Actions of Human Interleukin-4/B-Cell Stimulatory Factor-1 on Proliferation and Differentiation of Enriched Hematopoietic Progenitor Cells in Culture

By Yoshiaki Sonoda, Tsukasa Okuda, Shouhei Yokota, Taira Maekawa, Yukari Shizumi, Hikari Nishigaki, Shinichi Misawa, Hiroshi Fujii, and Tatsuo Abe

We studied the effects of recombinant human interleukin-4 (IL-4) on colony formation by enriched hematopoietic progenitors. IL-4 alone did not support colony formation at all. When IL-4 was combined with granulocyte colony-stimulating factor (G-CSF), the number of pure neutrophil colonies increased three times over that supported by G-CSF alone. IL-4 added 5 days after the addition of G-CSF failed to exert this synergistic effect, indicating that IL-4 acts on the early stage of proliferation. The mapping experiments (sequential observation of colony formation) have clearly shown that IL-4 did not initiate progenitor cell proliferation. Based on these data, IL-4 may possess a direct action on progenitor cells; however, it can only act as a costimulant with G-CSF. In contrast, IL-4 had possible inhibitory effects on macrophage colony formation supported by interleukin-3 (IL-3) and macrophage colony-stimulating factor (M-CSF). In other words, IL-4 may induce progenitor cells to become sensitive to G-CSF and thereby induce neutrophil differentiation. Delayed addition experiments demonstrated that human IL-4, unlike murine IL-4, could support neither proliferation nor survival of erythroid burst or mixed colony forming cells. Neutrophil colony forming cells only survived and recovered after addition of G-CSF and erythropoietin on day 5 of incubation. On the other hand, IL-3 supported neutrophil, erythroid burst, and mixed colony forming cells as reported previously (Sonoda et al, Proc Natl Acad Sci USA, 85:4360, 1988). These results led us to propose that IL-4 possibly acts with more mature progenitor cells than those of IL-3 or granulocyte-macrophage (GM)-CSF.

B-CELL STIMULATORY factor-1 (BSF-1), also termed interleukin-4 (IL-4), was defined as a costimulating factor for the anti-IgM antibody-induced proliferation of resting B cells.[1,2] Recently, this cytokine has been shown to have multiple functional activities when tested with B cells, T cells, and macrophages.[3-8] Independently, IL-4 was identified as a mast cell growth factor[9] and, more recently, as a specific factor for the proliferation of connective tissue type mast cells.[10]

In addition, Peschel et al[11] have reported that purified murine IL-4 derived from activated EL-4 cells has significant effects on hematopoietic progenitor cells. It enhances granulocyte/macrophage colony formation (CFU-GM) in the presence of granulocyte colony-stimulating factor (G-CSF), and erythroid-burst formation (BFU-E) and mixed colony formation (CFU-Mix) in the presence of erythropoietin (Ep). Rennick et al[12] have also reported that purified recombinant murine IL-4 enhances erythroid, granulocyte, macrophage, and mast cell colony formation in the presence of Ep, G-CSF, macrophage colony-stimulating factor (M-CSF), and interleukin-3 (IL-3). Most recently, Broxmeyer et al[13] found synergistic effects between purified recombinant human or murine IL-4 with G-CSF and Ep on the proliferation of myeloid and erythroid progenitor cells, respectively.

In this study, we have investigated the biologic activities of recombinant human IL-4 on enriched hematopoietic progenitor cells using human bone marrow null cells (1,000 per dish) as target cells in order to minimize in situ production of CSFs. IL-4 significantly enhanced pure neutrophil colony formation in the presence of G-CSF, as reported previously.[11-13] Unlike earlier reports, it did not show any ability to support the growth of erythroid bursts or mixed-colony forming cells. Our results indicate that IL-4 induces progenitor cells to become more sensitive to G-CSF and, therefore, predominantly induces neutrophil differentiation. We propose that IL-4 supports early stages of proliferation of hematopoietic progenitors, but that its primary targets are more mature than those of either IL-3 or GM-CSF.

MATERIALS AND METHODS

Recombinant factors. Purified bacterially derived recombinant human interleukin-3 (IL-3) was a generous gift of the Bacterial Expression Laboratories of the Genetics Institute (Cambridge, MA) and had a specific activity of 2×10^7 U/mg.[14] GM-CSF was purified from media conditioned by Chinese hamster ovary (CHO) cells engineered to express human GM-CSF, as described previously.[15] Purified CHO cell-derived Ep had a specific activity of 190,000 U/mg. CHO cell-derived M-CSF was 50% pure, and the source of G-CSF was a crude conditioned medium (CM) of CHO cells expressing human G-CSF. IL-4 was a crude CM produced by COS-cells that had been transfected with cDNA encoding human IL-4 and gave half-maximal activity in the chronic myelogenous leukemia (CML) blast proliferation assay at a final concentration of 1:13,000 dilution. These recombinant factors were kindly provided by Dr Steven C. Clark of the Genetics Institute. For part of the experiments, we used purified recombinant human IL-4 and G-CSF. Purified CHO cell-derived IL-4 was kindly supplied by Dr Kazuaki Hama of Ono Pharmaceutical, Osaka, Japan, and had a specific activity of 5×10^6 U/mg by human T cell line (Sez 627) proliferation assay.[16] Purified CHO cell-derived G-CSF was a generous gift from Dr Masayoshi Ono of Chugai Pharmaceutical, Tokyo, Japan, and had a specific activity of 1.14×10^8 U/mg.

Cell preparation. After informed consent was obtained, bone marrow cells were aspirated from the posterior iliac crest of healthy adult volunteers and placed in a 16 mL Falcon tissue culture tube

From the Department of Hygiene and the Third Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto; and the Kyoto First Red Cross Hospital, Kyoto, Japan.

Submitted September 22, 1989; accepted December 19, 1989.

Address reprint requests to Yoshiaki Sonoda, MD, Department of Hygiene, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyoku, Kyoto 602, Japan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

0006-4971/90/7508-0004$3.00/0

containing 400 U of preservative-free heparin (Shimizu Pharmaceutical, Osaka, Japan). Mononuclear cells were separated using Ficoll-Paque (Pharmacia, Piscataway, NJ) density gradient centrifugation, and nonadherent cells were recovered by overnight adherence to plastic dishes. The mononuclear nonadherent (MNNA) cell fractions were further enriched for null cell fractions using nylon fiber column (Wako Pure Chemical, Osaka, Japan) and rosette formation with neuraminidase-treated sheep erythrocytes as reported elsewhere.[17] For part of the experiments, peripheral blood null cell fractions were separated as bone marrow cells.

Clonal cell culture. Cultures were carried out in 35 mm Lux suspension culture dishes (#5221R, Miles Scientific, Naperville, IL) by using a modification of the technique originally reported by Iscove et al.[18] One milliliter of culture contained 1,000 bone marrow null cells or 1×10^4 peripheral blood null cells, 1.2% of 1,500 centipoise methylcellulose (Shinetsu Chemical, Tokyo, Japan), 30% fetal calf serum (FCS), 1% crystallized and deionized bovine serum albumin (BSA; Sigma Chemical Co, St Louis, MO), 5×10^{-5} mol/L mercaptoethanol (Sigma), and one or more recombinant human CSFs. The MNNA cell fractions were used for the titration experiment of recombinant human IL-4. Final concentrations of each CSF were as follows: IL-3, 100 U/mL; GM-CSF, 100 U/mL; G-CSF, 1:1,000 dilution of CHO cell CM; M-CSF, 1:1,000 dilution of CHO cell CM; and Ep, 2 U/mL. These concentrations supported maximal total colony formation, as we reported previously.[19] In part of the experiments, we used purified CHO cell-derived G-CSF. Ten nanograms of G-CSF supported maximal neutrophil colony formation in the titration experiment (data not shown). Dishes were incubated at 37°C in a fully humidified atmosphere flushed with a combination of 5% CO_2, 5% O_2, and 90% N_2. On day 12 to day 14 of incubation, all colonies were scored in situ on an inverted microscope. Colony types identified in situ were granulocyte (G), macrophage (M), granulocyte/macrophage (GM), eosinophil (EO), and erythrocyte-containing mixed colony (E-Mix). For morphologic analysis, granulocyte colonies were picked with a 3 μL Eppendorf micropipette and stained with May-Grunwald Giemsa.

Statistical analysis. The significance of the differences of means was determined using the two-tailed Student's *t* test.

RESULTS

Dose response of colony formation to IL-4. First we examined the optimal concentration of IL-4 by plating 1×10^4 bone marrow MNNA cells per dish in the presence of varying dilutions of CM containing IL-4. IL-4 alone did not support significant colony formation (data not shown). Then we tested the synergistic effects of IL-4 with G-CSF, the results are shown in Table 1. IL-4 induced neutrophil colony formation in the presence of G-CSF. On the other hand, concentrations up to 1:100 dilution of supernatant did not show any effect on colony formation supported by Ep alone (data not shown). Based on these data, we chose a 1:1,000 dilution of supernatant as the standard concentration of IL-4 in the following experiments.

Effects of IL-4 on colony formation supported by terminally acting CSFs and/or stage-specific CSFs. Next we examined the effects of IL-4 on colony formation supported by terminally acting CSFs such as G-CSF, M-CSF, and Ep[20] and combinations of these CSFs with IL-3. The results of representative experiments are shown in Table 2. IL-4 alone did not support significant colony formation. When G-CSF and IL-4 were combined, the number of neutrophil colonies was three times more than that with G-CSF alone or with IL-3 plus G-CSF or with a combination of five CSFs. IL-4 had no effect on macrophage colony formation in the presence of M-CSF. Also, the combination of IL-4 and Ep yielded almost the same number of erythroid bursts as that supported by Ep alone. Additionally, IL-4 and Ep did not support erythrocyte-containing mixed colonies. On the other hand, IL-3 and Ep supported almost the same number of erythroid bursts and mixed colonies as did a combination of five CSFs. Then we used peripheral blood null cells as a target population to determine if IL-4 has any effect on erythroid burst formation. The results are presented in Table 3. In this experiment, concentrations up to 1:100 dilution of supernatant containing IL-4 did not support any erythroid burst formation over that supported by Ep alone. However, in the same experiment, IL-3 exhibited potent burst-promoting activity (BPA). These results clearly demonstrated that IL-4 does not have significant BPA.

Next, we tested the effects of IL-4 on colony formation supported by stage-specific CSFs such as IL-3 or GM-CSF alone.[20] We found no effects on colony formation supported by these factors (data not shown).

Effects of IL-4 on colony formation supported by various combinations of CSFs. In order to delineate the actions of IL-4 on colony formation, we studied the effects of various combinations of CSFs with or without IL-4. As shown in Table 4, a combination of G-CSF and IL-4 again showed remarkable synergistic action on neutrophil colony forma-

Table 1. Colony Formation by Bone Marrow Cells Supported by Various Dilutions of Recombinant Human IL-4 in the Presence of G-CSF

IL-4 (Dilution)	Colony Types						
	G	M	GM	B	EO	E-Mix	Total
1:100	43 ± 2	1 ± 1	2 ± 1	0	0	0	45 ± 2
1:1,000	44 ± 4	1 ± 1	2 ± 1	0	0	0	47 ± 5
1:10,000	18 ± 1	1 ± 1	2 ± 1	0	0	0	20 ± 2
1:100,000	13 ± 3	1 ± 1	1 ± 1	0	0	0	16 ± 2
Mock CM*	13 ± 1	1 ± 1	1 ± 1	0	0	0	15 ± 1
CSFs†	17 ± 3	3 ± 2	9 ± 1	6 ± 2	3 ± 0	2 ± 2	40 ± 3

Data represent the mean ± SD of tripicate cultures containing 1×10^4 bone marrow MNNA cells per dish. All experimental groups contained G-CSF(1:1,000 dilution).

Abbreviations: B, erythroid burst; E-Mix, erythrocyte-containing mixed colony.

*Mock CM, conditioned medium from COS cells not transfected by a plasmid.

†CSFs contained IL-3 (100 U/mL), GM-CSF (100 U/mL), G-CSF (1:1,000 dilution), M-CSF (1:1,000 dilution), and Ep (2 U/mL).

Table 2. Effects of IL-4 on Colony Formation by Bone Marrow Null Cells Supported by Terminally Acting Recombinant Human CSFs: Comparison With Effects of IL-3

Factors	Colony Types						
	G	M	GM	B	EO	E-Mix	Total
IL-4	0	2 ± 1	0	0	0	0	2 ± 1
G-CSF	14 ± 1	4 ± 1	2 ± 1	0	0	0	20 ± 1
M-CSF	0	4 ± 2	0	0	0	0	4 ± 2
Ep	0	2 ± 1	0	5 ± 1	0	0	7 ± 2
Mock CM	0	2 ± 1	0	0	0	0	2 ± 1
IL-4 + G-CSF	49 ± 7	4 ± 1	5 ± 2	0	0	0	57 ± 6
IL-4 + M-CSF	0	3 ± 2	0	0	0	0	3 ± 2
IL-4 + Ep	0	2 ± 1	0	6 ± 3	0	0	8 ± 3
IL-3 + G-CSF	15 ± 2	9 ± 2	11 ± 1	0	8 ± 3	0	43 ± 6
IL-3 + M-CSF	2 ± 1	12 ± 1	6 ± 2	0	6 ± 2	0	25 ± 1
IL-3 + Ep	2 ± 1	8 ± 1	5 ± 1	22 ± 2	6 ± 2	3 ± 3	47 ± 4
CSFs*	15 ± 5	15 ± 4	14 ± 4	19 ± 2	8 ± 2	4 ± 4	74 ± 11

Data represent the mean ± SD of quadruplicate cultures containing 1,000 bone marrow null cells per dish. Abbreviations for colony types are defined in legend to Table 1.

*CSFs contained IL-3 (100 U/mL), GM-CSF (100 U/mL), G-CSF (1:1,000 dilution), M-CSF (1:1,000 dilution), and Ep (2 U/mL).

tion. To examine the nature of these neutrophil colonies, we performed morphologic analysis. Distributions of percentages of mature neutrophils in each colony supported either by G-CSF alone or G-CSF plus IL-4 were comparable, and none of the colonies contained blast cells (data not shown). IL-4 did not have BPA or mixed colony supporting ability, as shown in Tables 2 and 3. IL-4 did not affect erythroid burst formation supported by Ep and IL-3 or GM-CSF. In a separate experiment, we further tested the synergistic effects of IL-4 with GM-CSF. IL-4 did not synergize with GM-CSF in the absence of Ep. In addition, the numbers of erythroid bursts or erythrocyte-containing mixed colonies supported by IL-4, GM-CSF, and Ep were almost the same as those supported by GM-CSF and Ep (data not shown). On the other hand, IL-4 expressed significant inhibitory activity against pure macrophage colony formation supported either by a combination of IL-3 and M-CSF or a combination of five CSFs.

Effects of delayed addition of IL-4 or G-CSF on colony formation. In order to further clarify the mechanism of the synergistic action of IL-4 with G-CSF, we performed delayed addition experiments using two different approaches. Simultaneously, we mapped the growing colonies. First, 1,000 bone marrow null cells were plated in the presence of either IL-4 or G-CSF. On day 5 of incubation, G-CSF or IL-4 was carefully added to each culture dish. Cultures were continued for another 9 days and assayed. The results are shown in Table 5. Colony formation supported by day 0 G-CSF followed by day 5 IL-4 did not show synergistic action. However, when G-CSF was added on day 5 to cultures containing IL-4 from day 0, the number of neutrophil colonies increased three times higher than that supported by G-CSF alone, as described previously. To characterize this action in more detail, we carried out a "mapping experiment," which is sequential observation of colony formation in culture. In this experiment, we first identified small clusters of cells and recorded the area of the clusters in the dish. Subsequent growth of the clusters was recorded. The results are presented in Fig 1. In this experiment, dishes were examined on days 5, 7, 10, and 13 with an inverted microscope. From day 5 to day 10, over 40 small clusters containing fewer than 15 immature cells were identified; however, most of these disappeared by the next observation time. On day 13, only three small macrophage colonies were

Table 3. Effects of a Combination of Recombinant Human IL-4 and Ep on Colony Formation by Peripheral Blood Null Cells: Comparison With IL-3 and Ep

Factors	Colony Types						
	G	M	GM	B	EO	E-Mix	Total
IL-4 (1:1,000)	0	0	0	0	0	0	0
IL-3	0	1 ± 1	5 ± 1	0	6 ± 1	0	12 ± 2
Ep	0	0	0	15 ± 8	0	0	16 ± 8
Mock CM	0	0	0	0	0	0	0
Ep + IL-4 (1:100)	0	0	0	22 ± 2	0	0	22 ± 2
Ep + IL-4 (1:1,000)	0	0	0	21 ± 1	0	0	21 ± 1
Ep + IL-3	0	1 ± 1	1 ± 1	92 ± 9	5 ± 2	10 ± 3	110 ± 7
CSFs	7 ± 2	2 ± 2	8 ± 3	94 ± 10	6 ± 2	10 ± 4	127 ± 12

Data represent the mean ± SD of triplicate cultures containing 5×10^3 peripheral blood null cells per dish. CSFs and abbreviations as defined in legend to Table 1.

Table 4. Effects of IL-4 on Colony Formation by Bone Marrow Null Cells Supported by Recombinant Human CSFs Singly and/or in Combination

Factors	Colony Types						
	G	M	GM	B	EO	E-Mix	Total
IL-4	0	1 ± 1	0	0	0	0	1 ± 1
IL-3	1 ± 1	3 ± 2	3 ± 0	0	2 ± 1	0	8 ± 1
GM-CSF	1 ± 1	4 ± 2	2 ± 1	0	5 ± 2	0	13 ± 2
G-CSF	18 ± 1	4 ± 2	2 ± 2	0	0	0	24 ± 0
M-CSF	0	5 ± 2	0	0	0	0	5 ± 2
Ep	0	2 ± 1	0	2 ± 0	0	0	3 ± 1
Mock CM	0	1 ± 1	0	0	0	0	1 ± 1
IL-4 + G-CSF	42 ± 4	4 ± 1	4 ± 2	0	0	0	49 ± 4
IL-3 + G-CSF	17 ± 1	11 ± 2	6 ± 1	0	2 ± 2	0	36 ± 2
GM-CSF + G-CSF	15 ± 4	13 ± 5	7 ± 4	0	4 ± 3	0	39 ± 9
IL-3 + G-CSF + IL-4	25 ± 1	5 ± 2	6 ± 1	0	2 ± 1	0	38 ± 2
GM-CSF + G-CSF + IL-4	21 ± 1	8 ± 1	8 ± 3	0	2 ± 2	0	38 ± 3
IL-4 + M-CSF	0	1 ± 1	0	0	0	0	1 ± 1
IL-3 + M-CSF	0	12 ± 1*	1 ± 1	0	1 ± 2	0	15 ± 1
GM-CSF + M-CSF	1 ± 1	11 ± 2	2 ± 2	0	5 ± 3	0	19 ± 4
IL-3 + M-CSF + IL-4	0	5 ± 3*	2 ± 0	0	3 ± 1	0	9 ± 3
GM-CSF + M-CSF + IL4	0	7 ± 3	2 ± 1	0	5 ± 3	0	14 ± 3
IL-4 + Ep	0	1 ± 1	0	2 ± 1	0	0	2 ± 2
IL-3 + Ep	0	3 ± 1	1 ± 1	7 ± 3	2 ± 2	0	13 ± 3
GM-CSF + EP	1 ± 1	4 ± 1	1 ± 1	3 ± 2	5 ± 1	0	14 ± 3
IL-3 + Ep + IL-4	0	2 ± 2	2 ± 1	7 ± 1	1 ± 1	1 ± 1	13 ± 4
GM-CSF + EP + IL-4	1 ± 1	4 ± 2	1 ± 1	4 ± 0	3 ± 2	0	13 ± 4
CSFs	13 ± 1	12 ± 2†	11 ± 4	5 ± 0	5 ± 2	1 ± 1	47 ± 7
CSFs + IL-4	18 ± 2	6 ± 2†	8 ± 3	7 ± 3	2 ± 1	1 ± 1	42 ± 5

Data represent the mean ± SD of triplicate cultures containing 1,000 bone marrow null cells per dish. Numbers of M colonies significantly decreased after addition of IL-4 (*$P < .02$, †$P < .05$). Abbreviations defined in legend to Table 1.

seen; no other active cell proliferation was observed. If G-CSF was added to these cultures on day 5, however, significant proliferation of neutrophil colonies began (data not shown).

Next, we compared IL-3 and IL-4 for the ability to maintain erythroid progenitors (BFU-E) and multipotential progenitors (CFU-Mix) in culture. Peripheral blood null cells (1×10^4) were cultured for 5 days in the presence of either IL-4 or IL-3 alone. On day 5 of incubation, G-CSF and Ep were carefully overlayed, and colonies were enumerated on day 14. As shown in Table 6, approximately 80% of erythroid burst and all of mixed colonies survived in the presence of IL-3. By contrast, IL-4 could not support the survival of erythroid burst nor mixed colony forming cells. Only neutrophil colony forming cells survived and recovered after the delayed addition of G-CSF and Ep.

Colony formation supported by purified IL-4 in the presence of G-CSF or Ep. Although we used a plateau-range dilution of the IL-4–containing COS-cell supernatant, inhibitors for erythroid burst or mixed colony formation may have been present in the CM and may have affected the synergistic action. We therefore tested the effects of various concentrations (1 to 2,000 U/mL) of highly purified CHO cell-derived IL-4 on colony formation in the presence of G-CSF or Ep (Table 7). In this experiment, we also used purified CHO cell-derived G-CSF instead of a crude CM containing G-CSF. One unit per milliliter (0.2 ng/mL) of IL-4 again showed a distinct synergistic action with G-CSF

Table 5. Effects of Delayed Addition of IL-4 or G-CSF on Colony Formation by Bone Marrow Null Cells Supported by G-CSF or IL-4

Factors		Colony Types						
Day 0	Day 5	G	M	GM	B	EO	E-Mix	Total
G-CSF	—	4 ± 2	2 ± 1	0	0	0	0	5 ± 2
G-CSF	IL-4	6 ± 1	1 ± 1	1 ± 1	0	0	0	9 ± 2
IL-4	—	0	0	0	0	0	0	0
IL-4	G-CSF	19 ± 3	3 ± 1	0	0	0	0	22 ± 4
IL-4 + G-CSF	—	16 ± 1	1 ± 1	1 ± 1	0	0	0	18 ± 2
CSFs	—	5 ± 2	7 ± 3	8 ± 3	6 ± 2	2 ± 0	1 ± 1	27 ± 5
Mock CM	—	0	0	0	0	0	0	0

Data represent the mean ± SD of quadruplicate cultures containing 1,000 bone marrow null cells per dish. Abbreviations defined in legend to Table 1.

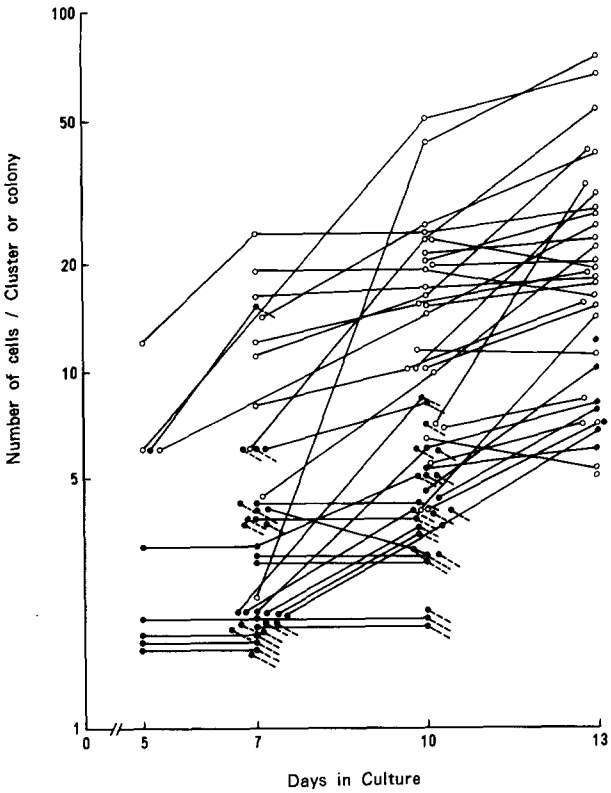

Fig 1. Changes in the number of cells in clusters or colonies. One dish was examined containing 1,000 bone marrow null cells in the presence of recombinant human IL-4 alone. ○, macrophage proliferation; ●, immature cell proliferation; ----, disappearances of the clusters.

on neutrophil colony formation. Over 10 U/mL (2 ng/mL) of IL-4 revealed the maximal synergistic effect with G-CSF. By contrast, concentrations up to 2,000 U (400 ng/mL) of IL-4 did not show significant BPA or mixed colony supporting ability in the presence of Ep.

DISCUSSION

Several investigators have documented the unique actions of IL-4 on hematopoietic progenitor cells. Peschel et al[11] reported the costimulating effects of purified murine IL-4 with G-CSF or Ep, generating enhanced proliferations of CFU-GM, CFU-E, BFU-E, and CFU-Mix. It is also effective as a megakaryocyte-stimulating factor in combination with IL-1, Ep, or supernatants of a T-cell hybridoma. Rennick et al[12] also reported the same enhancing effects of recombinant murine IL-4 on erythroid, granulocyte, macrophage, and mast cell colony formation, when used as costimulant with factors such as Ep, G-CSF, M-CSF, and IL-3. When highly purified Thy-1^{lo+} cells were used as target cells, the effect of IL-4 on colony formation supported by IL-3 has been somewhat controversial. In this culture system, IL-4 suppressed colony formation not only by CFU-GM, but also by CFU-Mix. Most recently, Broxmeyer et al[13] has reported that recombinant human IL-4 also enhanced neutrophil and erythroid colony formation supported by either G-CSF or Ep in relatively high density cultures, as has been reported in murine culture systems. They proposed that IL-4 augmented the release of G-CSF, CSF-1, or GM-CSF from macrophages and/or T cells at low concentration, and tumor necrosis factor-α from macrophages at higher concentration, suggesting an indirect mechanism of action for IL-4.

We investigated the effects of recombinant human IL-4 on colony formation by enriched human hematopoietic progenitor cells at low cell density. IL-4 alone did not support colony formation. However, IL-4 did markedly enhance pure neutrophil colony formation in the presence of G-CSF. As shown by the delayed addition experiment, this synergistic effect required the presence of IL-4 from the initial day of culture. IL-4 added 5 days after the addition of G-CSF failed to express this synergistic activity, indicating that IL-4 acts on an early stage of proliferation. The mapping study has clearly shown that IL-4 did not initiate significant proliferation of progenitor cells. Based on these data, IL-4 may possess direct action on progenitor cells, but only express a proliferative effect as a costimulant with G-CSF. On the other hand, IL-4 displayed inhibitory effects on macrophage colony formation supported by IL-3 and M-CSF, consistent with the earlier report by Rennick et al.[12] Recently, Peschel et al[21] reported that IL-4 induces the expression of an inhibitor of proliferation by bone marrow stromal cells, suggesting that its inhibitory effects might be indirect. IL-4

Table 6. Effects of Delayed Addition of G-CSF and Ep on Colony Formation by Peripheral Blood Null Cells Supported by IL-4 or IL-3

Factors		Colony Types						
Day 0	Day 5	G	M	GM	B	EO	E-Mix	Total
IL-4	—	0	0	0	0	0	0	0
IL-3	—	0	0	5 ± 1	0	3 ± 1	0	8 ± 1
G-CSF + Ep	—	5 ± 1	2 ± 1	2 ± 1	11 ± 5	0	0	20 ± 6
Mock CM	—	0	0	0	0	0	0	0
IL-4 + G-CSF + Ep	—	13 ± 3	1 ± 1	0	15 ± 6	0	0	29 ± 8
IL-3 + G-CSF + Ep	—	12 ± 4	3 ± 1	6 ± 1	69 ± 14	2 ± 2	7 ± 2	99 ± 20
IL-4	G-CSF + EP	13 ± 1	1 ± 1	1 ± 1	1 ± 1	0	0	15 ± 2
IL-3	G-CSF + Ep	9 ± 2	2 ± 1	7 ± 3	56 ± 3	2 ± 2	7 ± 2	84 ± 3
CSFs	—	11 ± 5	2 ± 1	5 ± 2	74 ± 4	4 ± 2	7 ± 2	104 ± 9

Data represent the mean ± SD of triplicate cultures containing 1×10^4 peripheral blood null cells per dish. CSFs contained IL-3, GM-CSF, G-CSF, M-CSF and Ep. Abbreviations defined in legend to Table 1.

Table 7. Colony Formation by Bone Marrow Null Cells Supported by Various Concentrations of Purified Recombinant Human IL-4 in the Presence of G-CSF or Ep

Factors	Colony Types						
	G	M	GM	B	EO	E-Mix	Total
G-CSF (20 ng/mL)*	15 ± 2	7 ± 1	3 ± 2	0	0	0	25 ± 3
G-CSF + IL-4 (U/mL)†							
1	28 ± 2	5 ± 1	6 ± 1	0	0	0	39 ± 2
10	57 ± 2	3 ± 2	5 ± 2	0	0	0	65 ± 3
100	63 ± 3	2 ± 1	4 ± 2	0	0	0	69 ± 2
1,000	59 ± 9	3 ± 2	6 ± 3	0	0	0	68 ± 9
2,000	59 ± 10	2 ± 1	5 ± 3	0	0	0	67 ± 9
Ep (2 U/mL)	0	8 ± 2	0	13 ± 1	0	0	20 ± 3
Ep + IL-4 (U/mL)							
1	0	3 ± 1	0	17 ± 3	0	0	20 ± 3
10	0	4 ± 1	0	15 ± 4	0	0	19 ± 4
100	0	4 ± 2	0	15 ± 4	0	0	19 ± 5
1,000	0	3 ± 1	0	10 ± 1	0	0	13 ± 1
2,000	0	3 ± 1	0	15 ± 3	0	0	17 ± 3
IL-4 (1,000 U/mL)	0	3 ± 2	0	0	0	0	3 ± 2
None	0	7 ± 1	0	0	0	0	7 ± 1
CSFs	15 ± 4	18 ± 5	16 ± 2	31 ± 3	9 ± 3	4 ± 1	92 ± 4

Data represent the mean ± SD of triplicate cultures containing 1,000 bone marrow null cells per dish. CSFs contained IL-3 (100 U/mL), GM-CSF (100 U/mL), G-CSF (20 ng/mL), and Ep (2 U/mL). Abbreviations defined in legend to Table 1.

*G-CSF, purified CHO cell-derived.

†IL-4, purified CHO cell-derived (5×10^6 U/mg).

in the murine system appears to directly increase the sensitivity of progenitor cells to G-CSF, thereby promoting differentiation along the neutrophil pathway. The other effects of IL-4 are likely to be indirect.

Our previous report suggested that the primary targets of IL-3 and GM-CSF are multipotential progenitors at the early and intermediate stages of development.[20] IL-3 can synergistically act with terminally acting CSFs such as G-CSF, Ep,[20] and IL-5[22] in support of neutrophil, erythroid, and eosinophil colony formation, respectively. In addition, IL-3 can initiate the proliferation of early multipotential progenitors.[20] In contrast, IL-4 could support neither proliferation nor survival of erythroid burst or mixed colony forming cells, as shown by the delayed addition experiment and mapping study and demonstrating apparent differences between functions of human IL-4 and IL-3. Broxmeyer et al,[13] however, have reported apparent BPA of recombinant human IL-4. This discrepancy may be due to the cell concentration in cultures. In their relatively high density culture, IL-4 may have stimulated macrophages or T cells to release cytokines with BPA, such as GM-CSF or IL-3. In most experiments, we used a COS-cell conditioned medium containing IL-4. Therefore, we tested the effects of highly purified IL-4 on colony formation supported by either G-CSF or Ep and found the same synergistic action of IL-4 with G-CSF. Again purified IL-4 did not synergize with Ep in support of erythroid burst or mixed colony formation.

Recently, Kishi et al[23] have shown that purified recombinant murine IL-4 directly acts on multipotential blast cell colonies to generate granulocyte/erythrocyte/macrophage/megakaryocyte (GEMM) colonies. However, we failed to find stimulatory effects of recombinant human IL-4 on erythroid burst and mixed colony forming cells. This suggests the possibility that IL-4 may behave differently in the human and murine systems. Alternatively, the discrepancies in the results may reflect differences in target populations, as well as the presence or absence of accessory cells that may modify the activities of IL-4 in culture.

ACKNOWLEDGMENT

The authors are grateful to Dr Steven C. Clark of the Genetics Institute for his critical reading of this manuscript and constructive advice. The authors also thank Yoriko Ohta for her excellent technical assistance.

REFERENCES

1. Howard M, Farrar J, Hilfiker M, Johnson B, Takatsu K, Hamaoka T, Paul WE: Identification of a T-cell-derived B cell growth factor distinct from interleukin-2. J Exp Med 155:914, 1982

2. Rabin EM, Ohara J, Paul WE: B-cell stimulatory factor 1 activates resting B cells. Proc Natl Acad Sci USA 82:2935, 1985

3. Grabstein K, Eisenman J, Mochizuki D, Shanbeck K, Conlon P, Hopp T, March C, Gillis S: Purification to homogeneity of B-cell stimulating factor. J Exp Med 163:1405, 1986

4. Peschel C, Green I, Ohara J, Paul WE: Role of B-cell stimulatory factor 1/interleukin 4 in clonal proliferation of B cells. J Immunol 139:3338, 1987

5. Brown M, Hu-Li J, Paul WE: IL-4/B cell stimulatory factor 1 stimulates T cell growth by an IL-2-independent mechanism. J Immunol 131:504, 1988

6. Widmer MB, Acres RB, Sassenfeld HM, Grabstein KH: Regulation of cytolytic cell populations from human peripheral

blood by B cell stimulatory factor 1 (interleukin 4). J Exp Med 166:1447, 1987

7. Fernandez-Botran R, Krammer PH, Diamantstein T, Uhr JW, Vitetta ES: B cell-stimulatory factor 1 (BSF-1) promotes growth of helper T cell lines. J Exp Med 164:580, 1986

8. Littman BH, Dastvan FF, Carson PL, Sanders KM: Regulation of monocyte/macrophage C2 production and HLA-DR expression by IL-4 (BSF-1) and IFN-r. J Immunol 142:520, 1989

9. Lee F, Yokota T, Otsuka T, Meyerson P, Villaret D, Coffman R, Mosmann T, Rennick D, Roehm N, Smith C, Zlotnik A, Arai K: Isolation and characterization of a mouse interleukin cDNA clone that expresses B-cell stimulatory factor 1 activities and T-cell– and mast-cell–stimulating activities. Proc Natl Acad Sci USA 83:2061, 1986

10. Hamaguchi Y, Kanakura Y, Fujita J, Takeda S, Nakano T, Tarui S, Honjo T, Kitamura Y: Interleukin 4 as an essential factor for in vitro clonal growth of murine connective tissue-type mast cells. J Exp Med 165:268, 1987

11. Peschel C, Paul WE, Ohara J, Green I: Effects of B cell stimulatory factor-1/interleukin 4 on hematopoietic progenitor cells. Blood 70:254, 1987

12. Rennick D, Yang G, Muller-Sieburg C, Smith C, Arai N, Takabe Y, Gemmell L: Interleukin 4 (B-cell stimulatory factor 1) can enhance or antagonize the factor-dependent growth of hemopoietic progenitor cells. Proc Natl Acad Sci USA 84:6889, 1987

13. Broxmeyer HE, Lu L, Cooper S, Tushinski R, Mochizuki D, Rubin BY, Gillis S, Willams DE: Synergistic effects of purified recombinant human and murine B cell growth factor-1/IL-4 on colony formation in vitro by hematopoietic progenitor cells. J Immunol 141:3852, 1988

14. Yang Y-C, Ciarletta AB, Temple PA, Chung MP, Kovacic S, Witek-Giannotti JS, Leary AC, Kriz R, Donahue RE, Wong GG, Clark SC: Human IL-3 (multi-CSF): Identification by expression cloning of a novel hematopoietic growth factor related to murine IL-3. Cell 47:3, 1986

15. Donahue RE, Wang EA, Kaufman RJ, Foutch L, Leary AC, Witek-Giannotti JS, Metzger M, Hewick RM, Steinbrink DR, Shaw G, Kamen R, Clark SC: Effects of N-linked carbohydrate on the in vitro properties of human GM-CSF, in Cold Spring Harbor Symposia on Quantitative Biology, vol 51. Cold Spring Harbor, NY, 1986, p 685

16. Maeda M, Noma T, Hama K, Honjo T: Application of a human T cell line derived from a Sezary syndrome patient for human interleukin 4 assay. Immunol Lett 18:247, 1988

17. Sonoda Y, Ogawa M: Serum-free culture of human hemopoietic progenitors in attenuated culture media. Am J Hematol 28:227, 1988

18. Iscove NN, Sieber F, Winterhalter KH: Erythroid colony formation in cultures of mouse and human bone marrow. Analysis of the requirement for erythropoietin by gel filtration and affinity chromatography on agarose-concanavalin A. J Cell Physiol 83:309, 1974

19. Sonoda Y, Yang YC, Wong GG, Clark SC, Ogawa M: Erythroid burst-promoting activity of purified recombinant human GM-CSF and interleukin-3: Studies with anti–GM-CSF and anti–IL-3 sera and studies in serum-free cultures. Blood 72:1381, 1988

20. Sonoda Y, Yang YC, Wong GG, Clark SC, Ogawa M: Analysis in serum-free culture of the targets of recombinant human hemopoietic factors: Interleukin 3 and granulocyte/macrophage–colony-stimulating factor are specific for early developmental stages. Proc Natl Acad Sci USA 85:4360, 1988

21. Peschel C, Green I, Paul WE: Interleukin-4 induces a substance in bone marrow stromal cells that reversibly inhibits factor-dependent and factor-independent cell proliferation. Blood 73:1130, 1989

22. Sonoda Y, Arai N, Ogawa M: Humoral regulation of eosinophilopoiesis in vitro: Analysis of the targets of interleukin-3, granulocyte/macrophage colony-stimulating factor (GM-CSF), and interleukin-5. Leukemia 3:14, 1989

23. Kishi K, Ihle JN, Urdal DL, Ogawa M: Murine B-cell stimulatory factor-1 (BSF-1)/interleukin-4 (IL-4) is a multi-CSF which acts directly on primitive hemopoietic progenitors. J Cell Physiol 139:463, 1989