Multi-colony Stimulating Activity of Interleukin 5 (IL-5) on Hematopoietic Progenitors from Transgenic Mice that Express IL-5 Receptor α Subunit Constitutively

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Summary

The interleukin 3 (IL-3), IL-5, and granulocyte/macrophage colony-stimulating factor receptors consist of a cytokine-specific α subunit and the common β subunit. Whereas IL-3 stimulates various lineages of hematopoietic cells, including multipotential progenitors, IL-5 acts mainly as an eosinophil lineage-specific factor. To investigate whether the lineage specificity of IL-5 is due to restricted expression of the IL-5 receptor α subunit (IL-5Rα), we generated transgenic mice that express the mouse IL-5Rα constitutively by phosphoglycerate kinase promoter. The transgenic mouse expressed IL-5Rα ubiquitously, and the bone marrow cells formed various types of colonies, including multi-lineage colonies, in response to IL-5. IL-5 also supported formation of both multi-lineage and blast cell colonies from dormant progenitors of the 5-fluorouracil–treated transgenic mice. The cells composing the blast cell colony gave rise to many colonies including multi-lineage colonies when they were replated in secondary culture containing either IL-5 or IL-3. There was no significant difference in replating efficiency or in types of secondary colonies between IL-5– and IL-3–stimulated cultures. Conversely, the cells from the IL-3–induced blast cell colonies of the transgenic mice proliferated in response to either IL-3 or IL-5. Thus, the development of the progenitors can be equally supported by either IL-5 or IL-3, suggesting that intracellular signals from the IL-3R can be replaced by those from IL-5. These results strongly suggest that the lineage specificity of IL-5 is mainly due to the restricted expression of IL-5Rα.

IL-3, IL-5, and GM-CSF exhibit similar functions on their common target cells such as eosinophils (1–3). These common functions are believed to be mediated by the shared receptor subunit (4–8). The high-affinity receptors for IL-3, IL-5, and GM-CSF are composed of a cytokine-specific α subunit (8–10) and the common β subunit; both are members of the class I cytokine receptor family. The α subunits bind their specific cytokine with only low affinity and form high-affinity receptor with the common β subunit (β_c)[1]. The β_c subunit by itself has no significant binding to any of these cytokines but is essential for high-affinity binding as well as signal transduction. Whereas there is only the common β subunit in the human receptors, the mouse has an additional IL-3–specific β subunit (β_{IL3}, also known as AIC2A) that is 91% identical to the mouse β_c (also known as AIC2B) at the amino acid level (11). The two β subunits are coexpressed on various hematopoietic cells. Interestingly, however, β_{IL3} itself binds mouse IL-3 with low affinity and interacts with only IL-3Rα subunit (IL-3Rα) (10). The physiological role of β_{IL3} in the mouse remains unknown.

Whereas IL-3, IL-5, and GM-CSF exhibit similar functions on target cells such as eosinophils, they also have their individual functions. IL-3 stimulates hematopoietic stem cells as well as various lineage-committed progenitors including granulocytes, macrophages, eosinophils, mast cells, megakaryocytes, and erythroid cells (12), so IL-3 is also known as multi-CSF. GM-CSF was originally defined as a factor that stimulates colony formation of granulocytes and macrophages. Subsequent studies, however, have shown that GM-CSF interacts with a much broader range of cells including uncommitted multipotential progenitors (13). In contrast, IL-5, originally found as a B cell differentiation factor that stimulates production of IgM and IgA in mouse (14, 15), has a major role in eosinophil development in both mouse and human, and primitive hematopoietic progenitors do not respond to

[1] *Abbreviations used in this paper:* β_c, common β subunit; β_{IL3}, IL-3–specific β subunit; BMC, bone marrow cell; Epo, erythropoietin; FU, fluorouracil; IL-3Rα, IL-3Rα subunit; PGK-1, phosphoglycerate kinase 1.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/03/0889/11 $2.00
Volume 181 March 1995 889–899

IL-5 (16). IL-5 supports only a few eosinophil colonies in semisolid cultures of mouse normal bone marrow cells (BMC) or spleen cells.

These functional differences may be due to different means of signal transduction among the three α subunits. Alternatively, because the three α subunits are equivalent in signaling, the differential response to cytokines may be due to differential expression of the α subunits. We have approached this question by generating transgenic mice expressing the IL-5Rα ubiquitously. BMC from the IL-5Rα transgenic mouse formed colonies of multiple lineages in response to IL-5, indicating that IL-5α has a potential similar to IL-3Rα when it is expressed on early progenitors. Thus, the lineage-restricted response of BMC to IL-5 is most likely due to the restricted expression of IL-5Rα on eosinophils.

Materials and Methods

Construction of the Phosphoglycerate Kinase 1-IL-5Rα Gene and Production of Transgenic Mice. An XhoI fragment carrying mouse IL-5Rα cDNA in pIL-5R.8 plasmid (9) was placed between the phosphoglycerate kinase (PGK) 1 promoter (17) and the SV40 early polyadenylation signal (Fig. 1). The plasmid vector for the PGK-1 promoter was provided by R. Murray (DNAX). A purified BamHI fragment containing PGK-IL-5Rα was microinjected into pronuclei of fertilized eggs of C57BL/6 × SJL F_2 hybrid mice as described (18) by DNX, Inc. (Princeton, NJ). Transgenic mice were screened by PCR and Southern blot analysis of tail DNA using an entire IL-5Rα cDNA fragment as a probe, as described previously (19).

PCR reaction was performed by using AmpliTaq DNA polymerase (Perkin Elmer-Cetus, Norwalk, CT), 20 pmol of primers, and 1 μg tail DNA for 30 cycles (94°C 1 min, 52°C 2 min, 72°C 3 min), followed by 10 min at 72°C. Oligonucleotide primers, P1 and P2, are shown in Fig. 1. The 5′ primer (P1: 5′ACGCTTCAAAAGCGCACGTCT3′) was in the PGK-1 promoter gene, and the 3′ primer (P2: 5′AACTTGAGCTAATCCAGTGGC3′) was in the IL-5Rα gene. PCR products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide. The expected size of the PCR product was 541 bp, and the identity of the PCR product was confirmed by digestion with either HindIII or XhoI.

RT-PCR. Total RNAs were isolated from various tissues and cells using the acid-guanidinium-phenol-chloroform protocol (20) (Clontech Laboratories, Palo Alto, CA). Polyinosinic acid (10 μg) was added as a carrier when RNA was extracted from blast cell colonies. In all cases, RNA preparations were subjected to a DNase I (GenHunter, Brookline, MA) digestion step before cDNA synthesis, thus eliminating any remaining genomic DNA. Total RNA was reverse transcribed using a First-Strand cDNA Synthesis Kit (Pharmacia, Piscataway, NJ) for 1 h at 37°C. PCR was performed by using P1 and P2 primers under the same conditions as described above. To check for genomic DNA contamination, a control reaction with heat-inactivated reverse transcriptase was always included.

Flow Cytometry. BMC from transgenic mice and their normal littermates were prepared after removing red blood cells using ammonium chloride buffer solution. A myeloid cell line, OTT1 (21), was maintained in RPMI medium containing 10% FCS (Sigma Chemical Co., St Louis, MO), 50 μM 2-ME (Sigma Chemical Co.) and 10 ng/ml mouse IL-3. Cells (10^6) in 50 μl of PBS containing 5% FCS were incubated with 1 μg of purified mAb, H7 (rat IgG2a), which recognizes IL-5Rα (22), or rat IgG2a (Pharmingen, San Diego, CA) as an isotype control for 30 min at 4°C. Cells were pelleted, washed with PBS, and incubated with PE-conjugated goat anti-rat IgG (H+L) (Boehringer Mannheim, Indianapolis, IN) for 30 min at 4°C. These cells were washed, resuspended in 1 ml PBS, and analyzed on a FACScan® (Becton Dickinson & Co., San Jose, CA).

Cell Preparation. Single-cell suspensions were prepared from bone marrow or spleen of 6–8-wk-old mice. BMC were flushed from femurs and tibiae into α-medium (GIBCO BRL, Gaithersburg, MD) by using a 26-gauge needle. Spleen cells were prepared by teasing the spleen in 3 ml of α-medium in a 35-mm suspension culture dish (model 171099; Nunc, Inc., Naperville, IL) and by repeated pipetting. Either BMC or spleen cells were passed through a 70-μm nylon cell strainer (model 2350; Becton Dickinson Labware, Franklin Lakes, NJ).

5-fluorouracil (FU) (Sigma Chemical Co.) was administered through tail veins of mice at a dosage of 150 mg/kg body weight (23). Spleen cells and BMC were harvested 4 d and 2 d after the 5-FU injection, respectively.

Growth Factors. Recombinant mouse IL-3 and GM-CSF were produced in silkworm and yeast, respectively (24, 25). Recombinant mouse IL-5, human IL-6, and human erythropoietin (Epo) were purchased from R&D Systems, Inc., (Minneapolis, MN). Unless otherwise specified, concentrations of growth factors used in this study were as follows: IL-3, 10 ng/ml; IL-5, 100 ng/ml; GM-CSF, 10 ng/ml; IL-6, 100 ng/ml; Epo, 2 U/ml.

Clonal Cell Cultures. Methylcellulose culture was carried out in 35-mm suspension culture dishes (model 171099, Nunc, Inc.). 1-ml of culture mixture consisted of 2 × 10^4 BMC from 5-FU-untreated mice, 5 × 10^4 BMC from 5-FU-treated mice, or 1 × 10^6 spleen cells from 5-FU-treated mice; α-medium; 0.9% 4000 centipoises methylcellulose (Fisher, Norcross, GA); 30% FCS (Hyclone Laboratories, Logan, UT); 1% deionized, fraction V BSA (Sigma Chemical Co.); 100 μM 2-ME; and hematopoietic growth factors. In a serum-free culture, FCS was replaced with a combination of 1% deionized crystallized BSA (Sigma Chemical Co.), 300 μg/ml 30% iron-saturated human transferrin (Boehringer Mannheim), 160 μg/ml soybean lecithin, 96 μg/ml cholesterol and 100 nM sodium selenite (all from Sigma Chemical Co.); fraction V BSA was omitted. Dishes were incubated at 37°C in a humidified atmosphere with 5% CO_2 in air. Colony types were determined on day 16 of incubation by in situ observation on an inverted microscope according to the criteria described previously (26). Except for megakaryocyte colonies, colonies consisting of ≥50 cells were scored. Abbreviations of colony types are as follows: GM, granulocyte/macrophage colonies; Mϕ, macrophage colonies; Eo, eosinophil colonies; GMM, granulocyte/macrophage/megakaryocyte colonies; GEM, granulocyte/erythrocyte/macrophage colonies; GEMM, granulocyte/erythrocyte/macrophage/megakaryocyte colonies; EM, erythrocyte/megakaryocyte colonies; Meg, megakaryocyte colonies; BFU-E, erythroid bursts; and Mast, mast cell colonies.

Replating Experiments. To determine the potential of the blast cell colonies, we carried out replating experiments of individual blast cell colonies developed in cultures of spleen cells from 5-FU-treated normal or transgenic mice. Individual blast cell colonies developed on day 7 of incubation in the presence of IL-5 or IL-3 were picked up with a micropipette (Eppendorf North America, Inc., Madison, WI) on an inverted microscope, resuspended in 100 μl of α-medium, gently pipetted, and divided into three parts. Each aliquot of the cell suspension was added to secondary culture medium containing IL-3 plus Epo, IL-5 plus Epo, or Epo alone. Replated cells were cultured and secondary colonies were scored in the same manner as primary cultures.

Results

Production of Mouse IL-5Rα Transgenic Mice. To express IL-5Rα in immature hematopoietic progenitor cells constitutively, we used the PGK promoter since PGK is a housekeeping enzyme that is expressed at a high level in virtually all cell types (27) and the PGK-1 promoter is highly active in embryonic tissues, especially in mouse embryonic stem (ES) cells (28). To generate transgenic mice, a 2.6-kb DNA fragment containing mouse PGK-1 promoter, mouse IL-5Rα cDNA, and SV40 early poly A tail (Fig.1) was microinjected into C57BL/6 × SJL F_2 hybrid mouse eggs. For screening transgenic mice, tail DNA was analyzed by PCR with oligonucleotide primers, P1 and P2 (Fig.1). Integration of the transgene was then confirmed by Southern blot analysis with entire mouse IL-5Rα cDNA as a probe (data not shown). Four founder mice were found to carry the mouse IL-5Rα transgene (Nos. 5, 12, 13, and 18) among 20 offspring. By crossing to C57BL/6 mice, two (Nos. 5 and 13) of them transmitted the transgene to half of their offspring, regardless of their sex.

Expression of mouse IL-5Rα Gene in Transgenic Mice. RT-PCR analysis was performed to examine expression of the transgene. To ensure that the PCR products were actually derived from RNA, the extracts from various tissues were treated with DNase I before reverse transcriptase reaction. PCR was performed for 30 cycles. Control PCR using heat-inactivated reverse transcriptase was also performed to check genomic DNA contamination. We used the same primers as those for the tail DNA screening, which covered both PGK-1 promoter and IL-5Rα cDNA. No PCR product was obtained in normal littermate mice (data not shown). The IL-5Rα transgene was expressed ubiquitously in transgenic mice derived from the No. 13 mouse (Fig. 2). Expression of the IL-5Rα transgene in BMC was also confirmed by using a mAb, H7 (22). Approximately 20% of the BMC of transgenic mice from No. 13 were stained with H7 (Fig. 3). In contrast, IL-5Rα expression was hardly detected in transgenic mice from No. 5 by RT-PCR and FACS® analysis (data not shown). We therefore analyzed hemizygote transgenic mice from the No. 13 female mouse in this study. In all experiments, we used as negative controls normal littermates whose genetic backgrounds were identical to those of the transgenic mice.

Both blood cell count and blood picture of the transgenic mice were normal. Neither eosinophilia nor lymphocytosis was observed in the transgenic mice.

Dose-dependent Effect of IL-5. BMC of the transgenic mouse formed colonies in an IL-5–dependent manner (Fig. 4). IL-5 stimulated only a few eosinophil colonies from BMC of normal mice. In contrast, various types of colonies were formed in response to IL-5 from the IL-5Rα transgenic mice. The number of colonies reached the maximal level at 100 ng/ml IL-5. At this concentration, the total number of colonies was comparable with that of colonies supported by the optimum con-

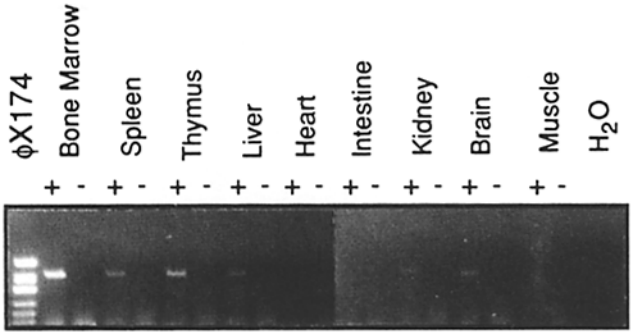

Figure 2. RT-PCR analysis of transgene expression. RNA was prepared from various tissues of IL-5Rα transgenic mice (No. 13 founder mouse). cDNA derived from 1 μg total RNA was used for PCR. PCR was performed for 30 cycles. Lane (−) is the PCR product with heat-inactivated reverse trancriptase in cDNA synthesis reaction. The PCR product without cDNA is also shown as H_2O. First lane is a DNA size marker (HinfI-digested *φX174*). Expression of the IL-5Rα transgene was observed in bone marrow, spleen, thymus, liver, kidney, and brain.

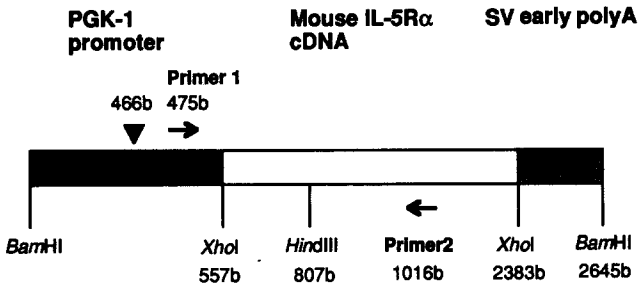

Figure 1. Structure of IL-5Rα transgene. The mouse PGK-1 promoter and the poly A addition site of the SV40 early gene were placed upstream and downstream of the mouse IL-5Rα cDNA, respectively, as described in Materials and Methods. The BamHI fragment was injected into fertilized eggs of C57BL/6 × SJL F_2 hybrid mice. (▼) The putative transcription start site. To distinguish the transgene product from the endogenous gene product, PCR primers 1 and 2 were chosen to cover both PGK-1 promoter and IL-5Rα sequences.

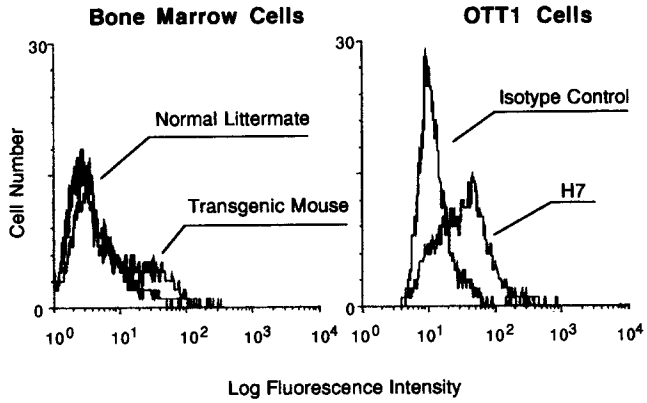

Figure 3. Cell surface expression of IL-5Rα. BMC of IL-5Rα transgenic mice derived from No. 13 mouse or their normal littermates were incubated with H7 and stained with PE-conjugated anti-rat IgG (H+L). Fluorescence was analyzed by FACScan®. OTT1 cells, a mouse myeloid cell line which responds to IL-3, GM-CSF, and IL-5 (22), were also stained with H7 as a positive control.

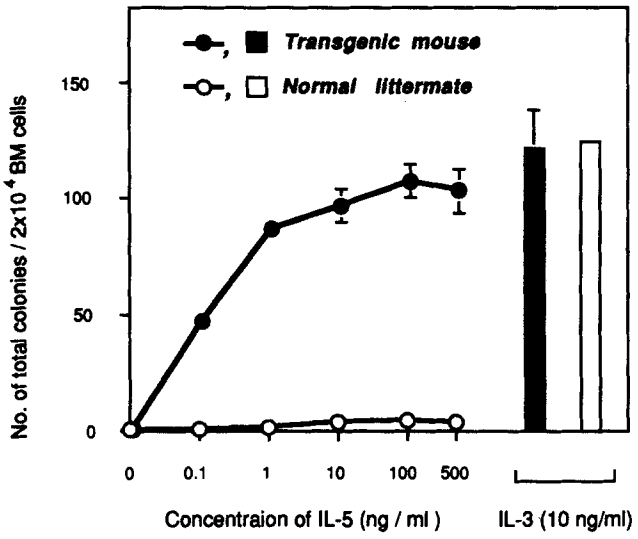

Figure 4. IL-5–dependent colony formation. Total number of colonies formed from 2 × 10^4 BMC of IL-5Rα transgenic mice or normal littermates were scored.

centration of IL-3. Thus, we employed 100 ng/ml IL-5 to analyze the effect of IL-5 more precisely on the hematopoietic progenitor cells of IL-5Rα transgenic mice in subsequent studies.

IL-5–dependent Hematopoietic Colony Formation. The number of GM and macrophage colonies supported by GM-CSF was almost the same between the transgenic mice and the normal littermates, ensuring that an equal number of progenitor cells was plated in a dish in this experiment (Fig. 5 *A*). As reported previously (12, 16), IL-5 supported the growth of a small number of eosinophil colonies from BMC of normal mice, whereas various types of colonies developed in the presence of IL-3. In contrast, both IL-5 and IL-3 equally stimulated development of GM, Mϕ GMM, Meg, and Mast colonies from BMC of transgenic mice (Fig. 5 *B*). Several eosinophil colonies were also formed in the culture of BMC of the transgenic mice in the presence of IL-5. Numbers and size did not exceed the eosinophil colonies derived from normal mice upon IL-5 stimulation. We could not find any erythroid lineage colonies in the culture of BMC of transgenic mice stimulated with IL-5 alone, indicating the inability of IL-5

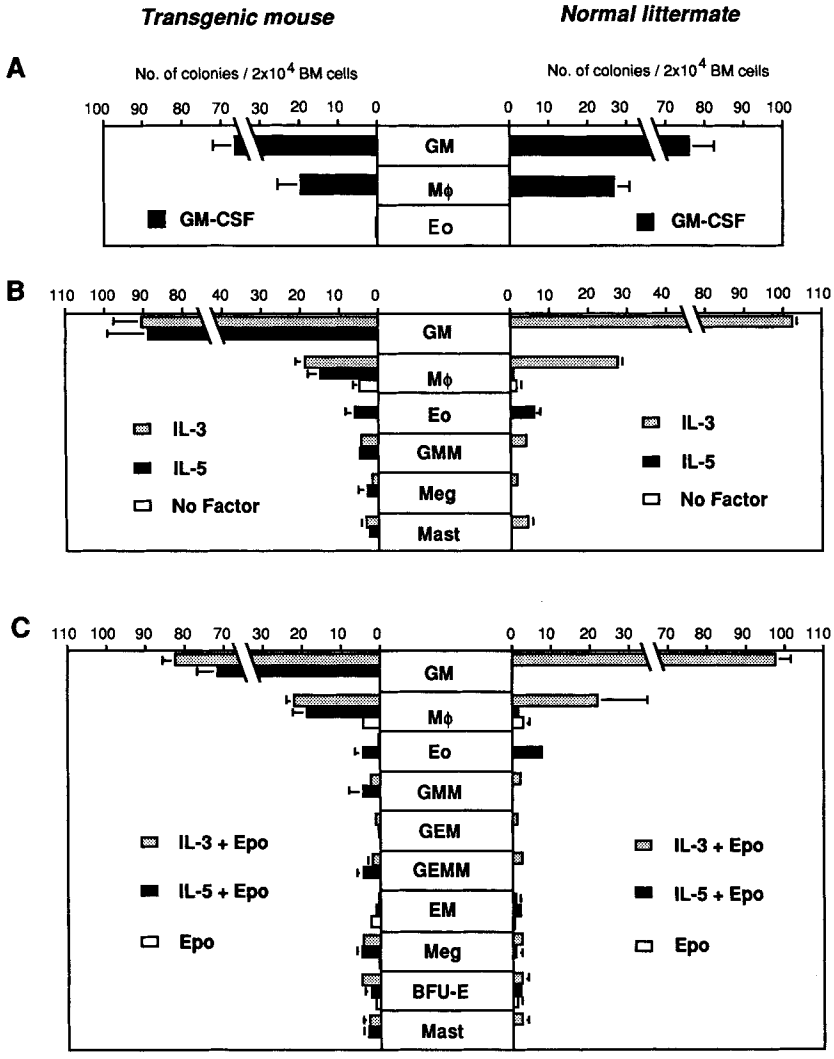

Figure 5. IL-5-induced colony formation. BMC (2 × 10^4 cells) from IL-5Rα transgenic mice or normal littermates were plated in the presence of FCS and various cytokines as indicated. The results are mean ± SD from triplicate cultures. Concentrations of growth factors are as follows: IL-3, 10 ng/ml; IL-5, 100 ng/ml; GM-CSF, 10 ng/ml; and Epo, 2 U/ml.

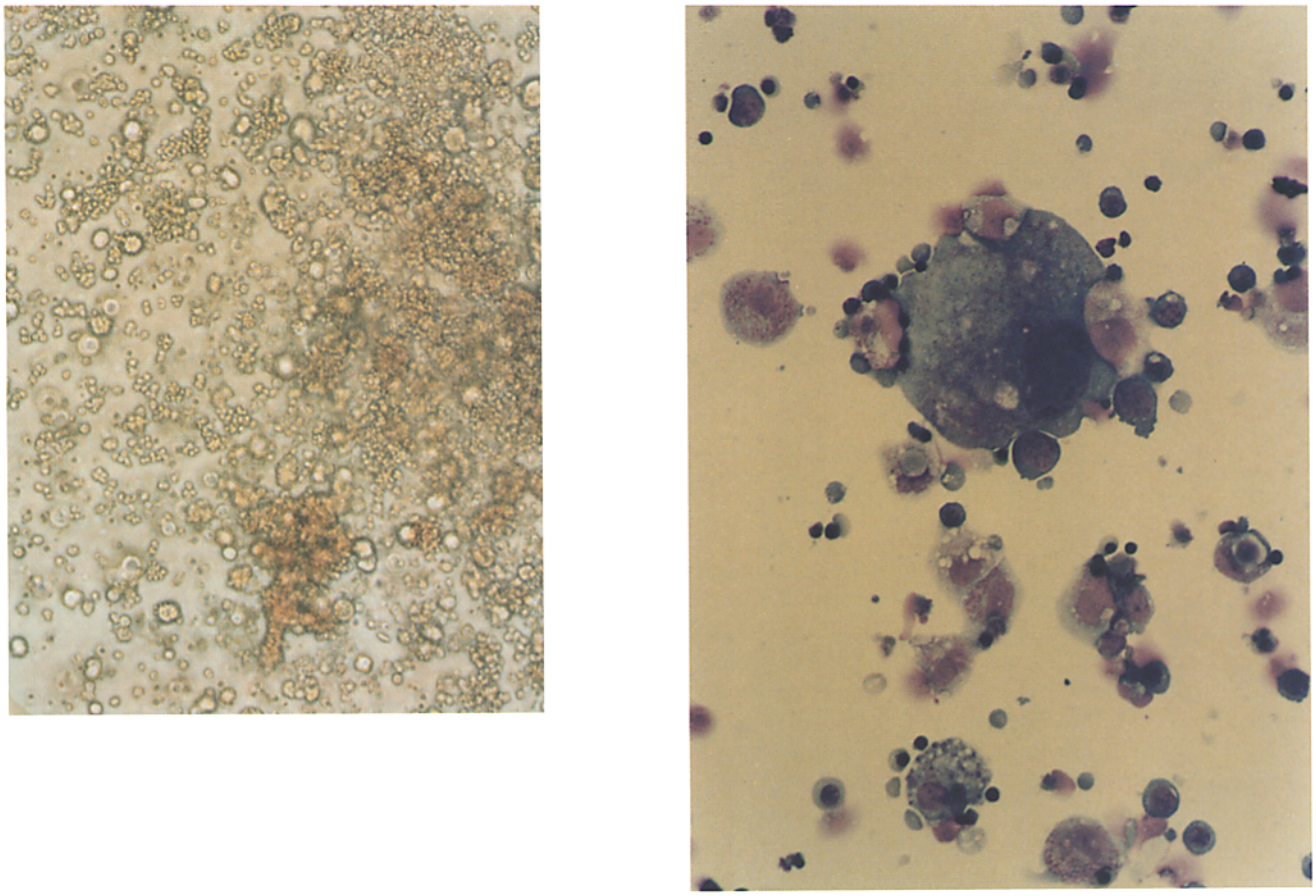

Figure 6. Morphology of a GEMM colony. (*Left*) A portion of a typical large GEMM colony from BMC of IL-5Rα transgenic mice on day 16 of incubation in the presence of IL-5 and Epo. ×100. (*Right*) A portion of a Wright Giemsa-stained smear of the colony shown at left, revealing immature myeloid cells, mast cells, erythroblasts, and megakaryocytes. ×400.

to substitute for Epo. In the presence of Epo, IL-5 as well as IL-3 supported the formation of BFU-E and multi-lineage colonies containing erythroid cells such as GEM, GEMM, and EM colonies (Fig. 5 *C* and Fig. 6). We also examined IL-5–induced colony formation from BMC of the transgenic mouse in serum-free condition, because FCS may contain various types of hematopoietic growth factors (29–32). Again, the effects of IL-3 on hematopoietic colony formation were completely replaced by IL-5 in the serum-free culture of BMC of the transgenic mice (Fig. 7).

Colony Formation from BMC of 5-FU–treated Mice. To investigate the effects of IL-5 on the development of primitive hematopoietic progenitors, we used BMC of 5-FU–treated mice. It is well established that the cell cycle of dormant progenitor cells is regulated by early acting cytokines such as IL-6, G-CSF, IL-11, leukemia inhibitory factor, IL-12, and stem cell factor (33). IL-3 is known to support the proliferation of multi-lineage progenitors when they exit from G_0 (12), and IL-3 supported formation of a few multi-lineage colonies from 5-FU–treated BMC of both normal and transgenic mice (Fig. 8). As reported previously (34, 35), a combination of IL-3 and IL-6 enhanced the formation of many multi-lineage colonies. Although IL-5 failed to support any colony formation from 5-FU–treated BMC of normal mice, a small number of multi-lineage colonies developed from 5-FU–treated BMC of transgenic mice in the presence of IL-5. IL-6 markedly synergized with IL-5 as well as IL-3 in support of multi-lineage colony formation from 5-FU–treated BMC of transgenic mice. These results indicate that the ability of IL-5 to support an early stage of hematopoietic development is comparable with that of IL-3 in transgenic mice.

Replating Experiment of Blast Cell Colonies. From spleen cells of 5-FU–treated mice, IL-3 supports formation of multipotential blast cell colonies which are highly enriched for progenitors (12, 36). When spleen cells from the transgenic mice treated with 5-FU were cultured in methylcellulose medium containing IL-3 or IL-5, small colonies consisting of blast cells with little sign of differentiation developed on day 7 of culture. In both cultures, approximately five blast cell colonies consisting of 50–200 blast cells were identified in a dish with 10^6 spleen cells. Expression of IL-5Rα transgene in these blast cell colonies was confirmed by use of RT-PCR (Fig. 9). Individual blast cell colonies were lifted on day 7, suspended in α medium, and divided into three parts. Each aliquot of the cell suspension was added to secondary culture medium containing IL-3 plus Epo, IL-5 plus Epo, or

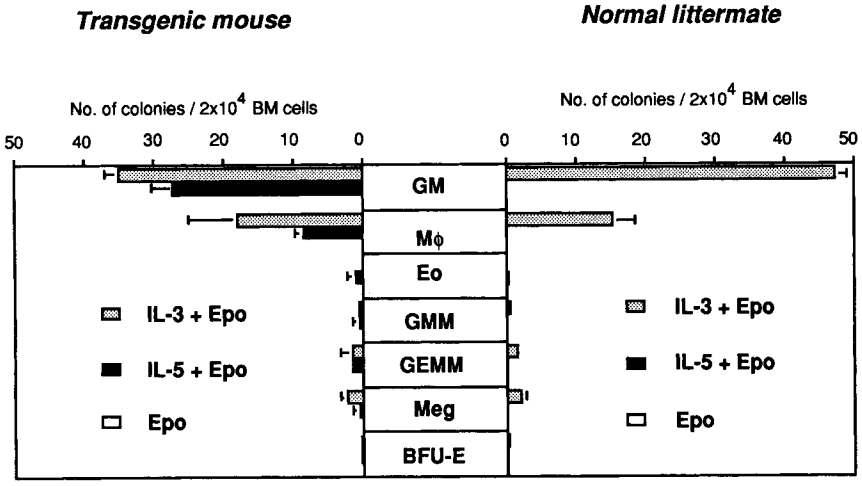

Figure 7. Colony formation from BMC of IL-5Rα trnasgenic mice or normal littermates in serum-free cultures. The results are mean ± SD from triplicate cultures. See legend to Fig. 5 for concentrations of growth factors.

Epo alone. Blast cell colonies supported by IL-5 responded not only to IL-5 but also to IL-3 and gave rise to secondary colonies (Table 1). Conversely, IL-3–induced blast cells responded to either IL-3 or IL-5. As previously reported (12, 36), very heterogeneous distribution of secondary colonies, including the incidence of GEMM colonies, was observed. On the other hand, the blast cells showed similar replating efficiency and similar distribution of secondary colony types when they were divided and individually replated in the secondary culture containing either IL-3 or IL-5. There was no significant difference in the fate of the blast cells regardless of whether IL-3 or IL-5 was used as a second stimulus.

In contrast to the transgenic mice, blast cell colonies were developed in the culture of spleen cells of the 5-FU–treated normal mice in the presence of IL-3, but not in the culture with IL-5. Normal mice-derived blast cell colonies supported by IL-3 gave rise to very few or no secondary colonies upon stimulation with IL-5, whereas IL-3 supported many secondary colonies including multi-lineage colonies from the same blast cells (Table 2).

These results indicate that 5-FU–resistant dormant hematopoietic progenitors of transgenic mice actually express both IL-3 and IL-5 receptors on their surface and that development of the progenitors can be equally supported by either IL-3 or IL-5. Results of replating studies also show that IL-5–induced formation of multi-lineage colonies results from direct action of IL-5 on progenitor cells, but not through accessory cells such as macrophages and lymphocytes, because blast cell colonies are devoid of any accessory cells or stromal cells.

Discussion

Whereas IL-3, GM-CSF, and IL-5 exhibit some similar functions on eosinophils and basophils (37, 38), there are also remarkable functional differences among them. In particular,

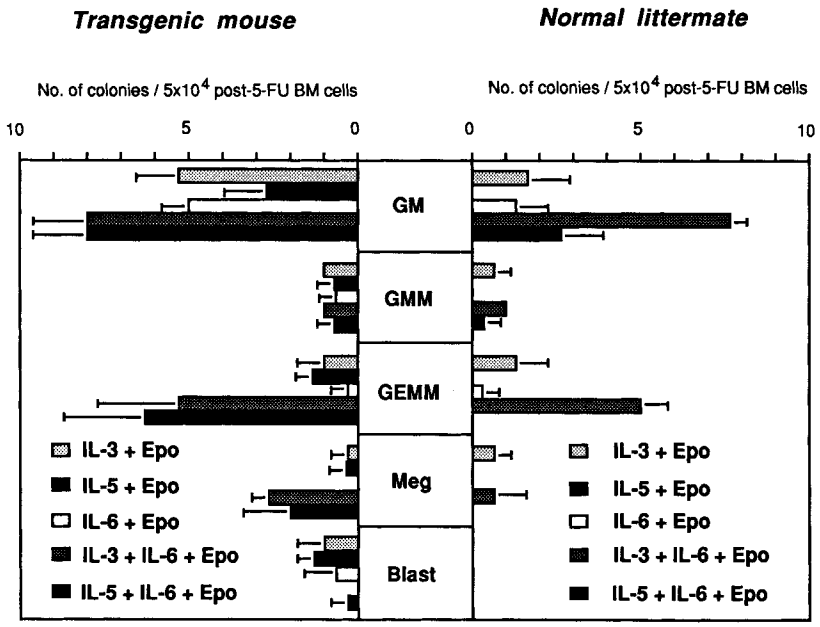

Figure 8. Colony formation from day 2 post-5-FU BMC of IL-5Rα transgenic mice or normal littermates. The results are mean ± SD from triplicate cultures. See legend to Fig. 5 for concentrations of growth factors. 100 ng/ml IL-6 was used in this experiment.

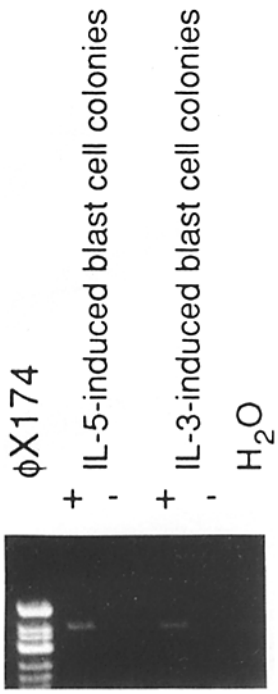

Figure 9. Expression of IL-5Rα mRNA in IL-5–induced blast cell colonies. Spleen cells from 5-FU–treated IL-5Rα transgenic mice were incubated with IL-3 or IL-5 in the absence of Epo. On day 7 of incubation, 50 blast cell colonies consisting of 100–200 cells were pooled, washed with PBS, and lysed in denaturing solutions with 10 μg polyinosinic acid as an RNA carrier. Using RNA from one quadrant of the lysate, cDNA was synthesized. PCR was performed for 35 cycles. Lane (−) is the PCR product with heat-inactivated reverse transcriptase in the cDNA synthesis reaction. The PCR product without any cDNA is also shown as H_2O. First lane is a DNA size marker (HinfI-digested *φX174*).

some functions of IL-3 and IL-5 are quite different. We have previously demonstrated that the functional high-affinity receptors for IL-3, IL-5, and GM-CSF are composed of α and β subunits (39). The α subunits are cytokine specific, each cytokine having its own specific α subunit. There are two distinct β subunits in the mouse; β_c is shared by these three receptors, whereas β_{IL3} is specific to the IL-3 receptor. Thus, the marked difference of the biological activities between IL-3 and IL-5 may be due to cellular signaling differences between cytokine-specific α subunits or between β_{IL3} and β_c. Alternatively, the difference may simply reflect the different pattern of expression of the α subunits.

These possibilities may be distinguished by analyzing receptor expression. Whereas a wide distribution of IL-3Rα in hematopoietic cells has been reported (10), anti-IL-5Rα antibody did not show significant staining of BMC (40) (Fig. 3). However, expression studies may not provide conclusive evidence to discriminate between the two possibilities, as the receptor expression can be measured only to a certain limit of sensitivity. Instead of studying receptor expression directly, we have approached the question by generating a transgenic mouse expressing the IL-5Rα constitutively. We have used the PGK promoter to express IL-5Rα, as PGK is a housekeeping enzyme that is present in various tissues constitutively. In fact, we detected IL-5Rα in various tissues of the transgenic mouse, where at least 20% of total BMC were stained with the anti-IL-5Rα antibody. IL-5Rα was expressed in blast cells from transgenic mice, which have the potential to differentiate to various lineages.

By using the BMC of transgenic mice, we have shown that IL-5 is able to support development of multi-potential progenitor cells without induction of preferential differentiation toward the eosinophil lineage in the IL-5Rα transgenic mouse. IL-5 supported formation of various types of colonies in methylcellulose culture of BMC from the IL-5Rα transgenic mice. These colonies included not only eosinophil colonies, but also GM, macrophage, GMM, Meg, and Mast colonies. There was no difference in number or size of IL-5–induced eosinophil colonies between the normal and the transgenic mice. IL-5 induced the colony formation of blast cells with no signs of terminal differentiation from 5-FU–treated spleen cells of the transgenic mice on day 7 of incubation. Our replating studies of individual blast cell colonies clearly demonstrated that proliferation of CFU blasts of the IL-5Rα transgenic mouse and their differentiation to terminal cells are equally supported by either IL-5 or IL-3, and that switching the cytokine from IL-5 to IL-3, or from IL-3 to IL-5 does not affect the differentiation of blast cells.

These results indicate that intracellular signals from the IL-3 receptors can be replaced with those from the IL-5 receptor and that the restricted response of the normal mouse BMC to IL-5 is likely due to the restricted expression of IL-5Rα. Thus, the progenitors do not appear to require IL-3Rα chain–specific or β_{IL3}-specific signals for their hematopoietic development, and β_c together with IL-5Rα is able to transduce essential signals for the development of multi-lineage hematopoietic progenitors. However, our results do not exclude the possibility that each α subunit and β_{IL3} plays a specific role in hematopoietic development, which may not be obvious by the in vitro colony assays employed in this study. To further investigate the role of β_{IL3} or β_c in the development of hematopoietic cells, knockout mice that lack either of the two β genes will be useful.

Recently, Dubart et al. (41) introduced the normal Epo receptor (EpoR) cDNA into mouse BMC using a retrovirus vector and analyzed growth of pluripotent progenitors upon Epo stimulation. Epo is a lineage-specific cytokine whose function is usually limited only to the proliferation and differentiation of late erythroid progenitors. They observed that the EpoR-infected BMC responded to Epo alone and gave rise to mixed colonies including erythrocytes, granulocytes, macrophages, and megakaryocytes. The mixed colonies contained the cells with a potential to form secondary colonies. These results indicate that EpoR is capable of stimulating the multipotential progenitors without restricting their differentiation potential to only an erythroid lineage. These results are consistent with our results using the IL-5Rα transgenic mouse and are also consistent with the results that EpoR and IL-3/IL-5/GM-CSF receptors share many common signaling pathways (42, 43).

However, there is a significant difference between EpoR-infected BMC and the IL-5Rα transgenic mouse. Epo alone supported erythrocyte development in the EpoR-infected BMC, whereas IL-3 or IL-5 alone did not support erythrocyte development. Whereas IL-5Rα is expressed constitutively in the transgenic mouse, IL-5 response absolutely depends on the presence of β_c. Thus, the difference may be due to the lack of β_c at a late stage of erythrocyte differentiation. Alternatively, there may be a difference in signaling pathways between EpoR and IL-3/IL-5/GM-CSF receptors. The mouse

Table 1. *Replating Studies of Transgenic Mouse Blast Cell Colonies*

Blast cell colony	Size	Stimuli		No. of secondary colonies						Replating efficiency
		Primary	Secondary	GM	GEM	GMM	GEMM	Mast	Total	
										%
			IL-3 + Epo	19	0	0	1	1	21	73.3
1	86	IL-3	IL-5 + Epo	13	0	0	6	2	21	73.3
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	21	0	0	3	0	24	82.8
2	87	IL-3	IL-5 + Epo	17	0	0	4	0	21	72.4
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	21	0	1	6	0	28	93.3
3	90	IL-3	IL-5 + Epo	18	0	1	5	0	24	80.0
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	17	0	0	3	0	20	50.0
4	120	IL-3	IL-5 + Epo	21	0	0	1	0	22	55.0
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	22	0	0	3	0	25	54.3
5	138	IL-3	IL-5 + Epo	15	0	0	2	0	17	37.0
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	18	0	1	9	0	28	50.0
6	168	IL-3	IL-5 + Epo	18	0	1	2	0	21	37.5
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	23	0	1	3	0	27	87.1
7	93	IL-5	IL-5 + Epo	18	0	0	3	0	21	67.7
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	28	0	1	2	0	31	93.9
8	99	IL-5	IL-5 + Epo	16	0	0	1	0	17	51.5
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	22	0	0	1	0	23	65.7
9	105	IL-5	IL-5 + Epo	19	0	0	2	0	21	60.0
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	29	0	1	2	0	32	78.0
10	123	IL-5	IL-5 + Epo	21	1	1	5	0	28	68.3
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	12	0	0	1	0	13	22.9
11	170	IL-5	IL-5 + Epo	11	0	0	1	0	12	21.2
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	41	0	3	12	0	56	99.3
12	188	IL-5	IL-5 + Epo	54	0	1	7	0	62	109.9
			Epo	0	0	0	0	0	0	0

Spleen cells from 5-FU–treated IL-5Rα transgenic mice or normal littermates (see Table 2) were incubated with IL-3 or IL-5 in the absence of Epo. On day 7 of incubation, blast cell colonies were individually lifted from the culture, suspended in α medium, gently pipetted, and divided into three parts. Each aliquot of the cell suspension was added to a secondary culture medium containing IL-3 + Epo, IL-5 + Epo, or Epo. Data represent the number of colonies on day 16 of the secondary culture. See legend to Fig. 5 for concentrations of growth factors.

Table 2. *Replating Studies of Normal Mouse Blast Cell Colonies*

Blast cell colony	Size	Stimuli		No. of secondary colonies						Replating efficiency
		Primary	Secondary	GM	GEM	GMM	GEMM	Mast	Total	
										%
			IL-3 + Epo	21	0	0	4	0	25	105.6
1	71	IL-3	IL-5 + Epo	1	0	0	0	0	1	4.2
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	8	0	0	10	0	18	62.1
2	87	IL-3	IL-5 + Epo	0	0	0	0	0	0	0
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	31	0	0	1	0	32	87.3
3	110	IL-3	IL-5 + Epo	0	0	0	0	0	0	0
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	8	0	0	2	0	10	19.7
4	152	IL-3	IL-5 + Epo	0	0	0	0	0	0	0
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	18	0	0	2	0	20	54.3
5	189	IL-3	IL-5 + Epo	0	0	0	0	0	0	0
			Epo	0	0	0	0	0	0	0
			IL-3 + Epo	36	0	0	2	0	38	57.0
6	200	IL-3	IL-5 + Epo	0	0	0	0	0	0	0
			Epo	0	0	0	0	0	0	0

Spleen cells from normal littermates. Please see Table 1 for details. Note that IL-5 could not support blast cell colony formation from spleen cells of normal littermates.

IL-3–dependent BaF3 transfected with the EpoR proliferated in response to either IL-3 or Epo, whereas only Epo induced globin synthesis (44, 45), indicating a difference in signaling pathways. Therefore it is possible that Epo-specific signaling is required for a late stage of erythrocyte differentiation. Whereas it is not clear from the report by Dubart et al. (41) whether Epo can support eosinophil differentiation in the EpoR-infected BMC, terminal differentiation of eosinophils may also require IL-5–specific signaling. These observations collectively suggest the possibility that cytokines such as IL-3/IL-5/GM-CSF or Epo induce proliferation signals in early hematopoietic progenitors expressing their receptors but may not instruct the direction of differentiation. Cytokine-specific signals may be required after commitment to a particular lineage such as erythrocytes or eosinophils.

It is well established that IL-3 and IL-6 synergistically stimulate formation of multi-lineage colonies (34, 35). Whereas IL-5 alone supported formation of a few multi-lineage colonies from BMC of the 5-FU–treated transgenic mice, a marked synergy between IL-5 and IL-6 was observed. As the multipotential progenitors appear to express IL-5Rα constitutively, synergy by IL-6 is probably not due to upregulation of the α subunit. Recently, McClanahan et al. (46) analyzed patterns of expression of various cytokine receptor genes in developing embryoid bodies from ES cells. They demonstrated that genes for IL-3Rα and GM-CSFRα are expressed constitutively in ES cells, as well as across the entire time course of embryoid body development. In contrast, β_c and β_{IL3} are upregulated in day 7 embryoid bodies. Thus, it is possible that IL-6 stimulates the transition of hematopoietic progenitors from the dormant G_0 stage to a cycling stage and that this transition is accompanied by upregulation of the β subunits. The marked synergy between IL-5 and IL-6 may result from enhanced formation of high-affinity receptors by association of IL-5Rα to the upregulated β subunits on the progenitors. BMC from transgenic mouse expressing not only an α subunit but a β subunit may provide a unique system for analyzing these questions.

We thank Drs. T. Nakahata, T. Yokota, and K. Arai (Institute of Medical Science, University of Tokyo) for discussion and critical reading of the manuscript.

The DNAX Research Institute of Molecular and Cellular Biology is supported by Schering-Plough Corporation.

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Received for publication 19 September 1994 and in revised form 18 October 1994.

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