PURIFIED INTERLEUKIN 5 SUPPORTS THE TERMINAL DIFFERENTIATION AND PROLIFERATION OF MURINE EOSINOPHILIC PRECURSORS

By YUJI YAMAGUCHI,* TOSHIO SUDA,* JUNKO SUDA,‡ MITSUOKI EGUCHI,‡ YASUSADA MIURA,* NOBUYUKI HARADA,§ AKIRA TOMINAGA,§ and KIYOSHI TAKATSU§

*From the *Division of Hematology, Department of Medicine, Jichi Medical School, Tochigi-Ken 329-04; ‡The Second Department of Pediatrics, Dokkyo University School of Medicine, Mibu, Tochigi-Ken, 321-02; and the §Department of Biology, Institute for Medical Immunology, Kumamoto University Medical School, Kumamoto, 860 Japan*

CSFs are essential for hemopoietic progenitor cells to proliferate and differentiate in vitro as well as in vivo. Recently, granulocyte/macrophage colony-stimulating factor (GM-CSF),[1] granulocyte-CSF (G-CSF), macrophage-CSF, IL-3, and IL-4 (B cell stimulatory factor 1) have been highly purified and characterized and their gene structures have been determined (1–6).

A B cell–active factor, T cell–replacing factor (TRF) derived from the B151 T cell hybridoma (7), has been shown to promote IgM secretion by the BCL_1 B cell line and to induce hapten-specific IgG secretion in vitro by in vivo antigen-primed B cells (7–9). More recently, purified TRF has been shown (9) to have B cell growth factor II (BCGF-II) activity, as well as B cell differentiation factor (BCDF) activity. BCGF-II activity was originally described by Swain and Dutton (10) as the ability to induce proliferation of BCL_1 cells. Recently, eosinophil differentiation factor (EDF) (11, 12) has also been shown to have BCGF-II activity and to induce both growth and differentiation of preactivated normal murine B cells. The recent molecular cloning of cDNA encoding TRF has confirmed that a single molecule is responsible for both TRF and BCGF-II activities (13), and most likely for EDF activity too. It has been proposed (13) that this molecule, recombinant murine TRF(rTRF), be called IL-5.

EDF has been defined as an activity that stimulates the production of functional eosinophils from bone marrow in liquid culture system (11, 14). However, it is not clear whether this factor is specific for the eosinophil differentiation, or whether it is analogous to CSFs described for other hemopoietic lineages.

Using clonal cell culture and liquid culture system, we studied the in vitro effect of IL-5 on murine hemopoietic cells at various stages of differentiation: bone marrow cells from normal mice, spleen cells of 5-fluorouracil (5-FU)-

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[1] *Abbreviations used in this paper:* BCDF, B cell differentiation factor; BCGF, B cell growth factor; EDF, eosinophil differentiation factor; 5-FU, 5-fluorouracil; sup, supernatant.

treated mice, blast cells derived from these cells, and mature eosinophils obtained from mice infected with parasites.

In this study, it was demonstrated that IL-5 was involved in the terminal differentiation and amplification of eosinophils.

Materials and Methods

Mice. Inbred female BDF_1 mice, 8–15 wk old, were purchased from the Shizuoka Experimental Animal Center (Shizuoka, Japan). Mice were administered 5-FU (Adria Laboratories, Inc., Columbus, OH) through the tail vein at a dosage of 150 mg/kg body weight. Spleen cells were harvested 4 d later, and we prepared single-cell suspensions from three to five mice.

Hemopoietic Factors. For murine rG-CSF we used supernatant of COS cells (COS-sup) transfected with the cDNA of murine G-CSF cloned by Tsuchiya et al. (2). COS-sup was kindly provided by Dr. S. Nagata (University of Tokyo). Human native G-CSF was generously provided by Chugai Pharmaceutical Co. (Tokyo, Japan), and had a sp act of $2.5–5.0 \times 10^7$ U/mg protein (15). Murine rGM-CSF was provided by Sumitomo Pharmaceutical Co. (Osaka, Japan). It had a sp act of 3.7×10^8 U/mg protein. We used supernatant of COS cells transfected with the cDNA of IL-3 provided by Dr. T. Yokota (DNAX, Palo Alto, CA) (6). We used rTRF as rIL-5, which synthesized according to the method described previously (13). Briefly, pSP6K-mTRF23 was cleaved with Sal I to linearize the plasmid DNA, and mRNAs were synthesized using SP6 RNA polymerase. The synthesized RNAs were injected into *Xenopus* oocytes, and their conditioned medium was collected after incubation for 36 h at 20°C, and purified using anti-TRF antibody-coupled affinity column (16). We used medium conditioned by mock-transfected *Xenopus* oocytes as the negative control. 1 U of IL-5 was defined as the reciprocal of the dilution yielding a response that is 50% of the maximal response to the stimulation activity of BCL_1 cells.

For the anti–IL-5 antibody, a monoclonal rat IgG_1 anti-IL-5 antibody was obtained from a B cell hybridoma, TB13, constructed by fusion between murine myeloma cells (P3-X63-Ag8.653) and rat spleen cells that had been immunized with HPLC-TRF from B151 supernatant. Ascitic fluid of mice injected with TB13 was applied to a protein A–coupled Sepharose CL-4B beads column, and the eluate from the column with 3M potassium isothiocyanate (pH 8.0) was used as anti-IL-5 antibody (16, 17).

Clonal Cell Culture. Cultures of 4×10^4 bone marrow cells per milliliter from normal mice or 1.2×10^6 spleen cells per milliliter from 5-FU-treated mice were prepared in 35-mm non–tissue culture dishes (Falcon Labware, Oxnard, CA) using methylcellulose medium. 1 ml of 1.2% methylcellulose (Fisher Scientific Co., Pittsburgh, PA) in α medium contained 30% FCS, 10 mg BSA (Sigma Chemical Co., St. Louis, MO), and appropriate amounts of rG-CSF, IL-3, and/or IL-5. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. The numbers of colonies were scored in the methylcellulose culture on day 7 or 14. Individual colonies were lifted with a 3-μl Eppendorf micropipette in medium containing 30% FCS. The samples were spun in a centrifuge (Cytospin; Shandon Southern Instruments Inc., Sewickley, PA) and stained with May-Grünwald-Giemsa.

Replating of Blast Cell Colonies. Spleen cells from 5-FU-treated mice were cultured in methylcellulose medium in 35-mm dishes in the presence of 100 U/ml IL-3 or 20 ng/ml human G-CSF. Blast cell colonies consisting of 50–100 cells were picked up, as described above, on day 7 of culture and were suspended in α medium containing 30% FCS (18). After washing twice, ~200 cells were cultured in methylcellulose medium containing G-CSF and/or IL-5. These cultures of replated cells were then incubated at 37°C in 5% CO_2 in air. After 7 d, each colony formed was lifted, and examined for morphology of the individual colonies.

Electron Microscopy. Several colonies in methylcellulose medium containing G-CSF and IL-5 were fixed in glutaraldehyde and osmium tetroxide, dehydrated, and embedded in epoxy resin for transmission electron microscopy.

Collection of Eosinophil-rich Peritoneal Exudate Cells. To obtain eosinophil-rich peritoneal exudate cells, we modified the methods described by Nawa et al. (19). Briefly, mice were given an intraperitoneal injection of 200 mg/kg cyclophosphamide (Shionogi Co., Osaka, Japan), and 2 d later were infected by oral administration of 500–1,000 *Toxocara canis* larvae. 12 d after infection, 1 ml of a 0.2 mg/ml (protein concentration) Anisakis extract was injected intraperitoneally, and the peritoneal lavage was collected 48 h later. *T. canis* larvae were provided by Dr. S. Kojima (University of Chiba, Chiba, Japan) and Anisakis extract was provided by Dr. Y. Hayashi and Dr. M. Torisu (University of Kyushu, Fukuoka, Japan) (20). We used the peritoneal exudate cells as cell suspensions of the liquid culture.

Liquid Cultures of Murine Peritoneal Exudates Cells. Cultures were performed using 24-well tissue culture plates (model 25820; Corning Glassworks, Corning, New York). Each well contained 2 ml α medium supplemented with 20% FCS, 10^6 of peritoneal exudate cells per milliliter, and IL-5 at various concentrations. Cultures were incubated at 37°C in a fully humidified atmosphere of 5% CO_2 in air. At various times, viable cells were counted using eosin exclusion, and differential counts of cytospin preparations stained with May-Grünwald-Giemsa carried out.

Results

Effect of IL-5, G-CSF, GM-CSF, and IL-3 on Colony Formation by Bone Marrow Cells from Normal Mice. The hemopoietic activities of each CSF and IL-5 were investigated using the methylcellulose culture method. As shown in Fig. 1*A*, an eosinophil colony was of compact type and consisted of medium-sized cells with dark hue. However, it was difficult by inverted microscope observations to identify the lineages of cells in mixed colonies, so individual colonies were lifted from the dishes and stained with May-Grünwald-Giemsa for the analysis of cell composition. Fig. 1*B* shows a portion of cytospun preparation, revealing that these cells were eosinophils. Electron microscopic studies confirmed the characteristic crystalloid structure of granules in mature eosinophils (Fig. 2). As shown in Table I, murine rIL-5 alone was able to support growth of a small number of colonies of untreated bone marrow cells. All the colonies formed were predominantly eosinophils (86–100%). These eosinophil colonies began to develop and increase in size after 5 d of culture. No significant dose-response relationship between the number of eosinophil colonies and the concentration (2–32 U/ml) of IL-5 was observed. Maximal number of eosinophil colonies was 3 ± 1 per 2×10^4 normal bone marrow cells at the concentration of 4–32 U/ml. G-CSF supported growth of only neutrophils and/or macrophage colonies, no eosinophils being detected in cultures thus treated. The effects of murine rG-CSF (dilution of COS-sup, 1:200) was compared with those of human native G-CSF (20 ng/ml), but there were no significant differences in their activities or lineage specificities. Since human G-CSF was highly purified compared with murine rG-CSF, we used human G-CSF in subsequent experiments.

Murine rGM-CSF supported growth of colonies containing neutrophils, macrophages, and/or a small number of eosinophils. IL-3 supported multilineage colonies containing neutrophils, macrophages, eosinophils, mast cells, and megakaryocytes. The incidence of colonies containing eosinophils was 23% of the total.

Addition of IL-5 to cultures containing G-CSF or GM-CSF led to the formation of colonies containing eosinophils, but did not alter the total number of colonies. In contrast, no significant increase of eosinophil colonies was observed in the

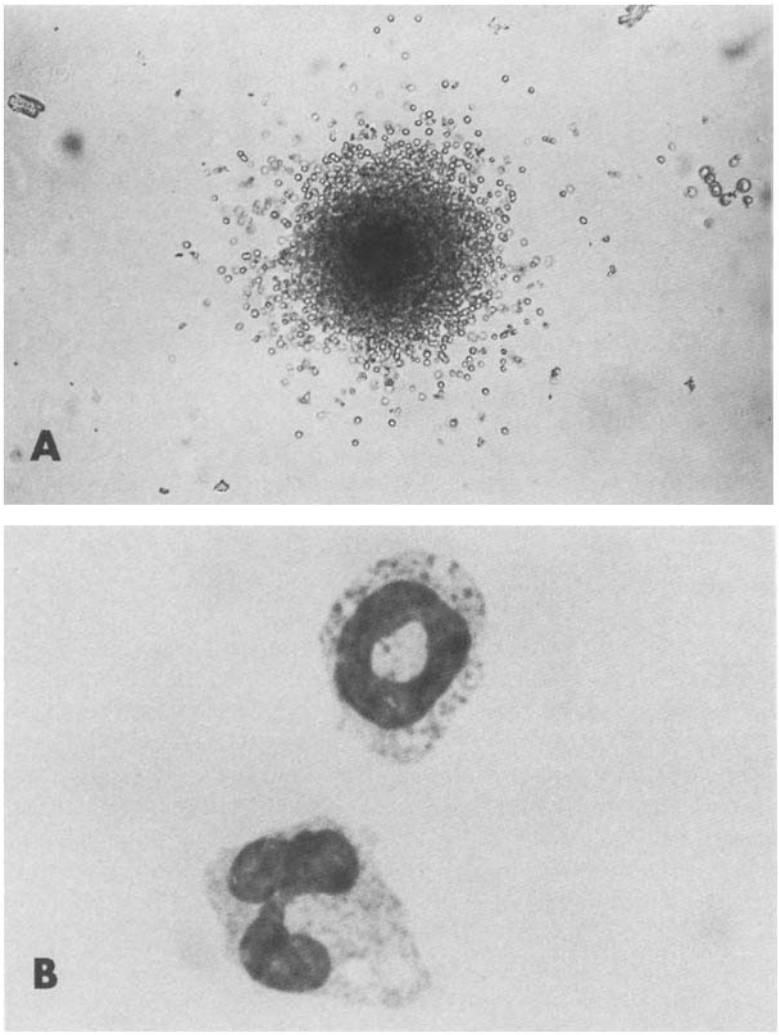

FIGURE 1. (*A*) Appearance of a typical colony containing eosinophils on day 14 of culture in the presence of rG-CSF and IL-5. (*B*) A portion of a May-Grünwald-Giemsa–stained smear of the colony shown *A*, revealing eosinophils.

dishes containing IL-3. The addition of the supernatant of *Xenopus* oocytes as negative control of IL-5 had no effect on colony formation. Since GM-CSF and IL-3 were able to induce by themselves formation of colonies containing eosinophils, we mainly examined the synergistic effects of IL-5 with G-CSF in the following experiments.

Synergistic Effect of IL-5 on Spleen Cells from 5-FU-treated Mice. We have previously reported that treatment of mice with a high dose of 5-FU resulted in enrichment of primitive hemopoietic progenitors (18), and that rG-CSF was able to support growth of multilineage colonies containing neutrophils, macrophages, mast cells, and megakaryocytes, but not eosinophils from spleen cells of 5-FU-treated mice (21). Using this system, we first investigated whether IL-5 promoted

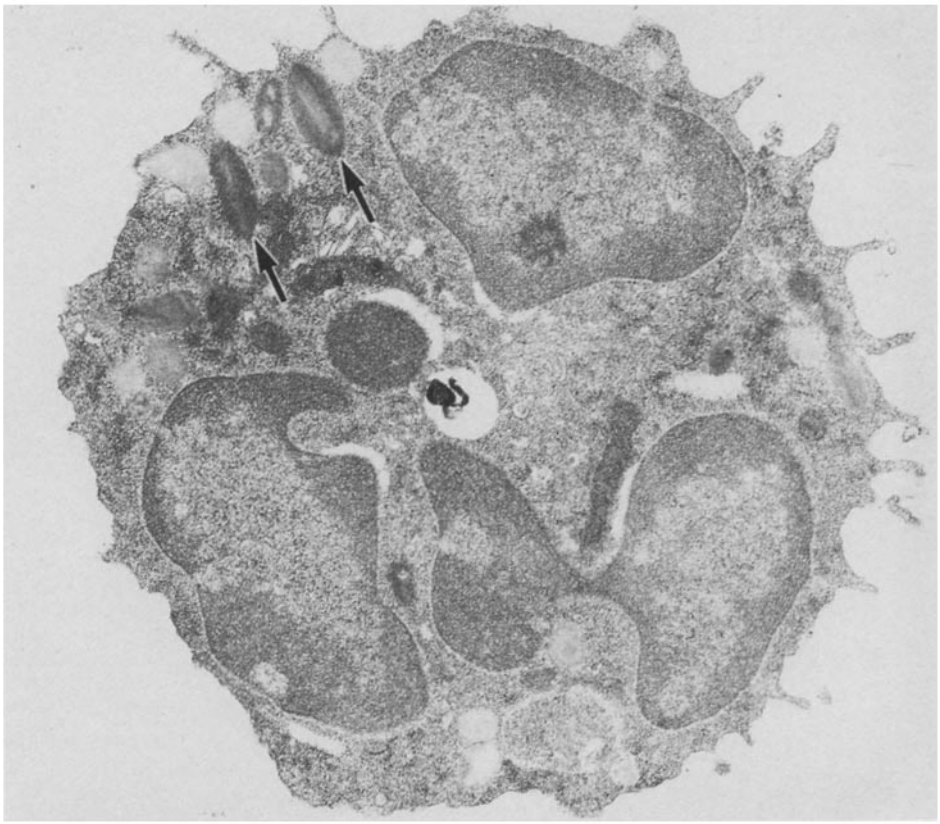

FIGURE 2. Ultrastructure of a mature eosinophil in culture. The crystalloid structure of the granules (indicated by the arrows) is characteristics of mature eosinophils. Magnification, ×20,400.

proliferation and differentiation of hemopoietic progenitor cells. rIL-5 alone did not support any colony formation at concentrations of 4 and 8 U/ml. On the other hand, in the presence of rG-CSF, various types of colonies were formed by these cells. These colonies were larger than those formed by the normal bone marrow cells described above, indicating that they were derived from the more primitive hemopoietic cells that had survived the treatment with 5-FU. Table II shows the IL-5 dose-response relationship for colony formation by spleen cells from 5-FU-treated mice. G-CSF alone failed to form the colonies containing eosinophils, but their appearance increased in a dose-dependent manner with the addition of up to 8 U/ml IL-5. At this concentration, 24 of 47 colonies contained eosinophils.

Table III shows the compositions of the various types of colonies formed in the presence of 20 ng/ml G-CSF and 8 U/ml IL-5. In mixed colonies containing eosinophils (Nos. 1–24), the proportion of eosinophils was different in each colony, ranging from 2 to 96%. ~60% of these colonies consisted of >40% eosinophils, clearly demonstrating that IL-5 had amplified the eosinophil population. Blast cells were defined as those without any signs of differentiation. On

TABLE I

Effect of IL-5 and Various CSFs (G-CSF, GM-CSF, IL-3) on Colony Formation by Bone Marrow Cells from Normal Mice

CSFs	IL-5 (8 U/ml)	G-CSF (20 ng/ml)	G-CSF + IL-5 (8 U/ml)	GM-CSF (100 U/ml)	GM-CSF + IL-5 (8 U/ml)	IL-3 (200 U/ml)	IL-3 + IL-5 (8 U/ml)
Number of colonies not containing eosinophils	0	34*	32	72	60	68	64
Number of colonies containing eosinophils¶	5‡ (86–100%)§	0	4 (5–93%)¶	4 (7–17%)¶	16 (1–100%)¶	20 (4–90%)¶	24 (2–96%)¶
Number of total colonies	5	34	36	76	76	88	88
Percent colonies containing eosinophils	100	0‖	11‖	5¶	21¶	23	27

* A total of 4×10^4 bone marrow cells from normal mice per dish were plated and cultured for 7 d. All colonies were lifted and examined for cell lineages using cytocentrifuge preparations stained with May-Grünwald-Giemsa.

‡ Colonies contained the majority of eosinophils: four colonies contained >99% eosinophils, one colony contained 86% eosinophils, 6% neutrophils, and 8% macrophages.

§ The range of percentage of eosinophils contained in a colony is shown.

‖,¶ All data were analyzed statistically by the χ^2 test, and significance was set at $p < 0.01$.

TABLE II

Dose-Response Relationship for IL-5's Effects on Colony Formation by Spleen Cells from 5-FU-treated Mice

CSFs	IL-5 alone		G-CSF alone (20 ng/ml)	G-CSF (20 ng/ml) + IL-5 (2 U/ml)	G-CSF (20 ng/ml) + IL-5 (4 U/ml)	G-CSF (20 ng/ml) + IL-5 (8 U/ml)	G-CSF (20 ng/ml) + IL-5 (16 U/ml)
	4 U/ml	8 U/ml					
Number of colonies not containing eosinophils	0	0	39*	41	31	23	47
Number of colonies containing eosinophils	0	0	0	3	11	24	13
Number of total colonies	0	0	39	44	44	47	60
Percent colonies containing eosinophils	0	0	0	7	26	51	22

* A total of 1.2×10^6 spleen cells from 5-FU-treated mice per dish were plated and cultured for 14 d. All colonies were lifted and examined for cell lineage using cytocentrifuge preparations stained with May-Grünwald-Giemsa.

day 15 of culture, immature colonies (e.g., Nos. 24 and 35) were still observed. These findings suggest that the development and maturation of individual colonies was different for each. We found no difference between the proportions of blast cells in dishes containing G-CSF alone and those containing G-CSF plus IL-5 (data not shown). Table IV shows the comparative data for colonies formed either in the presence of G-CSF alone or G-CSF plus IL-5. Other than those of the eosinophil lineage, the proportions of cells of all lineages, such as neutrophils, macrophages, and mast cells, were not different in cultures containing G-CSF alone and G-CSF plus IL-5.

As reported in our previous paper (22), GM-CSF was able to support very few colonies formed by spleen cells from 5-FU-treated mice, indicating that it did not act on more primitive stem cells.

IL-3 alone induced the development of multilineage colonies containing neutrophils, macrophages, mast cells, eosinophils, and megakaryocytes. Colonies

TABLE III

Composition of Multilineage Colonies Formed in the Presence of G-CSF (20 ng/ml) and IL-5 (8 U/ml)

Colony number	e*	n	m	Mast	M	Bl	Colony number	e	n	m	Mast	M	Bl
1	96	2	2				25		96	2	2		
2	92	4	4				26		81	1	10		8
3	91	5	3	1			27		74	11	8		7
4	73	13	12			2	28		73		12		15
5	70	23	7				29		68	5	12	2	13
6	68	15	17				30		62	21			17
7	63		37				31		55	19	22		4
8	62	10	6		1	21	32		54	44	2		
9	59	20	21				33		52	7			41
10	55	18	26			1	34		40	46			14
11	50	4	33			13	35		28	4			68
12	49	49	2				36		27	73			
13	48	28	20			4	37		24	2	65		9
14	47	20	33				38		17	83			
15	40	53	6			1	39		12	88			
16	21	45	2	6		26	40		8	91	1		
17	15	25	60				41		8	92			
18	13	51	36				42		8	81			11
19	11	51	36	2			43			100			
20	8	51	21	3		17	44			100			
21	7	47	40			6	45			100			
22	3	54	15	2		26	46			100			
23	2	63	13	22			47			100			
24	2	19	1			78							

All colonies in each dish were lifted and examined for morphology. Differential counts were performed on 200 cells stained with May-Grünwald-Giemsa.

* e, eosinophil; n, neutrophil; m, macrophage; mast, mast cell; M, megakaryocyte; and Bl, blast cell.

TABLE IV

Numbers of Different Types of Colonies Formed by Spleen Cells from 5-FU-treated Mice in the Presence of G-CSF Alone or G-CSF Plus IL-5

G-CSF (20 ng/ml)		G-CSF (20 ng/ml) + IL-5 (8 U/ml)	
m	5 (13%)	m + e, m	6 (13%)
n, m	24 (62%)	n, m + e, n, m	24 (51%)
n, mast	2 (5%)	n, mast	1 (2%)
n, m, mast	8 (20%)	n, m, mast + e, n, m, mast	14 (30%)
		n, m, mast, M + e, n, m, mast, M	2 (4%)
Total	39 (100%)		47 (100%)

e, eosinophil; n, neutrophil; m, macrophage; mast, mast cell; M, megakaryocyte. Differential counts were carried out on 200 cells.

TABLE V

Effect of IL-5, IL-3, and Anti-IL-5 Antibody on Colony Formation by Spleen Cells from 5-FU-treated Mice

CSFs	G-CSF (20 ng/ml) + IL-5 (8 U/ml)	G-CSF (20 ng/ml) + IL-5 (8 U/ml) + anti-IL-5 antibody (1:2000)	IL-3 (200 U/ml)	IL-3 (200 U/ml) + anti-IL-5 antibody (1:2000)
Number of colonies not containing eosinophils	19	23	18	20
Number of colonies containing eosinophils	3 (14%)	0	32 (64%)	30 (60%)
Number of total colonies	22	23	50	50

* A total of 1.2×10^6 spleen cells from 5-FU-treated mice per dish were plated and cultured for 14 days. All colonies were lifted and examined for morphology.

‡ Numbers in parentheses represents percentages of colonies containing eosinophils.

containing eosinophils made up 44% (28 of 64 colonies) of the total number. Although the addition of IL-5 to cultures containing IL-3 increased the formation of colonies containing eosinophils up to 69% (44 of 64 colonies), the proportion of eosinophils in total constituent cells of each colony was no different, being 2–26% in a dish containing IL-3 alone and 3–25% in a dish containing IL-3 plus IL-5.

Effects of Anti-IL-5 Antibodies. Table V shows the effect of anti-IL-5 antibody in the presence of IL-5 plus G-CSF, or IL-3 on colony formation by spleen cells from 5-FU-treated mice. By the addition of anti-IL-5 antibody (1:2,000) to the culture, formation of eosinophil colonies in the presence of G-CSF (20 ng/ml) and IL-5 (8 U/ml) was neutralized, whereas their formation was not affected in cultures containing IL-3 (200 U/ml). The same amount of ascites, used as a negative control for the antibody, did not have on colony formation.

Development of Eosinophil Colonies from Blast Cells. By the observation of spleen cells from 5-FU treated mice, it was demonstrated that IL-5 could support the proliferation and differentiation of eosinophils in the coexistence of G-CSF. However, in such cultures, dishes contained 1.2×10^6 cells/ml. To exclude the possibility of indirect effects through accessory cells such as macrophages and lymphocytes, we cultured the enriched population of hemopoietic progenitor cells at concentrations of 200 cells/ml. When spleen cells from mice pretreated in vivo with 5-FU were cultured in methylcellulose medium containing rIL-3, small colonies consisting of blast cells with little sign of differentiation developed on day 7 of culture. About 10 blast colonies consisting of 20–200 cells could be identified in a dish that received 10^6 spleen cells. Blast cell colonies were lifted on day 7, pooled, washed twice, and replated into secondary methylcellulose cultures in the presence of G-CSF with or without IL-5. In the secondary cultures containing G-CSF, about 200 blast cells formed 34 small colonies, each consisting of 50–500 neutrophils and/or macrophages. In the cultures containing G-CSF

TABLE VI
Effect of IL-5 on Colonies Formed by Secondarily Replated Blast Cells Supported by IL-3

CSFs	IL-5 alone (8 U/ml)	G-CSF alone (20 ng/ml)	G-CSF (20 ng/ml) + IL-5 (8 U/ml)
Small colonies (50–500 cells)	0	34*	29*
Large colonies (>500 cells)	2‡	0	9§
Total	2	34	38

Spleen cells from 5-FU-treated mice were incubated in culture medium containing 100 U/ml IL-3 for 7 d. On day 7 of culture, blast cell colonies consisting of 50–100 cells were pooled, washed, and replated (~200 cells/dish) in secondary cultures containing 20 ng/ml G-CSF.
* Colonies consisted of neutrophils and macrophages only.
‡ These two colonies were pure eosinophil colonies.
§ Colonies contained the majority of eosinophils; six colonies were pure eosinophil colonies, two colonies consisted of >95% eosinophils, and one colony consisted of 50% eosinophils.

and IL-5, the same number of blast cells formed 29 small colonies and 9 large ones, consisting of more than 500 cells each (Table VI). Examination of the morphology of these colonies revealed that all 29 small colonies consisted of neutrophils and macrophages, whereas the 9 large colonies contained eosinophils, 6 of which were pure eosinophil colonies. On the other hand, in the cultures containing IL-5 alone, two colonies consisting of >500 cells were formed, both of which were pure eosinophil colonies.

Similar experiments were done using primary culture of blast cells supported by G-CSF for 7 d instead of IL-3. ~200 cells obtained from blast cell colonies on day 7 were replated. In secondary dishes containing G-CSF alone, four colonies were formed, all of which were small and consisted of neutrophils and macrophages. In dishes containing G-CSF plus IL-5, five colonies were observed, two of which were large and consisted of neutrophils, macrophages, and eosinophils, the proportion of the latter being 81 and 98%. The other colonies were small and consisted of neutrophils and macrophages. In a dish containing IL-5 alone, one colony consisting of >1,000 cells was formed, and found to consist of only eosinophils.

Effects of IL-5 on Mature Eosinophils. We collected eosinophil-rich peritoneal exudate cells as described in Materials and Methods. The cells thus obtained consisted of 52% eosinophils, 42% macrophages, 4% lymphocytes, 1% neutrophils, and 1% mast cells.

Liquid culture was performed in α medium containing 20% FCS with or without IL-5. Fig. 3 shows that IL-5 was able to maintain mature eosinophils up to 15 d, whereas in its absence the number of eosinophils began to decrease on day 7 and few viable cells could be detected on day 13. Effects of IL-5 on the survival of eosinophils were detectable at concentrations ranging from 2 to 16 U/ml. On day 13, 84% of eosinophils were still viable in the presence of 8 U/ml IL-5, while only 2% survived in its absence.

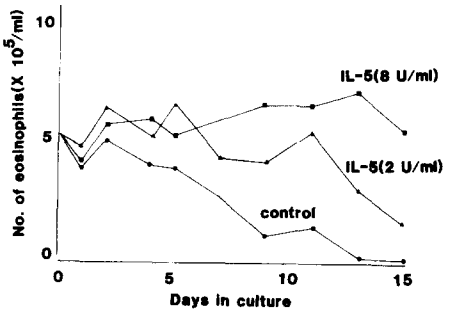

FIGURE 3. In vitro survival of mature eosinophils from eosinophil-rich peritoneal exudate cells in control and IL-5-containing cultures. At various times, viable cell numbers were counted using eosin exclusion, and differential counts carried out by examination of cytocentrifuge preparations stained with May-Grünwald-Giemsa. Each value is the mean of data of an experiment performed in duplicate cultures.

Discussion

The mechanisms of commitment to eosinophilic differentiation have not been well defined. Recently, Sanderson et al. (11) reported that supernatants of alloreactive T cell clones, which contained BCGF II activity, were able to support the growth of eosinophils in liquid culture. However, a liquid culture system could not define the clonal differentiation from pluripotent stem cells into mature cells. Therefore, a colony assay system was introduced into this study. Based on analysis of the differentiation potentials of single progenitors, and those of paired daughter cells (23, 24), it was proposed that the feature of differentiation and proliferation of hemopoietic progenitor cells is a stochastic process, indicating that each colony was very heterogenous in lineage expression. Thus, in situ identification or classification of colony type was insufficient to analyze hemopoietic differentiation. In this study, we lifted up all the colonies grown in dishes and examined the cell lineages by staining of cytospun preparations.

In the present study, we found that purified IL-5 alone could act on untreated bone marrow cells to support exclusively eosinophil colony formation. However, it did not support such colony formation by spleen cells from 5-FU-treated mice, in which only primitive stem cells had survived. Taking our previous findings into consideration (18, 21), we concluded that IL-3 and G-CSF were able to support the colony formation by spleen cells from 5-FU-treated mice, whereas GM-CSF, erythropoietin, and IL-5 were unable to act on such primitive hemopoietic cells. It was suggested that IL-5 itself did not influence the differentiation of lineages other than the eosinophil, as shown by the analysis of the cell composition of colonies formed in the presence of G-CSF with or without IL-5. The eosinophilopoietic activity of IL-5 was neutralized by anti-IL-5 antibody, but that of IL-3 was not. This suggests that at least two different pathways of eosinophil differentiation supported by IL-5 and by IL-3 may exist.

To exclude the possibility of interactions between cells in the same dish, we plated small numbers of enriched hemopoietic progenitors obtained from primary blast cell colonies that had been supported by IL-3 or G-CSF. A few eosinophil colonies were formed in the presence of IL-5 alone. Only small-sized neutrophil/macrophage colonies were formed in the presence of G-CSF alone. Addition of IL-5 to G-CSF-containing cultures induced formation of eosinophil colonies. These findings indicate that IL-5 specifically promoted terminal differentiation and amplification of the eosinophils. In this respect, IL-5 seems to resemble erythropoietin, which facilitates the terminal differentiation and am-

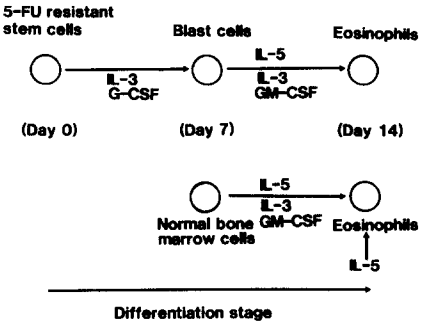

FIGURE 4. Interactions of the various CSFs with eosinophilopoiesis. 5-FU–resistant stem cells imply spleen cells obtained from mice administered 5-FU at a dosage of 150 mg/kg body weight intravenously. Blast cells imply blast cell colonies picked up from the methylcellulose medium cultured in the presence of IL-3 or G-CSF for 7 d. The interactions of the different CSFs on different stages of eosinophilic precursors are indicated. As shown in the figure, IL-5 acts on the late differentiation stage of eosinophilopoiesis, as discussed in the text. Furthermore, IL-5 maintains the survival of mature eosinophils in a liquid culture system.

plification of erythroid cells (25). Furthermore, when the proportion of eosinophils in colonies formed by spleen cells from 5-FU-treated mice was compared with their proportion in colonies formed by blast cells, the former were found to show a wide range (2–96%), while the latter showed a high eosinophil percentage (50–100%) in all colonies. These observations indicated that differentiation along the eosinophil pathway was restricted from pluripotent stem cells to monopotent progenitors. These data clearly showed that IL-5 amplified eosinophil numbers, whereas their differentiation potential was supported by G-CSF. We have to clarify whether or not G-CSF induces the expression of IL-5 receptors, and the in vivo significance of these synergistic interactions of hemopoietic cells. We summarized these data in Fig. 4. Blast cells induced by IL-3 or G-CSF from spleen cells of 5-FU-treated mice appear to be in the same differentiation stage as the majority of untreated bone marrow cells.

In our preliminary studies, murine IL-5 alone was able to support the eosinophil colony formation by human bone marrow cells as well as murine cells. It has been reported that the nucleotide and amino acid sequence homologies of the coding region of human and murine IL-5 are 77 and 70% (26), respectively. This human eosinophilopoietic activity of IL-5 seems to be similar to the so-called Eo-CSF reported by Metcalf et al. (27). IL-5 alone maintained mature eosinophils in liquid culture system. This effect was also lineage specific. This result is consistent with that reported by Begley et al. (14) in the human eosinophil culture system. These data suggested that mature eosinophils might have IL-5 receptors. To exclude the possibility of some influence by macrophages in the peritoneal exudates, we are now preparing highly enriched eosinophil fractions by Percoll gradient centrifugation.

In conclusion, IL-5 specifically facilitated the terminal differentiation and amplification of eosinophils. This mechanism of eosinophilopoiesis may be responsible for the urgent mobilization of eosinophils during helminthic infections and allergic responses.

Summary

Using a clonal culture system, we investigated the hemopoietic effects of purified recombinant IL-5 obtained from conditioned media of transfected *Xenopus* oocytes. IL-5 alone acted on untreated bone marrow cells and supported the formation of a small number of colonies, all of which were predominantly eosinophilic. However, it did not support colony formation by spleen cells from

5-FU-treated mice, in which only primitive stem cells had survived, while IL-3 and G-CSF did. Eosinophil-containing colonies were formed from these cells in the presence of IL-5 and G-CSF together. In contrast, G-CSF alone did not support any eosinophil colonies. The eosinophilopoietic effect of IL-5 was dose-dependent, and was neutralized specifically by anti-IL-5 antibody.

To exclude the possibility of interactions with accessory cells in the same culture dish, we replated a small number (200 cells/dish) of enriched hemopoietic progenitors, obtained from blast cell colonies, which were formed by cultivation of spleen cells from 5-FU-treated mice in the presence of IL-3 or G-CSF. From these replated blast cells, eosinophil colonies were induced in dishes containing IL-5 but not in those containing G-CSF alone.

From these findings, it was concluded that IL-5 did not act on primitive hemopoietic cells, but on blast cells induced by IL-3 or G-CSF. IL-5 specifically facilitated the terminal differentiation and proliferation of eosinophils. In this respect, the role of IL-5 in eosinophilopoiesis seems to be analogous to erythropoietin, which promotes the terminal differentiation and amplification of erythroid cells.

Moreover, IL-5 maintained the viability of mature eosinophils obtained from peritoneal exudate cells of the mice infected with parasites, indicating mature functional eosinophils carried IL-5 receptors.

The synergistic effects of IL-5 and colony-stimulating factors on the expansion of eosinophils is supposed to contribute to the urgent mobilization of eosinophils at the time of helminthic infections and allergic responses.

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