immunized with BATE-2 thymic stromal cells, and the immune serum was collected. The serum was heat inactivated and extensively absorbed with lymphoid cell lines (RPL-1, AKR-179, and 70Z/3), normal mouse thymocytes, and peripheral blood erythrocytes until the serum showed no reactivity against lymphoid lines by FACS analysis and by SDS-PAGE analysis of immunoprecipitated materials of radioiodinated lymphoid lines (data not shown). As a control, normal serum of Fischer rat was similarly treated. To test effects of the serum on the cell complex formation, varying dilutions of immune serum and normal serum were added to the monolayers of BATE-2 and AC-6 stromal cells. ⁵¹Cr-labeled indicator RPL-1 cells were loaded immediately thereafter, and the plate was incubated for 1 h at 37°C. After extensive washings, residual radioactivity was counted. As shown in Fig. 3, immune rat serum

significantly inhibited cell complex formation at dilutions from 1:10 to 1:80. This antibody also blocked the cell complex formation between RPL-1 and AC-6 stromal cells (shown with a open square). These results may indicate that both thymic and bone marrow stromal cells express a similar molecule(s) that can mediate the cell interaction between lymphocytes and stromal cells.

To investigate the time course of inhibition by the antibody, immune serum, normal serum, and control medium were added to the monolayers of BATE-2 stromal cells at various times before or after the initiation of culture. Cell complex formation was measured after 1 h of incubation. As shown

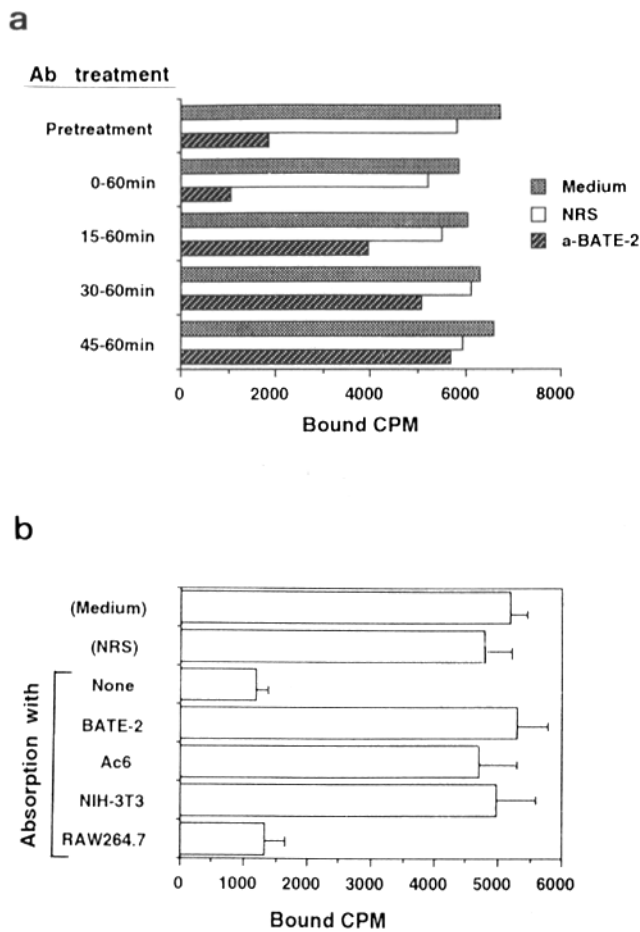


Figure 4. Inhibition of cell complex formation by antiserum and absorption of inhibitory activity by stromal cells. (a) Assay culture of cell complex formation between BATE-2 and ^{51}Cr -labeled RPL-1 cells was added with rat anti-BATE-2 serum (a-BATE-2) or normal rat serum (NRS) ($50\ \mu\text{l}$ of 1:10 dilution), or with medium alone at 0, 15, 30, or 45 min after the initiation of culture. A group of stromal cell monolayer was pretreated with antiserum for 30 min at room temperature and washed once with warm medium before adding ^{51}Cr -labeled RPL-1 cells (pretreatment). All cultures were assayed for RPL-1 binding 1 h after the incubation. (b) Rat anti-BATE-2 antiserum was absorbed extensively with BATE-2, Ac-6, NIH-3T3, or RAW264.7 cell lines. Absorbed, unabsorbed, and normal rat serum ($50\ \mu\text{l}$ of 1:10 dilution) were added to the assay culture of BATE-2 and ^{51}Cr -labeled RPL-1 cells at the initiation of culture. Cell complex formation was determined 1 h later.

in Fig. 4 a, while the pretreatment of stromal cells with immune rat serum significantly blocked cell complex formation, continuous presence of antibody exhibited the highest inhibition. The blocking activity diminished when antiserum was added at later stages of assay culture. These results suggest that the antibody inhibits only the initial step of lymphocyte-stromal cell interaction, i.e., the binding step of lymphocytes to stromal cells. To investigate whether specific antibody(ies) caused this blocking, we adsorbed the immune serum with various types of adherent cells (BATE-2, AC-6, NIH-3T3 or RAW264.7 cells), and then evaluated for their blocking effects on cell complex formation. As shown in Fig. 4 b, while nonadsorbed immune rat serum showed significant blocking, the serum absorbed with BATE-2, AC-6, or NIH-3T3 cell lines could not inhibit the cell complex formation. Macrophage line RAW264.7 was not able to absorb the blocking activity of the serum. These results indicate that the molecule(s) commonly expressed on BATE-2, AC-6, and NIH-3T3 lines but not on RAW264.7 line is(are) important in lymphocyte-stromal cell interaction.

Identification of a 107-kD Glycoprotein as a Molecule Mediating Stromal Cell-Lymphocyte Interaction. Using polyclonal rat anti-BATE-2 serum, we next tried to characterize the molecule(s) that may be involved in these lymphocyte-stromal cell interactions. Immunoprecipitation of surface radioiodinated BATE-2 cells was carried out with immune rat serum unabsorbed or absorbed with various stromal cell lines; the samples were analyzed by SDS-PAGE (Fig. 5). As shown in Fig. 5 a, immune rat serum immunoprecipitated several bands of molecules in reducing conditions, which included one major band of 107 kD, a minor band of 60 kD, and smear bands of ~90 and 130-150 kD. Under nonreducing conditions, although a weak band of 200 kD can be seen, most of the radioactivity is located at the top of the gel, indicating that the precipitated molecule mainly existed as a large molecular complex on the cell surface. After adsorption either with BATE-2, AC-6, or NIH-3T3, most of the bands, including the major band and the smear bands, except the 60-kD band, were absorbed out (Fig. 5 b). However, a macrophage line (RAW264.7) could not absorb the major band of the 107-kD molecule, though other bands (except the 60-kD band) disappeared completely. The 60-kD band appears to represent molecules nonspecifically bound to antibody-coupled Sepharose beads. As shown in Fig. 5 c, digestion of the 107-kD molecule with endo-glycosidase F reduced its molecular mass to 97 kD, indicating that the 107-kD molecule is a glycoprotein.

We next tried to raise a monospecific antiserum against isolated 107-kD molecules in order to confirm whether a similar binding inhibition activity could be observed with such serum as with the absorbed polyspecific antiserum against BATE-2 cells. To do this, preparative-scale SDS-PAGE of membrane extracts of the TSt-thymo cell line (stromal cell line expressing high levels of the 107-kD molecule) was performed, and the gel band corresponding to 107 kD was excised and injected into hamsters as described in Materials and Methods. After several immunizations, the serum was checked for monospecific reactivity by immunoprecipitation and SDS-PAGE analysis, and also tested for an effect on cell complex

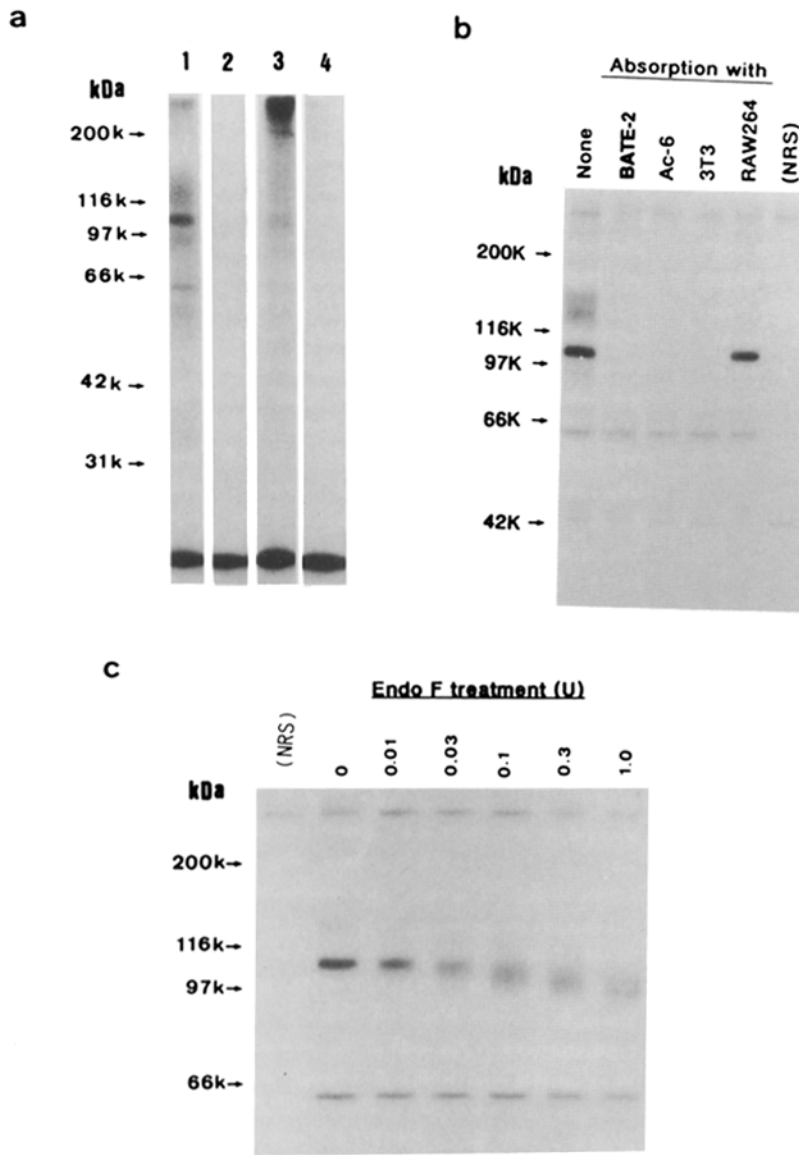


Figure 5. SDS-PAGE analysis of immunoprecipitated materials from stromal cell surface by rat anti-BATE-2 serum. (a) Radioiodinated lysate of BATE-2 cells was immunoprecipitated with normal rat serum or with rat anti-BATE-2 antiserum absorbed only with lymphoid cells, as shown in Fig. 3, and analyzed under reducing and non-reducing conditions by SDS-PAGE. Lane 1, anti-BATE-2 serum, reducing; lane 2, normal serum, reducing; lane 3, anti-BATE-2 serum, nonreducing; lane 4, normal serum, nonreducing. (b) Radioiodinated BATE-2 lysate was immunoprecipitated with rat anti-BATE-2 antiserum unabsorbed or absorbed with various stromal cells, as shown in Fig. 4 b, and analyzed by SDS-PAGE under reducing conditions. (c) After the immunoprecipitation of radioiodinated BATE-2 lysate with rat anti-BATE-2 serum, samples were digested with varying amounts of endoglycosidase F at 37°C for 2 h under reducing conditions, and analyzed by SDS-PAGE.

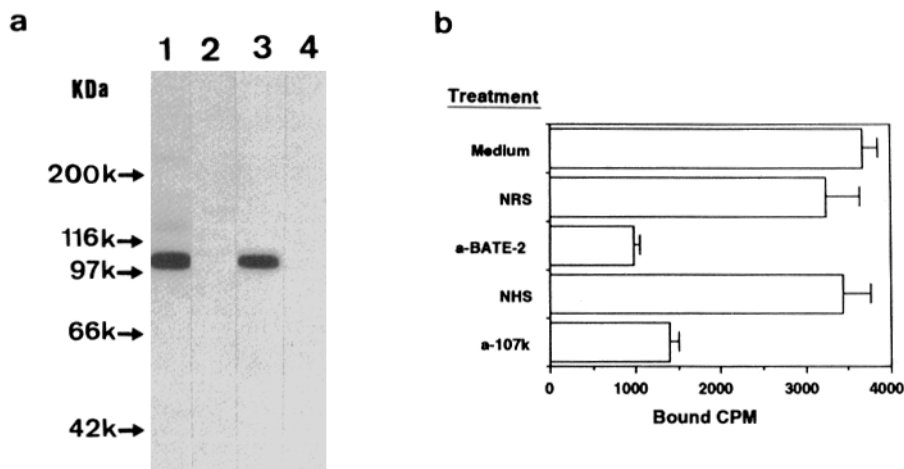


Figure 6. Production of monospecific antiserum against 107-kD molecule and blocking of cell complex formation by it. (a) Monospecific antiserum against the 107-kD molecule was raised in Syrian hamsters as described in Materials and Methods. Radioiodinated lysate of BATE-2 cells was immunoprecipitated with this hamster serum without secondary antibody. Conventional immunoprecipitation by using polyspecific rat anti-BATE-2 serum and secondary rabbit anti-rat IgG serum was also done for comparison. SDS-PAGE was run under reducing conditions. Lane 1, rat anti-BATE-2 serum, lane 2, normal rat serum, lane 3, hamster anti-107-kD serum, lane 4, normal hamster serum. (b) Monospecific hamster anti-107-kD serum (a-107-k), rat anti-BATE-2 serum, and control normal sera (50 μ l of 1:10 dilution of each serum) were added to the assay cultures of complex formation between BATE-2 and 51 Cr-labeled RPL-1 cells from the beginning of culture; bound RPL-1 cells were assayed 1 h later.

formation. As shown in Fig. 6, this serum immunoprecipitated the 107-kD band (Fig. 6 a), and showed comparable levels of cell complex blocking activity, as did polyspecific anti-BATE-2 serum (Fig. 6 b). Taken together, these results strongly suggest that the 107-kD glycoprotein (gp107) plays an important role in the mediation of stromal cell-lymphocyte interaction.

Discussion

In this paper, we investigated an adhesive interaction between lymphocytes and stromal cells of various origins. This cell interaction can generally be observed in LT BMC. We demonstrated that the interaction was an active process that required energy metabolism and cytoskeletal function. The presence of Mg^{2+} but not Ca^{2+} is necessary for the interaction to occur. We succeeded in raising an antiserum against the cell surface component of stromal cells that could block lymphocyte binding to stromal cells. The antiserum immunoprecipitates a 107-kD glycoprotein. Although the precise molecular nature is not yet clear, it appears that this subunit exists as oligomers on the stromal cell surface.

Adhesive interactions with stromal cells appear to be indispensable for lymphopoiesis as well as myelopoiesis in LT BMC. Preferential associations between hematopoietic precursors and stromal cells have been demonstrated in previous studies (1-8). These early studies demonstrated that the precursors first attached to the stromal cell surface and move underneath the membrane of stromal cells. Such interactions were commonly observed during the development of B lymphocyte and myeloid cells (2, 3) in LT BMC as well as in the culture of immature thymocytes with thymic stromal cells (12). This kind of cellular interaction may be important in vivo in that commitment of the multipotential stem cells to a particular lineage appears to be induced through these cell-to-cell interactions. In ontogeny, hematopoiesis occurs in particular tissues and organs, such as embryonic yolk sack and fetal liver, or bone marrow and other hematopoietic organs in the adult.

Soluble factors (cytokines) also appear to play essential roles for induction and maintenance of hematopoiesis (26). Although most of the soluble factors (such as G-CSF, M-CSF, and IL-7) support only restricted lineages of hematopoietic cells, some of the factors are shown to exert a variety of functions. It is well known that IL-3 is a multi-CSF (27), and IL-6 can exert heterogeneous functions both in vivo and in vitro (28, 29). Therefore, contacts between precursors and the cells that produce multifunctional factors allow these factors to function with specific progenitors.

Cell-to-cell adhesion is mediated by a variety of molecules that possess unique molecular characteristics. Cell adhesion molecules are grouped into three large families of molecules known as the integrin family, the immunoglobulin supergene family, and the selectin family (30-32). The integrin molecules consist of two subunits, α and β , which are non-covalently associated. Based on the structure of β subunit, this family is divided into three subfamilies ($\beta 1$, $\beta 2$, and $\beta 3$) (33). Among these subfamilies, the $\beta 2$ subfamily defines LFA-1

molecules, which are involved in lymphoid interactions (34). Treatment of RPL-1 cells with an anti-LFA-1 mAb (17/4.3) or continuous presence of the antibody in the culture did not affect the ability of RPL-1 cells to form the cell complex in our system (data not shown).

The immunoglobulin supergene family also defines molecules that are involved in cell adhesion. Among these, intercellular adhesion molecule 1 (ICAM-1) and neural cell adhesion molecule (N-CAM) may be involved in hematopoiesis (35, 36). The ICAM-1 molecule is a highly glycosylated cell surface protein of 85 kD and it is a ligand of LFA-1 (21). In humans, ICAM-1 is expressed on endothelial cells, fibroblasts, and lymphoid cells (37). However, analyses by a mAb against murine ICAM-1 molecule (38) and cDNA cloning of this molecule (39) indicated that ICAM-1 is mainly expressed on macrophages and myeloid cells as well as activated lymphocytes, but not on stromal cells in the mouse. This suggests that the ICAM-1-LFA-1 pathway may not function as a critical step in the interaction between hemato-lymphoid precursors and stromal cells. Although N-CAM is mainly expressed on cells of the nervous system (36), this molecule is found in a variety of nonnervous tissues and cells, including stromal cells (13) and a subset of early thymocytes (40). The stromal cells used in this study (BATE-2, BATE-10, AC-6, and ST2) also express this molecule. However, the mAb against murine N-CAM (H-28) (40) could not inhibit cell complex formation in our system (not shown).

There is another family of cell adhesion molecules, termed selectin (32) or LEC-CAM (41), to which lymphocyte homing receptor (MEL-14) (42), endothelial leukocyte adhesion molecule 1 (ELAM-1) (43), and granule membrane protein GMP-140 (32) belong. This family does not seem to be important in the cell interaction between stromal cells and lymphocytes, since cell adhesion with this family of molecules does not require active cell metabolism and depends on extracellular Ca^{2+} (32). SDS-PAGE analysis by using mAbs against Pgp-1 (IM7.8.1) (44) and heat-stable antigen (M1/69) (45) revealed that gp107 found in this study does not correspond to either of these molecules (data not shown).

The most likely candidate of an adhesion molecule that corresponds to our gp107 species is VCAM-1/INCAM-110 (46-49). VCAM-1/INCAM-110 is a counterligand for human VLA-4 ($\alpha 4\beta 1$) molecule (46-48). Elsewhere, we demonstrate that an anti-murine $\alpha 4$ inhibits $\alpha 4\beta 1$ -expressing hemato-lymphoid cell lines from adhering to LT BMC stroma (Miyake, K., I. L. Weissman, J. S. Greenberger, and P. W. Kincade, manuscript submitted for publication). A proposed receptor for this $\alpha 4\beta 1$ species on the stromal cells is also ~ 100 kD, which can be detected by mAbs M/K-1 and M/K-2 (Miyake et al., manuscript submitted for publication). Crossimmunoprecipitation experiments demonstrated that the identical cell surface molecule was immunoprecipitated from both thymic and bone marrow stromal cells with these mAbs and with our antiserum (unpublished results). Thus, at first sight, the most likely explanation for our gp107 is that it is related to or is identical to the murine VCAM-1/INCAM-110. However, a few discrepancies prevent that conclusion: we could not induce or upregulate the gp107 with cytokines/substances

known to induce VCAM-1 (TNF, IL-1 and endotoxin), and experiments with anti-murine $\alpha 4$ antibodies R1-2 (20) and PS/2 (Miyake et al., manuscript submitted for publication) did not inhibit RPL-1 binding to BATE-2 cells. Moreover,

some T cell lines that lack VLA-4 possess the ability to form cell complexes with thymic stromal cells (unpublished results). Nevertheless, direct identification will require the use of antibodies known to detect murine VCAM-1/INCAM-110.

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