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Selective Stimulation of Granulopoiesis *in vitro* by Established Bone Marrow Stromal Cells

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ABSTRACT. **Bone marrow is a major granulopoietic organ whose hematopoietic microenvironment is comprised of stromal cells. In the present work, we examined the regulation of in vitro granulopoiesis with an established line of bone marrow stromal cells. In coculture of the progenitor cells on the particular stromal cell lines from bone marrow, large granulocyte (G) colonies consisting of over 200 cells were formed without G-CSF for 5 days. Stromal cells supported development of Gr-1 (granulocyte specific surface marker)-negative progenitors into Gr-1 and myeloperoxidase positive granulocytes. Seventy percent of the large G-colonies were formed on the stromal layers even in the presence of anti-G-CSF antibody, which indicates the G-CSF independent pathway of granulopoiesis. Inhibition of the large G-colony formation by the addition of anti-adhesion molecules, such as very late activation antigen-4 (VLA-4) and CD31 (PECAM-1), suggested the role of cell-to-cell adhesion in stroma-supported granulopoiesis.**

Granulocytes constitute the major population of white blood cells and are produced in the bone marrow, where their progenitor cells derived from the stem cells proliferate and differentiate into mature granulocytes. During this process, the progenitor cells pass through several intermediate stages such as myeloblasts, promyelocytes, and myelocytes, and various granulocyte-specific enzymes such as myeloperoxidase and leukocyte elastase are produced at specific stages of their development (1, 2). Proliferation and differentiation of hematopoietic progenitor cells are regulated by a family of cytokines and at least three cytokines are involved in the production of granulocytes and macrophages, that is, IL-3, GM-CSF, and G-CSF (1–5). G-CSF specifically works on cells restricted to the granulocyte lineage (1, 3). This cytokine can stimulate colony formation of granulocytes in semisolid medium of bone marrow cells and induce granulopoiesis in vivo (3), indicating that G-CSF plays a major role in development of granulocytes in bone marrow.

In contrast to the accumulating evidence on the involvement of cytokines in granulocyte development, little is known about the regulation of granulopoiesis by the bone marrow hematopoietic microenvironment, or the roles of adhesion molecules among subsequent stages of granulocyte maturation. Since bone marrow is a major granulopoietic organ and the hematopoietic microenvironment is comprised of stromal cells (6, 7), it is conjectured that bone marrow stromal cells may regulate granulopoiesis. Thus, in the present work, we examined the effect of stromal cells on granulopoiesis in vitro using established lines of bone marrow stromal cells from T-antigen transgenic mice (8) and demonstrated that G-CSF-independent granulopoiesis was induced by the bone marrow stromal cells.

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MATERIALS AND METHODS

Antibodies and cytokines. Anti-B220 (RA3-6B2, kindly provided by Dr. H. Yagita, Juntendo University School of Medicine), anti-c-Kit antibody (rat monoclonal antibody ACK2, kindly provided by Dr. S.-I. Nishikawa, Kyoto University) (9), TER119 (erythroid lineage marker; kindly provided by Dr. T. Kina, Kyoto University), Mac-1 (M1/70, Caltag Laboratories, South San Francisco, CA), and 4 antibodies Gr-1 (RB6-8C5), L3T4 (GK1.5), Ly-2 (53-6.72), anti-mouse CD31 (anti-PECAM-1) rat monoclonal antibody (clone 390) from Pharmingen (San Diego, CA) were used. A rat monoclonal antibody PS/2, which recognizes mouse α4 subunit of very late activation antigen-4 (VLA-4), was a gift from Dr. H. Yagita (10, 11). Human recombinant G-CSF was purchased from R & D Systems (Minneapolis, MN), and anti-human G-CSF rabbit polyclonal antibody was purchased from Genzyme (Boston, MA). Recombinant murine IL-3 and GM-CSF were kindly provided by Kirin (Tokyo, Japan). All antibodies

added to the coculture were dialyzed against Dulbecco's phosphate-buffered saline (PBS).

Maintenance of bone marrow stromal cells. Mouse bone marrow stromal cell lines (TBR series) were established from temperature sensitive (ts) SV40 T-antigen transgenic mice as described previously (8). TBR cell lines were maintained in RITC 80-7 (Kyokuto Pharmaceutical Co., Ltd., Tokyo, Japan) (12) supplemented with 2% fetal bovine serum (FBS; Irvine Scientific, California, USA), 10 μg/ml transferrin (Sigma, St Louis, MO), 1 μg/ml insulin (Shimizu Pharmaceutical Co., Ltd., Shizuoka, Japan), and 10 ng/ml epidermal growth factor (EGF, recombinant; generously supplied by Wakunaga Pharmaceutical Co., Ltd., Tokyo, Japan). The cultures were incubated at 33°C, which is a permissive temperature for SV40 ts T-antigen.

Preparation of hematopoietic progenitor cells. Bone marrow cells were flushed from femurs of C57BL/6J mice with PBS containing 0.2% bovine serum albumin (PBS-BSA). The suspension was washed and passed through a nylon mesh (Falcon 2350, Becton Dickinson, Lincoln Park, NJ) to make a single cell suspension. Red blood cells were lysed with lysing buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA) followed by incubation with Iscove's modified Dulbecco's medium (IMDM) (Gibco-BRL) supplemented with 10% FBS for 1 hr to eliminate stromal cells that had adhered to culture substratum. To prepare lineage marker-negative (Lin^-) cells (13), bone marrow hematopoietic cells in suspension were washed with PBS-BSA, then incubated with a cocktail of monoclonal antibodies specific to lineage markers (Lin; B220, Mac-1, Gr-1 L3T4, Ly-2, and TER119). After 30 min of incubation on ice, the cells were washed twice with PBS-BSA. To eliminate lineage marker-positive (Lin^+) cells, the cells were incubated with immunomagnetic beads (Dynabeads M-450, coated with anti-rat IgG, Dynal AS, Oslo, Norway) for 30 min on ice, then positive cells were eliminated by a magnet. The fetal livers of 13.5-days ICR mice (Funabashi Farm Co., Ltd., Funabashi, Japan) were used as another source of the progenitor cells for some experiments. The livers were removed and a single cell suspension was prepared by flushing through 26 1/2-gauge needle followed by passing it through a nylon mesh (14). The fetal liver cells were lysed with lysing buffer. To eliminate Lin^+ cells, fetal liver cells were processed the same as the bone marrow cells described above.

Coculture of the hematopoietic progenitor cells on the stromal cell lines. For cocultivation of hematopoietic progenitor cells on stromal cell lines, confluent cell layers of TBR cell lines were precultured at 37°C by IMDM supplemented with 10% FBS. The hematopoietic progenitor cells were suspended in a semisolid medium containing IMDM, 30% fetal bovine serum, 0.8% methylcellulose, 1% BSA and 100 μM2-mercaptoethanol, and then incubated on the precultured stromal cell layers.

Analysis of expression of Gr-1. The hematopoietic cells were incubated with anti-Gr-1 antibody for 30 min on ice and washed with PBS-BSA. The cells were then incubated with FITC-conjugated anti-rat IgG for 30 min on ice. After washing with PBS-BSA twice, the cells were resuspended in PBS-BSA and analyzed by FACStar-plus (Beckton-Dickinson, Mountain View, CA).

RT-PCR method. Total RNA was isolated by the acid phenol procedure using Isogen (Wako Pure Chemicals, Ltd. Tokyo) according to the manufacture's protocol. Total RNA from 3×10^5 cells was incubated with 2,000 U of MMLV reverse transcriptase (BRL, Gaithersberg, MD), 25 U of ribonuclease inhibitor and 20 pM of random hexamer primers in a 20 μl reaction. One μl aliquot of 20 μl reverse transcribed solution was amplified by PCR amplification using 0.5 U of *Taq* polymerase (BMY, Tokyo, Japan) and 2 pM of each of the gene-specific primers for MPO (the sense primer 5′-CTTCA TGTTCCGCCTGAACAA-3′ and the antisense primer 5′-A TTGTGCCATCAGCTTCCGT-3′) for 25 cycles consisting of successive incubations at 95°C (1 min), 55°C (1 min) and 72°C (0.5 min).

RESULTS

Large granulocyte colony formation induced by the bone marrow stromal cells. We previously reported the ability of the established bone marrow stromal cell lines to support lineage-restricted myeloid progenitor cells, which were examined by the colony formation of the hematopoietic progenitors of fetal livers on the stromal cell layers in semisolid medium (8). Using typical stromal cell lines (TBR59 and TBR511), we examined how the stromal cells stimulate granulopoiesis in vitro. TBR511 restricted their ability to support granulopoiesis from progenitor cells in fetal liver (8). When the fetal liver progenitor cells were cocultured on the layers of the stromal cell lines in semisolid medium, they formed large adhering colonies of over 200 cells containing granulocytes after 5 days culture (Fig. 1); but without the stromal cell layers, no such colonies were observed even in the presence of G-CSF, except for forming small colonies of less than 30 cells designated as CFU-G after 6 days. Addition of G-CSF in the coculture only slightly stimulated the large granulocytic (G)-colony formation (Table 1). When bone marrow progenitor cells were cocultured on the stromal cell layers, essentially the same result was obtained, although there were differences in colony numbers between bone marrow progenitors and fetal liver progenitors (Table I). Thus, these bone marrow stromal cell lines can stimulate the large G-colony formation from the progenitor cells of bone marrow and fetal liver without addition of G-CSF.

Large G-colony formation from Gr-1 (a granulocyte-specific surface marker) negative progenitor cells. To determine which stage of the progenitor cells responded to form the large G-colonies on the stromal cells, we examined the expression of Gr-1, a granulocyte lineage-

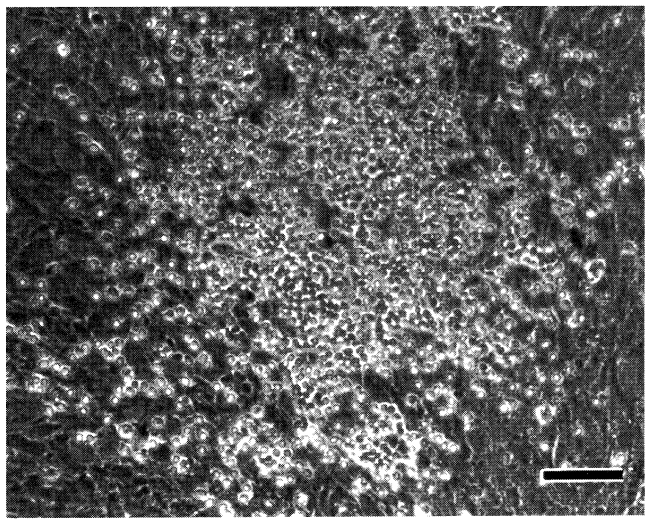

Fig. 1. Large granulocyte colonies formed on the stromal cell layers. Fetal liver progenitor cells were cultured for 5 days on TBR511 monolayer in semisolid medium. Bar indicates 100 μm.

specific marker, by FACS analysis in the progenitor cells from bone marrow and fetal liver. Expecting that the large G-colonies would be formed from immature granulocyte progenitors, Gr-1$^+$ cells were eliminated from fetal liver or bone marrow cells. Because of the abundance of erythroid progenitors in fetal livers, TER119$^+$ (an erythroid-specific surface marker positive) cells were depleted together. Although most cells were Gr-1$^-$ in 13.5-day fetal liver (Fig. 2B), the TER119$^+$ and Gr-1$^+$ cells of the 13.5-day fetal liver were depleted by magnetic beads after lysing treatment. Even after removing the Gr-1$^+$ cells, the large G-colony formation was observed on the TBR stromal cell layers from the Gr-1$^-$ cells in the absence of G-CSF (Table II). As a result, the ability of progenitor cells to form large G-colonies in fetal liver was enriched 5-fold to 10-fold in Gr-1$^-$/TER119$^-$ cells. In bone marrow, in which half of the hematopoietic cells are Gr-1$^-$ (Fig. 2A), the lineage marker-negative (Lin$^-$) progenitor cells (TER119$^-$, Mac-1$^-$: macrophage-specific marker, CD4$^-$, CD8$^-$: T-cell-specific markers, B220$^-$: B-lymphocyte-specific marker, in addition to Gr-1$^-$) were prepared from bone marrow cells. In the absence of stromal cells, the Lin$^-$ progenitor cells from bone marrow formed less G-CSF dependent G-colonies than those from the unfractionated bone marrow cells (Table I, II), but they formed more than 3-fold large G-colonies on TBR511 cell layers in the absence of G-CSF (Table II). Addition of G-CSF in the coculture showed no apparent enhancement in colony formation either in bone marrow or in fetal liver.

G-CSF independent colony formation on the stromal cells. Addition of G-CSF on the coculture was less effective on the colony formation and these stromal cell lines did not produce a measurable activity of G-CSF as previously reported (8). To show clearly the stimulation of G-CSF independent colony formation by the stromal cells, anti-G-CSF antibody was added to the coculture. The inhibition was saturated at 30% by its concentration of 10 μg/ml when an increasing amount of anti-G-CSF antibody was added (Fig. 3). Thus, approximately 70% of the large G-colonies were stroma dependent. Ten μg/ml of anti-G-CSF antibody was sufficient to inhibit CFU-G formation completely (Table III). An addi-

Table I. LARGE GRANULOCYTE COLONY FORMATION OF THE PROGENITOR CELLS FROM FETAL LIVER AND BONE MARROW ON THE STROMAL CELL LAYERS.

Addition		Number of Large G-colonies/2×10^4 Cells	
		G-CSF (−)	G-CSF (+)
Fetal Liver Cells after Lysing			
without stromal cells	Exp. 1	0	0
	Exp. 2	0	0
TBR59 Cell Layer	Exp. 1	17.7±2.9	19.3±2.9
	Exp. 2	14.7±4.2	19.3±1.9
TBR511 Cell Layer	Exp. 1	27.7±2.5	33.0±5.0
	Exp. 2	31.0±7.5	34.0±4.2
Bone Marrow Cells after Lysing			
without stromal cells		0	(14.5±0.7)*
TBR511 Cell Layer		42.3±2.1	35.3±8.4

Progenitor cells were derived from 13.5-day old fetal livers, or bone marrow cells from 8-week old C57BL/6 mouse. After lysing treatment, 2×10^4 hematopoietic cells were cultured in the semisolid medium with (1 ng /ml) or without G-CSF as described in Materials and Methods and the formed colonies were counted on the 5th day. Number of large G-colonies (average of 3 wells) are shown. * Parentheses indicate the number of small hanging colonies in semisolid medium distinguished from large G-colonies. Exp.; independent experiment.

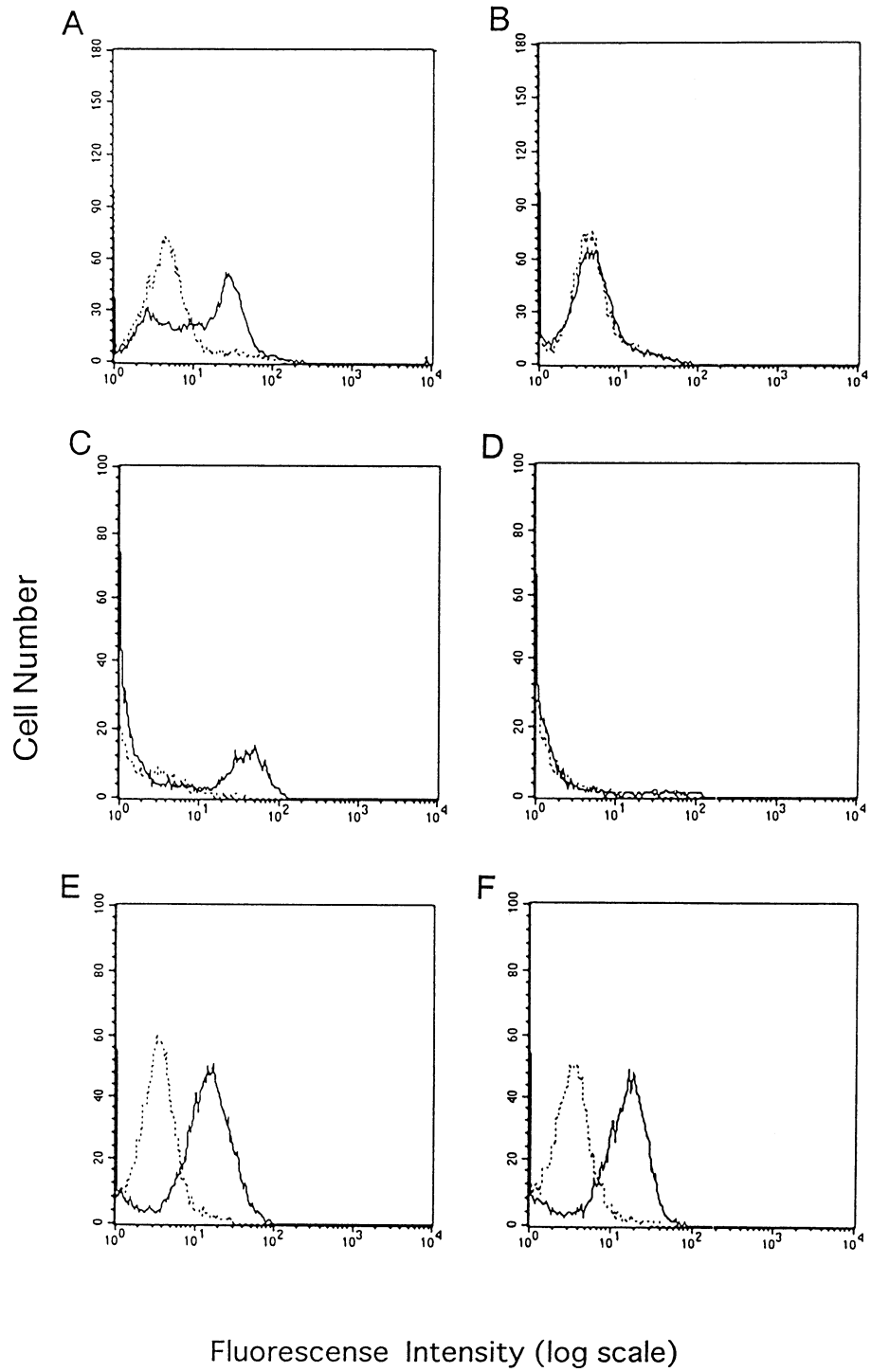

Fig. 2. Expression of Gr-1. To follow the expression of Gr-1, a granulocyte-specific surface marker, the cells were stained with Gr-1 monoclonal antibody coupled with FITC and analyzed by FACS. TER119$^-$/Gr-1$^-$ fetal liver cells of 13 day-old embryos were fractionated by magnetic beads with TER119 and Gr-1 antibodies, and cultured for 4 days with or without TBR511 stromal cell layers. (A) Expression in unfractionated bone marrow cells; Gr-1$^+$ cells were 57%. (B) Expression in unfractionated fetal liver cells; Gr-1$^+$ cells were not detectable. (C) Fetal liver cells after 4 days culture in the presence of G-CSF (1 ng/ml). (D) TER119$^-$/Gr-1$^-$ fetal liver cells after 4 days culture in the absence of G-CSF, (E) TER119$^-$/Gr-1$^-$ fetal liver cells after 4 days culture on the TBR511 stromal cell layers in the presence of G-CSF (1 ng/ml), (F) TER119$^-$/Gr-1$^-$ fetal liver cells after 4 days culture on the TBR511 stromal cell layers in the absence of G-CSF. Dotted lines show the patterns without the antibody.

Table II. LARGE GRANULOCYTE COLONY FORMATION OF THE Gr-1$^-$ PROGENITOR CELLS FROM FETAL LIVER AND BONE MARROW ON THE STROMAL CELL LAYERS.

		Number of Large G-colonies/2 × 10^4 Cells	
		G-CSF (−)	G-CSF (+)
Gr-1$^-$/TER119$^-$ Fetal Liver Cells			
without stromal cells	Exp. 1	0	0
	Exp. 2	0	0
TBR511 Cell Layer	Exp. 1	203.7±22.7	225.7±15.3
	Exp. 2	186.0±31.1	200.3±31.2
TBR59 Cell Layer	Exp. 1	106.0±22.7	96.0±31.9
	Exp. 2	172.3± 3.8	127.7±33.6
Lin$^-$ Bone Marrow Cells			
without stromal cells		0	(5.0±3.0)*
TBR511 Cell Layer		140.7±38.7	162.3±43.7

After lysing, bone marrow cells from 8-weeks C57BL/6 mouse were incubated with a cocktail of monoclonal antibodies of Lin markers followed by incubation with magnetic beads to eliminate Lin$^+$ cells. After the treatment, 2 × 10^4 Lin$^-$ bone marrow cells were cultured on stromal cells in the presence (1 ng/ml) or absence of G-CSF for 5 days in the semisolid medium as described in Materials and Methods. For preparation of fetal liver progenitor cells, the lysing treated cells were incubated with TER119 and Gr-1 followed by incubation with magnetic beads to eliminate erythroid progenitor cells and Gr-1$^+$ cells. Finally, recovery of the TER119$^-$/Gr-1$^-$ cells were 15 to 20% from the fetal liver cells after lysing treatment. In the TER119$^-$/Gr-1$^-$ fetal liver cells, because of enrichment of the colony-forming cells, 4,000 cells were seeded and the number of colonies was counted on the 4th day. The number of colonies was expressed as number/2 × 10^4 cells based on the recovery of the cells. All numbers were derived from three wells. * Parentheses indicate number of small hanging colonies in semisolid medium distinguished from large G-colonies. Exp.; independent experiment.

tion of anti-G-CSF antibody did not decrease the size of the large G-colonies (data not shown). Thus, bone marrow stromal cells supported granulocyte development of the Gr-1$^-$ progenitors independent of G-CSF. The colony forming activity of conditioned medium from TBR511 was assayed, and the magnitude of collective activity of secreted soluble factors in the conditioned media was very low (Table IV).

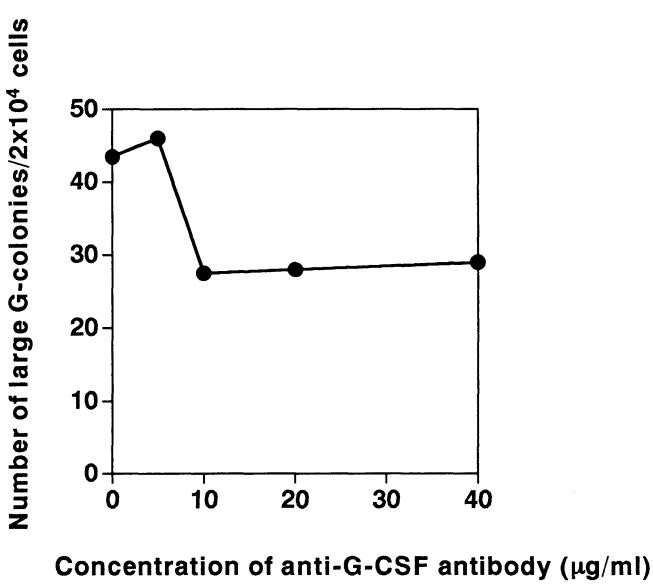

Fig. 3. Dose response effect of anti-G-CSF polyclonal antibody on G-colony formation. The fetal liver progenitor cells after lysing were seeded on the TBR511 cell layers at 1 × 10^4 cells/well. Polyclonal rabbit anti-C-CSF antibody was added in increased amount and the number of G-colony formation was examined. All points were derived from the results of 2 wells.

Expression of granulocyte-specific markers. Formation of the large G-colonies may be due to the growth expansion of the progenitor cells on the stromal cell layers as reported in the stroma-supported erythropoiesis (14–16). Induction of terminal differentiation in the stroma-supported granulopoiesis was followed by expression of Gr-1 during in vitro culture (Fig. 2). Without stroma cell layers, G-CSF induced production of Gr-1$^+$ cells from the Gr-1$^-$ progenitor cells of 13.5-day old fetal liver. On the TBR511 cell layers, Gr-1$^+$ cells were produced in the absence of exogenously added G-CSF, and addition of G-CSF did not stimulate expression of Gr-1. When expression of myeloperoxidase (MPO) gene as a granulocyte-specific gene was measured by RT-PCR, it was induced by cocultivation with the stromal cells as well as by G-CSF (Fig. 4).

Molecules involved in the stroma-supported granulopoiesis. The stromal cells may support granulopoiesis through cell-to-cell interaction, thus we examined the effect of antibodies against the adhesion molecules. CD34, VLA-4, and PECAM-1 are expressed during the early stages of myeloid maturation (17, 18), and c-Kit is known to be involved in the stroma-supported erythropoiesis (15) and to be expressed on immature hematopoi-

Table III. EFFECT OF ANTI-G-CSF ANTIBODY ON LARGE GRANULOCYTE COLONY FORMATION.

		Number of Large G-colonies/2×10^4 Cells		
		G-CSF (−)	G-CSF (+)	anti-G-CSF
without stromal cells	Exp. 1	0	(17.0±4.3)*	0
	Exp. 2	0	(0.3±0.6)*	0
TBR511 Cell Layers	Exp. 1	56.0±4.4	63.0±2.0	47.7±3.1
	Exp. 2	45.3±8.7	40.0±8.7	25.7±3.1

The fetal liver progenitor cells after lysing were seeded on the TBR511 cell layers. Anti-G-CSF antibody (10 μg/ml) was added to the culture. All numbers were derived from 3 wells. * Parentheses indicate numbers of small hanging colonies in semisolid medium distinguished from large G-colonies. Exp.; independent experiment.

Table IV. EFFECT OF CONDITIONED MEDIUM FROM TBR511 ON FEEDER DEPENDENT GRANULOCYTE COLONY FORMATION.

conditioned media concentration	colony number			
	0%	10%	20%	30%
TBR511 conditioned medium on TBR511	25.0	21.5	21.0	24.5
TBR511 conditioned medium without stromal cells*	0.0	0.0	0.5	3.0

Bone marrow derived Lin^- progenitor cells were cultured on TBR511 cells with conditioned medium of TBR511 in semisolid medium. Conditioned medium was prepared from 3 days culture of confluent monolayer culture of TBR511. All numbers were the average of two wells derived from 1×10^3 Lin^- cells. * Conditioned media enhanced growth of stromal cells from Lin^- cells and adhered cells grew well in conditioned media. Most of the colonies in feeder-less-wells were accompanied by stromal cells.

Table V. EFFECT OF ANTIBODIES FOR THE LARGE GRANULOCYTE COLONY FORMATION ON THE TBR511 CELL LAYERS.

	no addition	anti-c-Kit	anti-VLA-4	anti-PECAM-1
Exp. 1#	38.2±3.0 (100%)	29.9±1.0 (78%)	31.7±2.4 (83%)	24.2±1.3 (63%)
Exp. 2	33.3±1.5 (100%)	24.3±1.5 (73%)	21.7±9.5 (65%)	17.3±5.7 (52%)
Exp. 3	41.7±2.5 (100%)	36.3±3.2 (87%)	N.D.	21.7±3.8 (52%)
Exp. 4	45.3±4.5 (100%)	30.6±3.8 (66%)	27.7±4.2 (61%)	N.D.
Exp. 5	56.0±4.4 (100%)	34.7±4.5 (62%)	29.3±2.5 (52%)	N.D.

Antibodies were added to the culture at 10 μg/ml and the colonies formed were counted on 5th day. Exp.; independent experiment. All numbers were derived from three wells. #: Unrelated rat monoclonal antibody against a surface molecule of spleen stromal cell line (clone 11D) was added to the culture at 10 μg/ml. N.D.; not determined.

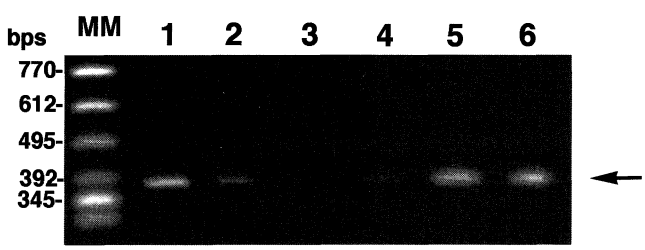

Fig. 4. Expression of myeloperoxidase gene. Expression of granulocyte-specific myeloperoxidase was examined by RT-PCR from each preparation of cells as described in Materials and Methods. Total cellular RNA in each fraction was reverse-transcribed and amplified with 25 cycles of PCR reaction and the DNA product was analyzed in 2% agarose gel electrophoresis. The arrow indicates the size (379 bps) of the expected product. Lane 1, unfractionated bone marrow cells; lane 2, Lin^- bone marrow cells; lane 3, Lin^- cells after 4 days of culture in the absence of G-CSF; lane 4, Lin^- cells after 4 days of culture in the presence of G-CSF (1 ng/ml); lane 5, Lin^- cells after 4 days of culture with TBR511 cells in the absence of G-CSF; lane 6, Lin^- cells after 4 days of culture with TBR511 cells in the presence of G-CSF (1 ng/ml). The left end lane indicates size markers.

etic cells promoting their proliferation (19, 20). Addition of anti-c-Kit antibody reduced the large G-colony formation 60–80% at 10 μg/ml which is sufficient for inhibition of stroma dependent lymphopoiesis (13). Addition of anti-VLA-4 antibody reduced it to 50–80% at 10 μg/ml which is sufficient for inhibition of stroma dependent lymphopoiesis (11, 13), and addition of anti-PECAM-1 reduced it to 50–60% (Table IV). Thus, both adhesion molecules are involved in the stroma-supported granulopoiesis.

DISCUSSION

We have established many bone marrow stromal cell lines from T-antigen transgenic mice and examined their ability to support the hematopoietic progenitor cells of myeloid lineage. We demonstrated that various bone marrow stromal cell lines selectively stimulate large colony formation of granulocytes, macrophages and erythrocytes (8). Except for the erythrocytes, which required erythropoietin in addition to the stromal cell

layers, no exogenously added cytokines were required for stimulation of the large colony formation of granulocytes and macrophages. Therefore we conjectured that cytokine-independent pathways of granulopoiesis might occur in bone marrow, and that the stromal cells may mediate information necessary for induction of proliferation and differentiation of granulocytes through cell-to-cell interaction. In the present study, we demonstrated that bone marrow stromal cells support granulopoiesis of the Gr-1$^-$ progenitor cells through an independent pathway of G-CSF. The stromal cell lines used in this study did not produce G-CSF (8) and exogenously added G-CSF did not enhance the G-colony formation. Addition of anti-G-CSF antibody caused only partial reduction in the colony formation, suggesting the presence of progenitors that respond to the stromal cells independent of G-CSF. Although growth and differentiation of the granulocytic progenitor cells occurred in the G-CSF-dependent pathway and the stroma-dependent pathway, both pathways may induce very similar differentiation programs (2, 4, 21) as shown by induction of Gr-1 and MPO. At present, it is not known which population of progenitor cells respond to the signals from the stromal cells.

We previously reported that the fetal liver (16) and spleen (14) stromal cells supported erythropoiesis and that the bone marrow stromal cells supported the development of the sorted hematopoietic stem cells (13). SCF/c-Kit interaction was required for both processes (13, 22), but c-Kit was down regulated after erythroid maturation (15, 23), thus c-Kit seems to be functional only at a restricted immature stage of the hematopoietic cells. In granulopoiesis, however, the inhibitory effect of anti-c-Kit antibody was weaker than those in erythropoiesis or the stem cell development. Lund-Johansen and Terstappen (17) reported that VLA-4 is expressed in immature granulocytes, but downregulated after maturation. Inhibition of the stroma-supported granulopoiesis by anti-VLA-4 was weaker than those in B-lymphopoiesis (10, 13), the stroma-supported erythropoiesis (22) and development of the sorted stem cells on the stromal cells (13) at the same antibody concentration. VLA-4/VCAM-1 interaction may not be strongly involved in granulocytic development, while it affects development of other lineages of progenitor cells. Since PECAM-1 was expressed in immature granulocytes and also shown to play a crucial role in transendothelial migration of mature granulocytes (17, 24, 25), we examined the effect of anti-PECAM-1 antibody on the stroma-supported granulopoiesis. The result indicated that PECAM-1 was more strongly involved in the stroma-supported granulopoiesis than VLA-4. These results on the effects of several antibodies against surface molecules expressed in the hematopoietic cells suggested that stromal cells supported granulopoiesis in a different way compared with other lineages of hematopoietic cells, in which rather similar molecules such as c-Kit and VLA-4 are involved in the stromal supported hematopoietic development.

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