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.

1 Abbreviations used in this paper: αc, common β subunit; βIL3, IL-3-specific β subunit; BMC, bone marrow cell; Epo, erythropoietin; FU, fluorouracil; IL-3Rα, IL-3Rα subunit; PGK-1, phosphoglycerate kinase 1.
IL-5 (16). IL-5 supports only a few eosinophil colonies in semi-solid 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-5Rα 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-IL5Rα 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) 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 x SJL F2 hybrid mice as described previously (18) by DNX, Inc. (Princeton, NJ). Transgenic mice were screened by PCR and Southern blot analysis of tail DNA using an entire 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 x SJL F2 hybrid mice as described previously (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 AmpliTag 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‘ACGCTTCAAAAGGCCACGTCTG3) was in the PGK-1 promoter gene, and the 3’ primer (P2: 5‘ACCTGACCTAATCCAGTGG3) 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.

RTFPCR. 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 ng) was added as a carrier when RNA was extracted from blast cell colonies. In all cases, RNA preparations were subjected to a DNase I digestion 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, OTTI (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 (105) 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 (F+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 Laboratoryware, 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. Methyllumcellulose culture was carried out in 35-mm suspension culture dishes (model 171099, Nunc, Inc.). 1-ml of culture mixture consisted of 2 × 104 BMC from 5-FU-untreated mice, 5 × 104 BMC from 5-FU-treated mice, or 1 × 103 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 mM sodium selenite (all from Sigma Chemical Co.); fraction V BSA was omitted. Dishes were incubated at 37°C in a humidified atmosphere with 5% CO2 in air. Colony types were determined on day 16 of incubation by in situ observation on a 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/macroage colonies; Mφ, macroage colonies; Eo, eosinophil colonies; GMM, granulocyte/macroage/megakaryocyte colonies; GEM, granulocyte/erythrocyte/macroage colonies; GEMM, granulocyte/erythrocyte/macroage/megakaryocyte colonies; EM, erythrocyte/megakaryocyte colonies; Meg, megakaryocyte colonies; BFU-E, erythroblast 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 IL5Rα Transgenic Mice. To express IL5Rα 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 F2 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 littersmates 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 4. IL-5-dependent colony formation. Total number of colonies formed from $2 \times 10^4$ BMC of IL-5Rα transgenic mice or normal littermates were scored.

Figure 5. IL-5-induced colony formation. BMC ($2 \times 10^4$ 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 G0 (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 10⁶ 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...
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,
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; β5 is shared by these three receptors, whereas β1,3 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 β1,3 and β5. 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 mice, 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 differenti-
| Blast cell colony size | Stimuli | No. of secondary colonies | Replating efficiency % |
|------------------------|---------|---------------------------|------------------------|
|                        | GM      | GEM | GMM | GEMM | Mast | Total |          |
| 1                      | 86      | IL-3 + Epo | 19  | 0   | 0   | 1    | 1      | 21 | 73.3 |
| 2                      | 87      | IL-3 + Epo | 13  | 0   | 0   | 6    | 2      | 21 | 73.3 |
|                        | IL-5 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | Epo     | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | IL-3 + Epo | 21  | 0   | 0   | 3   | 0     | 24    | 82.8 |
| 3                      | 90      | IL-3 + Epo | 17  | 0   | 0   | 4    | 0      | 21 | 72.4 |
|                        | IL-5 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | Epo     | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | IL-3 + Epo | 18  | 0   | 0   | 5   | 0      | 24    | 80.0 |
|                        | IL-5 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
| 4                      | 120     | IL-3 + Epo | 17  | 0   | 0   | 3    | 0      | 20 | 50.0 |
|                        | IL-5 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | Epo     | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | IL-3 + Epo | 22  | 0   | 0   | 3   | 0      | 25    | 54.3 |
|                        | IL-5 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
| 5                      | 138     | IL-3 + Epo | 15  | 0   | 0   | 2    | 0      | 17 | 37.0 |
|                        | IL-5 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | Epo     | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | IL-3 + Epo | 18  | 0   | 0   | 9   | 0      | 28    | 50.0 |
| 6                      | 168     | IL-3 + Epo | 18  | 0   | 0   | 2    | 0      | 21 | 37.5 |
|                        | IL-5 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | Epo     | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | IL-3 + Epo | 23  | 0   | 0   | 3   | 0      | 27    | 87.1 |
|                        | IL-5 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
| 7                      | 93      | IL-5 + Epo | 18  | 0   | 0   | 3    | 0      | 21 | 67.7 |
|                        | IL-3 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | IL-5 + Epo | 28  | 0   | 0   | 2   | 0      | 31    | 93.9 |
| 8                      | 99      | IL-5 + Epo | 16  | 0   | 0   | 1    | 0      | 17 | 51.5 |
|                        | IL-3 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | IL-5 + Epo | 22  | 0   | 0   | 1   | 0      | 23    | 65.7 |
| 9                      | 105     | IL-5 + Epo | 19  | 0   | 0   | 2    | 0      | 21 | 60.0 |
|                        | IL-3 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | IL-5 + Epo | 29  | 0   | 0   | 1   | 0      | 32    | 78.0 |
| 10                     | 123     | IL-5 + Epo | 21  | 1   | 1   | 5    | 0      | 28 | 68.3 |
|                        | IL-3 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | IL-5 + Epo | 12  | 0   | 0   | 1   | 0      | 13    | 22.9 |
| 11                     | 170     | IL-5 + Epo | 11  | 0   | 0   | 1    | 0      | 12 | 21.2 |
|                        | IL-3 + Epo | 0   | 0   | 0   | 0   | 0     | 0     | 0     | 0   |
|                        | IL-5 + Epo | 41  | 0   | 3   | 12   | 0      | 56    | 99.3 |
| 12                     | 188     | IL-5 + Epo | 54  | 0   | 1   | 7    | 0      | 62 | 109.9 |

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 |
| 1 71                  | IL-3         |                           | 21 | 0   | 0   | 4    | 0    | 25    | 105.6 |
| 2 87                  | IL-3         |                           | 1  | 0   | 0   | 0    | 0    | 1     | 4.2   |
| 3 110                 | IL-3         |                           | 8  | 0   | 0   | 10   | 0    | 18    | 62.1  |
| 4 152                 | IL-3         |                           | 0  | 0   | 0   | 0    | 0    | 0     |       |
| 5 189                 | IL-3         |                           | 8  | 0   | 0   | 2    | 0    | 10    | 19.7  |
| 6 200                 | IL-3         |                           | 18 | 0   | 0   | 2    | 0    | 20    | 54.3  |
|                       | IL-5 + Epo   |                           | 0  | 0   | 0   | 0    | 0    | 0     |       |
|                       | IL-5 + Epo   |                           | 0  | 0   | 0   | 0    | 0    | 0     |       |
|                       | IL-5 + Epo   |                           | 36 | 0   | 0   | 2    | 0    | 38    | 57.0  |

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, βc and βIL-3 are upregulated in day 7 embryoid bodies. Thus, it is possible that IL-6 stimulates the transition of hematopoietic progenitors from the dormant G0 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.

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