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Identification of a unique membrane-bound molecule on a hemopoietic stem cell line and on multipotent progenitor cells

(stem cell enrichment/growth factor receptors)

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ABSTRACT **Hemopoietic stem cells are a distinct population of cells that can differentiate into multilineages of hemopoietic cells and have long-term repopulation capability. A few membrane-bound molecules have been found to be preferentially, but not uniquely, present on the surface of these primitive cells. We report here the identification of a unique 105-kDa glycoprotein on the surface of hemopoietic stem cell line BL3. This molecule, recognized by the absorbed antiserum, is not present on the surface of myeloid progenitors 32D and FDC-P1 cells, EL4 T cells, and NIH 3T3 fibroblasts. This antiserum can also be used to block the proliferation of BL3 cells even in the presence of mitogen-stimulated spleen cell conditioned medium, which is known to have a stimulating activity on BL3 cells. It can also inhibit development of *in vitro*, fetal liver cell-derived multilineage colonies, but not other types of colonies, and of *in vivo* bone marrow cell-derived colony-forming unit spleen foci. These data suggest that gp105 plays an important role in hemopoietic stem cell differentiation.**

True hemopoietic stem cells (HSCs) are those that have extensive self-renewal ability, are capable of giving rise to multilineage hemopoietic cells, and are able to reconstitute lethally irradiated recipients over the long term (1). Using isoenzymes or retroviral markers, several investigators have shown that multiple clones of stem cells exist as an individual. However, only one or a few of them are active at one time, and the activity of each of these clones to become dominant or quiescent is not predictable (2–4). It has been reported that a single stem cell is sufficient and capable of reconstituting the whole hemopoietic system (5). Therefore, many investigators are attempting to isolate or purify this type of stem cell. Sophisticated cell sorting analysis with a combination of surface markers suggests that primitive long-term repopulating stem cells can be enriched manyfold (6–8).

We have established a hemopoietic cell line, BL3, from a lethally irradiated recipient mouse that was reconstituted with fetal liver cells transduced with a rearranged retroviral genome (9). BL3 cells possess stem cell properties in that they can reconstitute lethally irradiated recipients, can give rise to precursor colony-forming unit, spleen (pre-CFU-S), and colony-forming cells, and are able to associate with stromal cells to develop "cobblestones" characteristic of primitive hemopoietic stem cells (9). They also express GATA-1, a transcription factor known to be expressed in HSCs, and commonly known stem cell surface markers such as Thy-1 and Sca-1, but not lineage-specific markers such as Mac-1, B220, and Gr-1 (9). That BL3 cells are able to reconstitute lethally irradiated recipients over the long term and to develop cobblestones with stromal cells suggests that they are very primitive cells and therefore may possess other surface markers not recognized previously. In addition, BL3 cells are embryonic in origin—that is, they are derived from fetal liver cells of 12-day-old mouse embryos. It has been shown that fetal/embryonic HSCs are different from adult HSCs in quantity and quality (ref. 2; P.M.C.W., unpublished data). Specifically, their cell surface markers might also be different (7, 8). For these reasons, we proceeded to develop monospecific polyclonal antibodies to see if a molecule unique to BL3 cells is also present on multipotent hemopoietic progenitor cells from either fetal liver or adult bone marrow. Our data suggest that a glycoprotein of 105 kDa is present on BL3 cells, on some fetal liver cell-derived CFU-mix colonies, and some bone marrow cell-derived CFU-S foci.

MATERIALS AND METHODS

Cell Lines and Mice. The details of maintaining BL3 cells, use of laboratory mice, and collection of fetal liver and bone marrow cells have been described (9).

Western Blot Analysis. Cells were harvested, washed twice with PBS containing 2% fetal calf serum (P2), and lysed in 10 μl of lysis buffer containing 10 mM Tris·HCl (pH 8.0), 50 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, and 2 μg of aprotinin per ml. The lysate was placed on ice for 30 min and was then spun at 16,000 × *g* for 5 min at 4°C. The supernatant was collected and mixed with 2× sample buffer that contained 100 mM Tris·HCl (pH 6.8), 2% (vol/vol) glycerol, 0.02% bromophenol blue, 2% SDS, and 2% 2-mercaptoethanol. The mixture was boiled for 3–5 min before loading onto a SDS/7% polyacrylamide gel. Biotinylated SDS/PAGE standards (Bio-Rad) were used as molecular weight markers. Electrophoresis was carried out in the presence of Tris-glycine buffer with a Tall Mighty Small vertical slab gel unit (Hoefer). After electrophoresis, the gel was soaked in Tris-glycine buffer containing 20% (vol/vol) methanol for 30 min and the proteins were transferred in the same buffer onto the PVDF membrane (Millipore). The membrane was blocked for >60 min at room temperature with blocking solution (GIBCO/BRL) and incubated for another 60 min at room temperature with primary antibodies at a concentration of 5 μg/ml in blocking solution. IgG from all sera was purified through a recombinant protein G-agarose column (BRL). Next, the membrane was washed three times with washing solution containing 50 mM Tris·HCl (pH 7.5), 200 mM NaCl, and 0.05% Tween 20 for 5 min each time. It was then incubated with 1:2000 biotinylated goat anti-rabbit IgG in blocking solution (GIBCO/BRL) for 30 min at room temperature. After being washed three times with washing solution, the membranes were incubated with 1:2500 streptavidin–alkaline

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Abbreviations: HSC, hemopoietic stem cell; CFU-S, colony-forming unit, spleen; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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phosphatase conjugate (Boehringer Mannheim) for 30 min at room temperature followed by another four washes. They were then visualized by staining with Lumi-Phos 530 (Boehringer Mannheim) and exposed to x-ray film.

Immunoprecipitation. Twenty million cells were harvested and washed twice with P2. The cell pellet was resuspended with 0.5 ml of P2 and incubated with 10 μg of IgG for 2 h at 4°C. The cells were washed twice with P2 and lysed in the same lysis buffer described for Western blotting. The cell lysates were placed on ice for 30 min and spun, and the supernatants were transferred into the tubes containing 40 μl of protein A-agarose suspension (Boehringer Mannheim). They were incubated for a further 2 h at 4°C. Complexes of antigen–antibody–protein A-agarose were collected and washed three times with lysis buffer The pellets were resuspended with 40 μl of 2× sample buffer, boiled for 3 min, and spun for 2 min at room temperature. Supernatants were collected and separated by SDS/7% PAGE. The proteins were visualized as described for Western blots.

Glycosylation Studies. After immunoprecipitation, the protein–antibody-protein A-agarose complex was resuspended with 6 mM Tris·HCl, pH 6.8/0.2% SDS, boiled for 5 min, and spun for 2 min at room temperature. The supernatant was mixed with an equal volume of 2× glycosidase buffer, which contains 200 mM sodium phosphate buffer (pH 6.6), 50 mM EDTA (pH 7.5), 2% Triton X-100, 0.1% SDS, and 300 mM 2-mercaptoethanol. The mixture was incubated with 0.4 unit of N-glycosidase F (Boehringer Mannheim) in a total reaction volume of 22 μl for 12–20 h at 37°C. Next, another 20 μl of 2× sample buffer as in Western blot analysis was added to each sample. The samples were then boiled for 3 min before they were subjected to gel electrophoresis, which was done according to the procedure for Western blot analysis.

Preparation of Antisera. One adult New Zealand female rabbit was injected with 1.2×10^8 BL3 cells intravenously and was booster injected 3 weeks later with another dose of 1×10^8 cells. Subsequently, the animal was injected every 6–8 weeks. Blood was collected 10 days postinjection and serum collected was heat inactivated at 56°C for 30 min. The serum was then absorbed with 0.1 vol packed with WEHI-3 cells twice, 0.1 vol packed with EL4 cells twice, and 0.1 vol packed with murine red blood cells once. WEHI-3 and EL-4 cells were chosen for absorption because they were negative for gp105 in Western blot analysis. The adsorptions were performed by rotating the tubes at room temperature for 1 h. The absorbed serum was collected by centrifugation at 1000 × *g* for 10 min. For titer determination, 1×10^4 BL3 cells or WEHI-3 cells in a final 100-μl vol were incubated with or without various dilutions of antiserum and in the presence of rabbit complement at a predetermined optimal concentration (1:15) (Accurate Chemicals). After 45 min of incubation at 4°C, an equal volume of 0.4% trypan blue was mixed with treated cells and viability was determined. Specific cytotoxicity was determined as described (10, 11). In this way, the specific killing for WEHI-3 cells was 58% at a 1:4 dilution of the antiserum before absorption and 0% at a 1:4 dilution and 12% at a 1:2 dilution after absorption, whereas the specific killing for BL3 cells ranged from 95% to 100% at all dilutions examined from 1:0 to 1:128 before and after absorption. Based on this assay, we were able to establish that the anti-BL3 antiserum has a titer of 1:1000, which is defined as the dilution at which 50% specific killing of BL3 cells occurred.

Preparation of Conditioned Medium (CM). To prepare CM of BL3 cells (BL3CM), cells were expanded until they were in logarithmic phase. They were then resuspended in fresh RPMI 1640 medium supplemented with 10% fetal calf serum (R10) at a concentration of 2×10^6 cells per ml for 36 h. The spent medium was collected by centrifugation at 16,000 × *g* at 4°C. Supernatants were stored at −20°C until use. CM of mitogen-stimulated spleen cells (SCM) was prepared as described (12).

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) Proliferation Assay. Exponentially growing BL3 cells were washed three times with R2 and the cell concentration was usually adjusted to 4×10^5 cells per ml in R2. The cells were then mixed with serially diluted sera or CM preparations and deposited into individual wells of a 96-well microtiter plate. Usually four or five replicates for each experimental point were set up. Incubation was carried out in a 37°C humidified 5% CO_2/95% air incubator for 3–4 days. At the end of the incubation, a MTT assay was performed. To do that, 10 μl of MTT (5 mg/ml) (Sigma) (13) was added into each well. The plates were incubated at 37°C for another 4 h, and then 100 μl of acid isopropanol was added to dissolve the formazan product of MTT reduction. The extent of coloring of each reaction mixture was then determined by a microplate reader with 570/630 dual wavelength (Bio-Tek Instrument, Burlington, VT; LE311).

RESULTS

One adult female rabbit was first injected with 1.2×10^8 BL3 cells and then injected with 1×10^8 BL3 cells every 2 months. The serum was tested positive by a complement-mediated cytotoxicity assay in which specific killing was observed when BL3 cells were used as target cells. Its titer, as determined by the serum dilution at which there is 50% specific killing of BL3 cells, was 1:1000.

That the antiserum is cytotoxic to BL3 cells in the presence of complement suggests its recognition of a molecule present uniquely on their cell surface. A series of Western blot and immunoprecipitation analysis confirms this contention. For Western blot analysis, 10^6 BL3 cells, EL4 cells, 32D cells (14), and 3T3 cells were lysed in buffer, electrophoresed, and blotted onto PVDF membrane, which were then incubated with either the antiserum or the preimmune serum. A band with a mass of 105 kDa was present distinctly in lysates of BL3 cells only (Fig. 1*A*). For immunoprecipitation, 20×10^6 live cells were incubated with either the antiserum or preimmune serum before cell lysis (13), and cell lysates were then incubated with protein A-agarose. The complexes were washed and boiled to release the proteins, which were then analyzed as described for Western blot analysis. The results indicated that immunoprecipitation with the antiserum, but not with the preimmune serum, still produced a predominant 105-kDa band (Fig. 1*B*). Again, this band was observed on the surface of BL3 cells only and not on that of EL4 cells, 32D cells, or 3T3 fibroblasts (Fig. 1*B*).

To determine whether p105 is a glycoprotein, we treated the protein with N-glycosidase F (Boehringer Mannheim). Fig. 2 indicates that after treatment with 0.4 unit of N-glycosidase F overnight at 37°C, a predominant band with a mass of 85 kDa in the immunoprecipitates was observed, indicating that the size of the unprocessed protein is 85 kDa and its glycosylated form is 105 kDa. We have not tested whether p105 contains O-linked sugar moieties. Also, further analysis indicated that it is not autophosphorylated on tyrosine residues, suggesting that it does not contain tyrosine autophosphokinase activity (data not shown).

Specific recognition of gp105 by the antiserum on BL3 cells suggests that it has a biological function on BL3 cells and therefore on HSCs. We started by asking whether gp105 is a receptor to which BL3 cells themselves may be producing a corresponding ligand. In addition to several important stem cell properties they possess, BL3 cells do not respond to many cytokines that are known to have an effect on HSCs, added to the culture either singly or in combination (9). However, by a proliferation assay with BL3 cells themselves as responding cells, we showed that BL3 cells respond to their own CM prepared at high cell density (Fig. 3). A stimulating activity is also present in SCM (Fig. 3*C*). The response is dose dependent

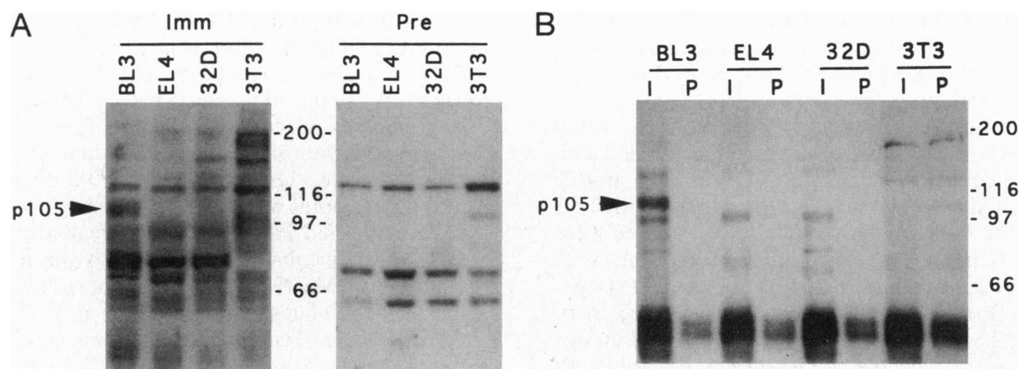

FIG. 1. Anti-BL3 antibody recognizes a p105 molecule specifically present on BL3 cells. (*A*) Western blot analysis. One million cells of each type were used and transferred to PVDF membrane and were then incubated with primary antisera: anti-BL3 antiserum (Imm) or preimmune serum (Pre). EL4 is a T-cell line, 32D is a myeloid progenitor cell line, and 3T3 is NIH 3T3 fibroblast cell line. Note the presence of a p105 molecule only in BL3 lysate blotted with anti-BL3 antiserum. (*B*) Immunoprecipitation. Twenty million live cells were incubated with either anti-BL3 antiserum (lanes I) or preimmune serum (lanes P). Cells were then lysed and total lysates were further incubated with protein A-agarose; complexes were collected, washed, and boiled in buffer; and the supernatant was subjected to SDS/7% PAGE. Note the presence of a distinct p105 molecule recognized by the antibody.

(Fig. 3). For unknown reasons, the activity detected in BL3CM varied from batch to batch, whereas the activity from SCM was very consistent. Since we have shown that BL3 cells do not produce many known cytokines, whereas mitogen-stimulated spleen cells do, it is possible that additional molecules are required to stabilize this stimulatory factor(s).

Because of the unique responsiveness of BL3 cells to this factor(s) and their possession of gp105, we asked whether anti-gp105 antibody can block this stimulation. Fig. 3*B* indicates that addition of the antiserum can specifically inhibit proliferation of BL3 cells but not that of myeloid progenitor FDC-P1 cells and v-abl-transformed MM1 mast cells (15, 16). Furthermore, the antiserum can also block the stimulatory activity found in SCM (Fig. 3*C*). The inhibition is also dose dependent, and complete inhibition can be achieved at a dilution of 1:100. In the presence of the antibody, BL3 cells maintain a survival curve not different from that of cells maintained at low cell concentration that is nonpermissive for growth. This suggests that the antibody is not toxic to BL3 cells because otherwise one would observe an accelerated rate of cell death.

Since BL3 cells retain many aspects of HSC properties (9), we next tested whether growth and differentiation of multipotent hemopoietic progenitors isolated from normal fetal liver or bone marrow can be affected in the presence of the antibody. We first carried out an *in vitro* clonogenic assay with fetal liver cells, which were preincubated with the antiserum or preimmune serum at 1:10 or 1:100 dilution before they were plated in the semisolid methylcellulose cultures. Six to 8 days later, the numbers and types of colonies were recorded. Strikingly, the typical multilineage (E-mix) colonies were not observed in cultures containing anti-BL3 antiserum at 1:10 dilution, contrasting their presence in cultures of preimmune

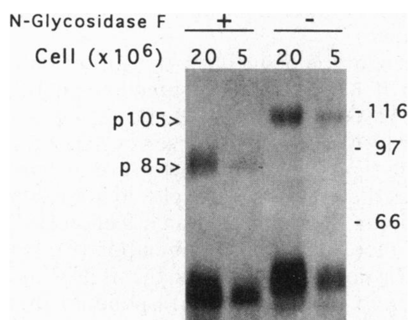

FIG. 2. p105 is an N-linked glycosylated protein.

serum (Table 1). No differences in the numbers and types of colonies were observed in cultures of both preimmune and immune sera at 1:100. The total number of colonies in cultures with anti-BL3 antiserum remained largely unchanged (Table 1). In addition, small blast-like colonies could be observed in culture with a 1:10 dilution of the antiserum. Replating cells from 10 such colonies in the absence of the antibody yielded formation of 10 E-mix colonies, 2 macrophage CFU colonies, and mast cell-like colonies. Therefore, the antibody can inhibit specifically the development of, but is not cytotoxic to, fetal liver cell-derived multipotent progenitor cells *in vitro*.

CFU-S-forming cells are multipotent hemopoietic progenitors capable of reconstituting lethally irradiated recipient mice over the short term. We therefore performed CFU-S assay with donor adult bone marrow cells, with or without treatment of anti-BL3 antibody plus complement. Before engraftment into irradiated recipients, bone marrow cells were incubated with preimmune or anti-BL3 immune antiserum at a predetermined concentration in the presence of complement. Cells recognized by the antibody will be lysed by the complement. After treatment, the same number of live cells was injected into each recipient. Thirteen days later, the number of foci was recorded. In two experiments, there was a consistent reduction in the number of CFU-S foci in recipient spleens of bone marrow cells treated with the antiserum compared with those treated with no serum or preimmune serum (Table 2). The reduction was proportional to the amount of antibody used.

DISCUSSION

We have successfully raised polyclonal antibodies specifically directed against BL3 cells. It recognizes with high affinity and specificity the gp105 glycoprotein, which is present on BL3 cells in abundance. This antibody can neutralize the activity of a factor present in SCM or BL3CM in stimulating BL3 cell growth. These data suggest that the factor in the CM is either the ligand of gp105 or gp105 is a subunit of a receptor to which the ligand binds. However, it is also possible that gp105 represents a general surface molecule totally independent from the ligand–receptor complex such that its recognition by the antibody inhibits cell growth in a dominant fashion. Two reasons may not favor the latter possibility. First, we showed that gp105 is present specifically on BL3 cells that retained many properties of HSCs and therefore it could not be a general molecule such as one present in all types of cells for transmitting growth inhibition signals. Second, incubation of the antibody with BL3 cells resulted in specific cell death (Fig.

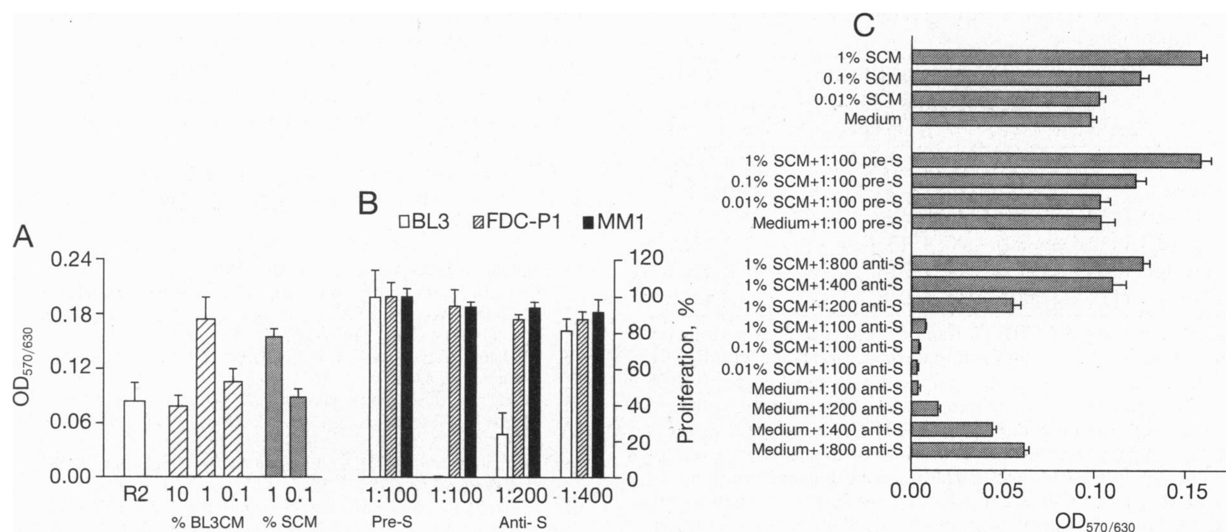

FIG. 3. (*A*) The presence of stimulatory activity in BL3CM or SCM. Exponentially growing BL3 cells at a concentration of 2×10^4 cells in a final vol of 100 μl were incubated with 1:10 dilutions of BL3CM or SCM. Dilutions were made with RPMI 1640 medium supplemented with 2% fetal calf serum (R2). At the end of 3 days, the MTT assay was performed. Four replicates were set up for each experimental point. Error bars indicate SD. (*B*) Anti-BL3 antibody specifically inhibited proliferation of BL3 cells. Exponentially growing BL3 cells (open bars), FDC-P1 cells (hatched bars), or MM1 cells (solid bars) at a concentration of 2×10^4 cells in a final vol of 100 μl were incubated with either 1:100 dilution of preimmune antiserum (pre-S) or various dilutions (1:100–1:400) of anti-BL3 antiserum (anti-S). Dilutions were made with R2. After 3 days of incubation, MTT was added and incubation continued for 4 h. All readings of each cell line are normalized with those of the corresponding cells incubated with preimmune serum, whose readings are expressed at 100% but whose actual average MTT readings are 0.147 for BL3, 0.541 for FDC-P1, and 0.903 for MM1 cells. Error bars indicate SD. (*C*) Blockage of stimulatory activity by anti-BL3 antiserum. BL3 cells (2.5×10^4) in a final vol of 100 μl containing either R2, BL3CM, or SCM, with or without pre-S or anti-S, were incubated for 4 days. The MTT assay was performed at the end of the incubation. Four replicates were set up for each experimental point.

3), whereas incubation with fetal liver multipotential cells apparently resulted in growth arrest and not cell death. This pleiotropic effect is more in line with characteristics of receptor–ligand molecules. Using this antibody, we have recently isolated a phage clone from a BL3-derived λgt11 cDNA phage library. Sequence analysis of >1 kb of DNA of this clone indicates that the gene is unique but has homology to several genes encoding growth factor receptors or growth factors.

That addition of anti-gp105 antibody to BL3 cell culture results in cell death is consistent with the hypothesis that this neutralizing antibody is blocking the effect of an autocrine growth factor. Death of BL3 cells is due to the minimum culture conditions in which they were maintained and to their unique property of not being able to respond to many different known growth factors (9). On the other hand, addition of the antiserum to clonogenic assay of cultures of fetal liver cells suggests that multipotent progenitor cells were growth arrested only, and, upon replating in the absence of the antibody, they could still continue to develop multilineage colonies. In this culture condition, an optimal growth-stimulating amount of SCM—a source of many growth factors—was included. Fetal liver multilineage progenitor cells are known to respond to these factors singly or in combination. Many of these factors can also maintain cell survival. Thus, using three different biological assays, specific stem cell effects were observed as a result of recognizing gp105 by the antiserum on their cell surface.

Various molecules are known to be present on HSCs and have been used for various analyses and purification. For example, as few as 100 Thy-1$^+$, Lin$^-$, Sca-1$^+$ cells can rescue 95% of lethally irradiated recipients (38). Thy-1 and Sca-1 have masses of 30 and 8 kDa under nonreducing conditions and of 30 and 18 kDa under reducing conditions, respectively (18). A CD34 antigen is found to be on human HSCs, and its murine counterpart has been biochemically characterized to have a mass ranging from 90 to 110 kDa, depending on cell types (19). Its molecular mass is therefore similar to that of gp105. However, it is expressed in NIH 3T3 cells, PA6 stromal cells,

Table 1. Hemopoietic colony formation in the presence of anti-BL3 antiserum

		No. of colonies/3×10^4 cells plated					
	Dilution	BFU-E	M-CFU	E-mix	GM-CFU	Others	Blast
Preimmune serum	1:10	16 ± 1	49 ± 6	24 ± 4	25 ± 6	9 ± 6	0
Antiserum	1:10	12 ± 2	68 ± 6	0	25 ± 2	5 ± 1	26 ± 6
Preimmune serum	1:100	14 ± 3	42 ± 2	25 ± 6	19 ± 2	8 ± 3	0
Antiserum	1:100	14 ± 1	49 ± 2	26 ± 4	21 ± 4	10 ± 1	0

Fetal liver cells from 12-day-old mouse embryos were collected as described (17). Cells (6×10^5) in 0.1 ml of R2 medium (9) were mixed with 0.1 ml of serum. Immune or preimmune serum was also diluted with R2 medium. The mixtures were then incubated for 2 h at 4°C. At the end of the incubation, another 0.1 ml of serum was added to each of the mixtures and then they were plated in the methylcellulose culture medium, which had a final vol of 2 ml and contained 1% SCM (12), erythropoietin (1 unit/ml) (Boehringer Mannheim), and 0.9% methylcellulose. Colonies were examined 6 days later and the numbers and types of colonies were recorded. Duplicates were set up for each experimental point. Values shown are means ± SD. Colony types were scored based on previous criteria (12) except "others" is defined as colonies of granulocytic or mast cell lineages; Blast is defined as small colonies of <100 cells with blast cell phenotype, which was observed only in culture containing anti-BL3 antiserum. Three additional experiments were done and similar results were obtained. BFU-E, burst-forming units, erythroid; M-CFU and GM-CFU, macrophage and granulocyte/macrophage CFU.

Table 2. Reduction of CFU-S after treatment with anti-gp105 antibody and complement

	Treatment	No. of CFU-S colonies/individual recipients	No. of CFU-S colonies, mean ± SD
Exp. 1	Anti-BL3 (15 μg/ml)	6, 13, 6, 8, 7, 5	7.5 ± 2.9
	None	16, 13, 20, 17, 19	17.0 ± 2.7
Exp. 2	Anti-BL3 (50 μg/ml)	4, 4, 5, 5	4.5 ± 0.6
	Anti-BL3 (12.5 μg/ml)	10, 9, 13, 6, 8	9.2 ± 2.6
	Pre-IgG (50 μg/ml)	17, 16, 18, 14, 16	16.2 ± 1.5
	Pre-IgG (12.5 μg/ml)	16, 16, 17, 15	16.0 ± 0.8

In Exp. 1, 7-week-old C57BL/6 females were used. Bone marrow cells were taken by flushing femurs and were washed twice with R2 (9). Twenty million cells per ml were incubated with purified anti-gp105 antibody (15 μg/ml) at 4°C for 45 min followed by incubation with 1:15 diluted low-toxin rabbit complement at 37°C for 45 min. After a viability check by trypan blue exclusion, 1×10^5 live cells in 0.5 ml of R2 were injected into each lethally irradiated mouse (each received 10 cGy delivered by a ^{137}Cs source irradiator). Spleens of recipients were removed 13 days posttransplantation and fixed in Bouin's solution; CFU-S colonies were scored. In Exp. 2, 8-week-old BALB/c females were used. CFU-S assay was performed the same as in the first experiment except different amounts of antibody were used.

and M1 leukemic cells (19, 20). In our study, by Western blot analysis and immunoprecipitation, gp105 is not present in lysates of NIH 3T3 cells, 32D, and FDC-P1 myeloid progenitor cells (Fig. 1). Since anti-gp105 is a polyclonal antibody, which probably recognizes several epitopes on the molecule, it is therefore unlikely that gp105 is the murine CD34. In addition, hyperphosphorylation of CD34 has been observed (21), whereas gp105 on BL3 cells is not phosphorylated under normal experimental conditions (X.-D.H. and P.M.C.W., unpublished data). An important regulator, c-kit (22–24), which is a tyrosine kinase receptor for stem cell factor (25, 26) has been shown to be on HSCs and has been used for enrichment of human and mouse stem cells (27–31). By reverse transcription-PCR and the MTT cell proliferation assay, we showed that BL3 cells do not express and produce either c-kit or stem cell factor (9).

Another potential candidate molecule on HSCs is AA4.1. This antigen has been shown initially to be on pre-B cells and later on lymphomyeloid stem cells (7, 32, 33). To our knowledge, the gene encoding AA4.1 and its biochemical properties are unknown. A recent study indicates that instead of its continuous presence on HSCs, AA4.1 expression may be related to a particular stage in the cell cycle, suggesting that it may not be specific for HSCs (34). BL3 cells are weakly positive with anti-AA4.1 antibody and the positive cells are heterogeneous; this heterogeneity may also be related to the cell cycle (14). By contrast, gp105 is present in abundance on BL3 cells, suggesting nonidentity of these two molecules.

A few years ago, a *Flk2* cDNA was isolated from AA4.1-enriched HSCs and was reported to be expressed with restriction in HSCs/progenitor cells (2). Independent cloning and analysis of *Flt3*, now considered the same gene as *Flk2*, suggest that the *Flt3/Flk2* gene is expressed in several different types of cells and tissues (35, 36). The gene encoding its ligand has been isolated and *Flt3* has been shown to stimulate proliferation of hemopoietic progenitor cells from fetal liver and adult bone marrow (37). Our preliminary reverse transcription-PCR analysis suggests that both *Flt3/Flk2* ligand and receptor are not expressed in BL3 cells, and thus they are unlikely to be the same as gp105 (14).

In summary, our data suggest that a gp105 molecule is present specifically on BL3 cells, on multilineage hemopoietic progenitor cells from either fetal liver or bone marrow. Because BL3 cells have biological properties resembling those of primitive HSCs, and because the antiserum was raised easily by injecting BL3 cells into one rabbit, gp105 is likely to be present in even higher abundance in totipotent primitive HSCs. Its expression appears to decrease progressively in more differentiated progenitor cells. Examination of the regulation of this molecule on true HSCs and enrichment of the early stem cells by means of this surface molecule might be valuable.

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