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Negative regulation of early B lymphopoiesis by interleukin 3 and interleukin 1α

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ABSTRACT **We recently developed a two-step methyl cellulose culture system for murine lymphohemopoietic progenitors that are capable of differentiation along the myeloid and B-lymphoid lineages. In this system, two-factor combinations, which include steel factor plus interleukin (IL) 6, IL-11, or granulocyte colony-stimulating factor effectively supported the lymphomyeloid potential of primary colonies. Interestingly, IL-3 could neither replace nor act synergistically with steel factor in maintaining the B-lymphoid potential of the primary colonies although the frequency of colony formation was the same with IL-3 and steel factor. We now report that addition of IL-3 or IL-1α to a permissive system suppresses the B-lymphoid potential of primitive progenitor cells in primary culture in dose-dependent fashion. *In vivo* transfer of the primary colonies to *scid* mice confirmed the suppressive effects of IL-3 and IL-1α. In addition, IL-1α inhibited pre-B-cell colony formation in the secondary culture. Once pre-B-cell colonies had formed in secondary culture, neither factor affected the proliferation of the pre-B cells. These results suggest negative regulatory roles for IL-3 and IL-1α in early stages of B lymphopoiesis.**

Studies in cell culture have provided evidence that proliferation of primitive hemopoietic progenitors is regulated by interacting groups of cytokines (1). Interleukin (IL) 3 (2), IL-4 (3), and granulocyte/macrophage colony-stimulating factor (GM-CSF) (4, 5) form a group of cytokines that support continued proliferation of multipotential progenitors. These factors, however, do not appear to trigger cell cycling of dormant primitive progenitors. Rather, they support proliferation of multipotential progenitors only after they exit from the dormant state. IL-6 (6), granulocyte colony-stimulating factor (G-CSF) (7), IL-11 (8, 9), IL-12 (33), and leukemia inhibitory factor (LIF) (10, 11) form a group of cytokines that work synergistically with IL-3, IL-4, and GM-CSF to support the proliferation of multipotential progenitors from cell-cycle-dormant progenitors. Steel factor (SF) is unique in that it interacts with both groups of cytokines, except IL-4, in the triggering of cell division and in the support of continued proliferation of multipotential progenitors (12, 13).

In contrast to hemopoietic (myeloid) regulation, the mechanisms controlling the early stages of lymphopoiesis have not been clarified, partly because appropriate culture assays for lymphohemopoietic progenitors have only recently become available. Independently, investigators in three laboratories described culture assays for lymphohemopoietic progenitors. Cumano *et al.* (14) and Baum *et al.* (15) described coculture systems using murine stromal cell lines for fetal murine and fetal human lymphohemopoietic progenitors that have the ability to yield myeloid and B-lymphoid differentiation. We have established (16) a two-step methyl cellulose culture assay for murine lymphohemopoietic progenitors in which both myeloid and B-lymphoid cells are induced from single progenitors. In this assay, isolated marrow progenitors were plated in the primary culture and the resulting myeloid colonies were replated in secondary lymphoid culture containing SF and IL-7. Initially, we used a combination of SF and medium conditioned by pokeweed-mitogen-stimulated spleen cells (PWM-SCM) in the primary culture. Subsequent studies revealed that IL-6, IL-11, or G-CSF can replace PWM-SCM and that SF plus one of these factors can effectively support the B-cell potential of the primary colonies. Interestingly, however, cytokine combinations containing IL-3 did not support the B-cell capacity of primary colonies even though the number and size of primary colonies was comparable to that of colonies supported by the SF-containing combinations. Because of the unexpected effects of IL-3, we carried out a survey of lymphohemopoietic cytokines and characterized in more detail the negative effects of these factors in the early stages of B-cell development. We now report that IL-3 and IL-1α, when added under permissive culture conditions, strongly inhibit the B-cell potential of lymphohemopoietic progenitors.

MATERIALS AND METHODS

Growth Factors. Medium conditioned by CHO cells transfected with an expression plasmid containing murine SF cDNA was provided by D. Donaldson of the Genetics Institute, Cambridge, MA. Purified recombinant human IL-7 and murine IL-4 were provided by C. Faltynek of Sterling Drug, Malvern, PA. Purified recombinant murine GM-CSF and the medium conditioned by CHO cells that had been genetically engineered to produce murine IL-3 at a high titer (≈70,000 units/ml) were gifts from T. Sudo of Biomaterial Research Institute, Yokohama, Japan. Purified recombinant murine IL-3 was purchased from R & D Systems. Purified bacterially derived human G-CSF was provided by L. Sousa of Amgen, Thousand Oaks, CA. Recombinant human IL-6 was a gift from M. Naruto of Toray Silicon, Osaka. Recombinant human IL-2 was a gift from Shionogi, Osaka. Recombinant murine tumor necrosis factor α was provided by P.-H. Lai of Protein Institute, Broomall, PA. Purified recombinant human IL-11 was provided by P. Schendel, Genetics Institute. Purified recombinant human LIF and Cos-cell-conditioned medium containing human IL-1α, murine IL-5, or murine IL-9 were provided by K. Turner, Genetics Institute. Purified recombinant human IL-1α was provided by Y. Hirai, Otsuka Pharmaceutical, Tokushima, Japan. Purified recombinant human erythropoietin (Ep) was provided by the Genetics Institute Clinical Manufacturing Group. Unless specified otherwise, the concentrations of cytokines used were as follows: SF, 5 units/ml; IL-1α (COS-cell conditioned

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Abbreviations: IL, interleukin; GM-CSF, granulocyte/macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; LIF, leukemia inhibitory factor; SF, steel factor; Ep, erythropoietin.

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medium), 2 units/ml, IL-1α (purified), 1.0 ng/ml; IL-2, 200 units/ml; IL-3 (CHO-cell conditioned medium), 200 units/ml; IL-3 (purified), 10 ng/ml; IL-4, 10 ng/ml; IL-5, 100 units/ml; IL-6, 100 ng/ml; IL-7, 5 ng/ml; IL-9, 5 units/ml; IL-11, 100 ng/ml; Ep, 2 units/ml; G-CSF, 1.0 ng/ml; GM-CSF, 160 ng/ml; LIF, 100 ng/ml.

Primary and Secondary Methyl Cellulose Cultures. Bone marrow cells were harvested from BDF1 mice 2 days after i.v. injection of 5-fluorouracil (Adria Laboratories) at 150 mg/kg (body weight). Cells were enriched by density-gradient separation, depleted of lineage-marker-positive cells, and sorted for Ly-6A/E-positive cells as described (17). Fifty enriched cells were plated in a 35-mm suspension culture dish (Nunc) containing α-medium, 1.2% (1500 centipoieses) methyl cellulose (Shinetsu Chemical, Tokyo), 30% (vol/vol) fetal calf serum (HyClone), 1% deionized fraction V bovine serum albumin (Sigma), 1×10^{-4} M 2-mercaptoethanol (Eastman Organic Chemicals, Rochester, NY), IL-7 (5 ng/ml), Ep (2 units/ml), and designated combinations of growth factors. Dishes were incubated at 37°C in a humidified atmosphere flushed with 5% CO_2. On designated days between 5 and 17 days of incubation, the total colony number was scored, and 20 colonies were picked, pooled, and washed. Aliquots of 1/20 or 1/40 of the pooled cells were plated in secondary methyl cellulose culture containing SF (5 units/ml) and IL-7 (5 ng/ml). The number of pre-B-cell colonies was counted on day 11 of the secondary culture by using the criteria described in ref. 16.

Timed Exposure to Growth Factors. Fifty enriched marrow cells were incubated in each well of a 96-well microtiter plate in suspension culture. The culture contained α-medium, 30% fetal calf serum, 1% bovine serum albumin, 1×10^{-4} M 2-mercaptoethanol, SF, IL-11, IL-7, Ep, and either IL-3 or IL-1α. After incubation for 1, 2, 4, or 6 days, the cells were washed and recultured with SF, IL-11, IL-7, and Ep again in a 96-well microtiter plate. After a total of 10 days in suspension culture, a 1/80 aliquot of each sample was plated in methyl cellulose culture with SF and IL-7 to test the B-cell potential of the samples.

Effects of Growth Factors in Secondary Culture on the Proliferation of B-Cell Progenitors. Forty primary colonies induced by a combination of SF, IL-11, IL-7, and Ep were picked, pooled on day 10, and enriched for Mac-1$^-$ and Gr-1$^-$ cells by using negative immunomagnetic selection with anti-Mac-1 (M1/70.15.11.5.HL, ATCC), anti-Gr-1 (RB6-8C5, a gift from R. L. Coffman, DNAX), and Dynabeads M-450 sheep anti-rat IgG (Dynal, Great Neck, NY). Aliquots of 1/80 of the cells were replated with SF, IL-7, and designated growth factors. The number of pre-B-cell colonies was scored on day 11 of the secondary culture.

Analysis of Proliferation of Pre-B Cells. Pre-B-cell colonies that formed in response to a combination of SF, IL-11, IL-7, and Ep in primary culture followed by a combination of SF and IL-7 in secondary culture were individually picked on day 10 of secondary culture, pooled, washed, and suspended in α-medium. After eliminating Mac-1$^+$, Gr-1$^+$ cells by use of immunomagnetic-negative selection, 2×10^4 cells in 0.2 ml were cultured with SF, IL-7, and a test factor in each well of 96-well microtiter plates. Two days later the number of recoverable cells were counted.

***In Vivo* Transfer of Primary Colonies.** Enriched marrow cells from BDF1 mice (*Igh-6$^{b/non\text{-}b}$, H-2$^{b/d}$*) were cultured in methyl cellulose (50 cells per dish) under three conditions: in the presence of SF, IL-11, plus IL-7; SF, IL-11, IL-7, plus IL-3; or SF, IL-11, IL-7, plus IL-1α. On day 8, 160 primary colonies were picked from each culture group, pooled, and washed. Total cell number from 160 colonies of each group was 2.3×10^6, 5.0×10^6, and 2.5×10^6, respectively. One-eighth of the pooled cells (equivalent to 20 colonies) was injected intravenously into each of four 7-week-old female C.B.-17 *scid* mice (*Igh-6^b, H-2^d*; Taconic Farms). One-eightieth of pooled cells (equivalent to 2 colonies) from cultures lacking IL-1α or IL-3 was also injected. Four control mice did not receive injections. Before and serially after cell injection, serum levels of non-b haplotype (donor-derived) IgM were measured by ELISA using anti-mouse non-b haplotype IgM, DS-1 (PharMingen). On day 114, all mice were sacrificed and 2×10^5 spleen cells were stained with AF6-88.5.3 (anti-mouse H-2K^b, IgG2a; American Type Culture Collection) and fluorescein isothiocyanate-conjugated goat anti-mouse γ-chain-specific antibody (Cappel) followed by biotin-conjugated goat anti-mouse μ-chain-specific antibody (Cappel) plus streptavidin-phycoerythrin (Becton Dickinson).

RESULTS

Inhibitory Effects of Lymphohemopoietic Factors in Primary Culture. We have observed (16) that two-factor combinations containing SF, such as SF plus IL-11, SF plus IL-6,

Table 1. Colony formation and B-cell potential of the primary colonies supported by combinations of two or three cytokines

Test cytokines in primary culture	No. of primary colonies	Replated on day 8		Replated on day 11	
		Mean cell no. of primary colonies ($\times 10^{-4}$)	Average no. of pre-B-cell colonies	Mean cell no. of primary colonies ($\times 10^{-4}$)	Average no. of pre-B-cell colonies
SF, IL-11	21 ± 3	2.0	27 ± 6	4.8	44 ± 1
SF, IL-11, IL-3	19 ± 3	5.9	0	6.7	0
SF, IL-11, IL-4	23 ± 2	1.4	46 ± 5	5.5	3 ± 1
SF, IL-11, IL-6	19 ± 2	1.5	14 ± 5	6.0	32 ± 7
SF, IL-11, G-CSF	14 ± 4	2.0	19 ± 2	5.4	54 ± 6
SF, IL-11, GM-CSF	18 ± 3	5.6	50 ± 4	3.6	1 ± 1
SF, IL-6	20 ± 3	1.4	18 ± 1	6.4	7 ± 0
SF, IL-6, IL-3	21 ± 1	4.1	0	8.6	0
SF, IL-6, IL-4	16 ± 2	1.2	7 ± 0	2.4	0
SF, IL-6, G-CSF	22 ± 4	2.0	13 ± 5	8.0	2 ± 1
SF, IL-6, GM-CSF	17 ± 4	2.5	15 ± 5	4.8	1 ± 1
SF, G-CSF	15 ± 5	0.31	6 ± 1	6.2	96 ± 20
SF, G-CSF, IL-3	24 ± 2	2.1	0	4.8	0
SF, G-CSF, IL-4	1 ± 2		ND		ND
SF, G-CSF, GM-CSF	17 ± 4	1.3	7 ± 1	6.2	39 ± 9

Fifty enriched marrow cells were cultured in the presence of Ep (2 units/ml), IL-7 (200 units/ml), and designated cytokine combinations. Primary colonies were scored on day 8. The number of pre-B-cell colonies was derived from 5% of 20 pooled colonies. ND, not done.

Table 2. Effects of addition of IL-1α or IL-3 on the size and B-cell potential of the primary colonies supported by SF plus IL-11

Test cytokine in primary culture	No. of primary colonies	Mean cell no. of primary colonies ($\times 10^{-4}$)	No. of pre-B-cell colonies
None	17 ± 2	3.8	122 ± 2
IL-1α	22 ± 6	1.6	0
IL-3	20 ± 3	5.8	0

Fifty enriched marrow cells were cultured in the presence of SF, IL-11, IL-7, Ep, and the cytokine to be tested. Primary colonies were scored and replated on day 10. The number of colonies is expressed as the mean ± SD of triplicate cultures. The number of pre-B-cell colonies was derived from 1/40 of 20 pooled colonies.

and SF plus G-CSF, effectively support the B-cell potential of primary colonies, whereas two-factor combinations containing IL-3 do not. This prompted us to examine the effects of IL-3 and other lymphohemopoietic factors in three-factor combinations on the B-cell potential of the primary colonies (Table 1). We studied the cytokines that are known to regulate early stages of hemopoiesis (2–13). As we reported (16), SF plus IL-11 effectively supported the B-cell potential of the primary colonies harvested on day 8 or 11. Although addition of IL-4, IL-6, G-CSF, or GM-CSF to SF plus IL-11 somewhat changed the optimal time of primary culture for maintenance of the B-cell potential, it did not abrogate the B-cell potential of the primary colonies. In contrast to these factors, addition of IL-3 to SF plus IL-11 abrogated the B-cell potential of the primary colonies although the number and size of the primary colonies were unaffected. Addition of single factors to the combination of SF and IL-6 or the combination of SF and G-CSF also yielded similar results with the exception of IL-4. Addition of IL-4 appeared to suppress the B-cell potential of the primary colonies supported by SF plus IL-6 and almost totally suppressed the primary colony formation supported by SF plus G-CSF. Addition of IL-3 in both cases abrogated the B-cell potential of the primary colonies, whereas the number and size of the primary colonies were unaffected.

Effects of Other Cytokines on the B-Cell Potential of the Primary Colonies. Because of the unexpected inhibitory effects of IL-3, we tested other lymphohemopoietic cytokines including IL-1α, IL-2, IL-5, IL-9, tumor necrosis factor α, and LIF. Of these factors, IL-1α strongly inhibited the B-cell potential of the primary colonies supported by SF and IL-11. A representative analysis of the inhibitory effects of IL-1α and IL-3 is shown in Table 2. Again, IL-3 abrogated the B-cell potential but not the proliferative ability of the primary colonies.

Dose Effects and Kinetics of Suppression of B Lymphopoiesis by IL-3 and IL-1α. IL-3 and IL-1α suppressed B lymphopoiesis in a similar dose-dependent manner. An example of a dose–response study with IL-1α is shown in Fig. 1. Purified IL-3 at 1.0 ng/ml and purified IL-1α at 1 ng/ml suppressed completely the B-cell potential of the primary colonies. Either cytokine at 0.1 ng/ml showed >90% inhibition. To test the possibility that IL-3 and IL-1α altered the optimal time for maintenance of the B-cell potential in primary colonies, we replated the colonies after various days of incubation. Addition of IL-3 or IL-1α to a combination of SF, IL-11, IL-7, and Ep in the primary culture abrogated the B-cell potential of the primary colonies regardless of the date of replating. A representative analysis with IL-3 is shown in Fig. 2.

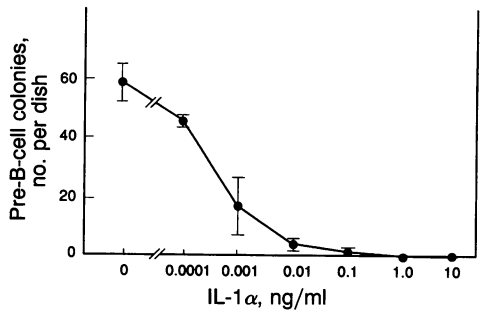

FIG. 1. Dose–response curve for the effect of IL-1α on pre-B-cell colony formation. Fifty enriched cells were cultured with SF, IL-11, IL-7, Ep, and IL-1α. On day 11, 20 colonies were picked, pooled, washed, and assayed for pre-B-cell colony formation.

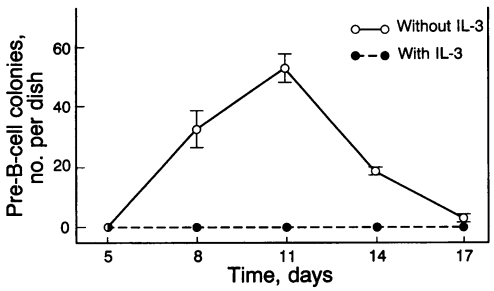

FIG. 2. Effect of IL-3 on the time course of pre-B-cell colony formation. Fifty enriched cells were cultured in methyl cellulose with SF, IL-11, IL-7, and Ep in the presence or absence of IL-3. Five to 17 days later, groups of 20 colonies were serially picked, pooled, washed, and assayed with SF and IL-7 in methyl cellulose culture for pre-B-cell colony formation.

To further define the developmental stages in which IL-3 and IL-1α act as inhibitors, we carried out timed-exposure experiments. As shown in Table 3, exposure for >2 days to IL-3 or IL-1α significantly suppressed the B-cell potential of the early progenitors.

Effects of Growth Factors in Secondary Culture on the Proliferation of B-Cell Progenitors. We then tested the possible inhibitory effects of lymphohemopoietic factors on proliferation of pre-B-cell progenitors by replating pooled primary colonies. Addition of IL-2, IL-5, IL-9, IL-11, G-CSF, LIF, or tumor necrosis factor α to secondary culture with SF and IL-7 did not suppress formation of pre-B-cell colonies (data not shown). Pre-B-cell colony formation was inhibited by IL-1α and IL-4 to ≈5% of control cultures without IL-1α or IL-3. When IL-3, IL-6, or GM-CSF was

Table 3. Effects of timed exposure to IL-3 or IL-1α on early stages of lymphopoiesis

Test cytokine in primary suspension culture	Duration of exposure, days	No. of pre-B-cell colonies
None	1	39 ± 4
	2	35 ± 0
	4	40 ± 0
	6	39 ± 3
IL-3	1	25 ± 1
	2	69 ± 1
	4	5 ± 2
	6	1 ± 1
IL-1α	1	38 ± 3
	2	14 ± 5
	4	3 ± 1
	6	0

Fifty enriched cells were incubated with SF, IL-11, IL-7, Ep, and IL-1α or IL-3 in suspension culture, washed, and recultured in suspension without IL-1α or IL-3. Ten days after initiation of suspension culture 1/80 samples were analyzed for pre-B-cell colony formation.

Table 4. Serum non-b haplotype IgM levels of *scid* mice injected with pooled primary colony cells

Primary colonies		Serum non-b IgM level, μg/ml				
Induction of primary colonies	No. of primary colonies injected	Preinjection	4 weeks	8 weeks	12 weeks	15 weeks
	None	<1	<1	<1	<1	<1
SF, IL-11, IL-7	20	<1	14	227	159	299
SF, IL-11, IL-7	2	<1	5	55	63	93
SF, IL-11, IL-7, IL-3	20	<1	<1	<1	<1	<1
SF, IL-11, IL-7, IL-1α	20	<1	<1	<1	<1	<1

Enriched marrow cells from BDF1 mice (*Igh-6*$^{b/non\text{-}b}$) were cultured (50 cells per dish) in methyl cellulose with SF, IL-11, and IL-7 in the presence or the absence of IL-3 or IL-1α for 8 days. Then primary colonies were picked, pooled, washed, and injected intravenously into C.B.-17 *scid* mice (*Igh-6*^{b}). Serum non-b haplotype IgM was measured serially by ELISA and expressed as the mean value from four mice.

tested, overgrowth of myeloid cells made it impossible to score pre-B-cell colonies accurately.

Effects of Cytokines on the Proliferation of Pre-B Cells. Cells constituting the established pre-B-cell colonies can proliferate further in the continued presence of SF and IL-7 without changing their surface marker phenotypes. The cells continue to express B220 and Thy1.2 but not surface immunoglobulin, other T-cell markers, or myeloid lineage markers (data not shown). We tested the effects of lymphohemopoietic factors on the late stages of B-cell development by using suspension culture of pre-B-cell colony cells in the presence of SF and IL-7. None of the lymphohemopoietic growth factors tested, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, G-CSF, GM-CSF, and LIF, inhibited the proliferation of pre-B cells (data not shown).

***In Vivo* Transfer of Primary Colonies.** To confirm the inhibitory effects of IL-3 and IL-1α on the early stages of B-cell development, we studied the ability of primary colonies to reconstitute the immune system of *scid* mice. As shown in Table 4, all mice injected with equivalents of 2 and 20 pooled primary colonies derived from cultures containing SF, IL-11, and IL-7 but not IL-1α or IL-3 revealed high serum levels of non-b haplotype IgM. However, serum non-b IgM was not detectable in the four mice that were injected with 20 primary colonies cultured in the presence of IL-1α or IL-3.

On day 114 of cell transfer, mice were sacrificed and their spleen cells were stained with anti-H-2K^{b} and anti-IgM. The H-2 haplotype of DBA/2 mice, a parent of BDF1 mice and *scid* mice is type d. Because the haplotype of C57BL/6, the other parent of BDF1 mice, is type b and, therefore, distinguishable from type d by anti-H-2K^{b} monoclonal antibody AF6-88.5.3, it is possible to demonstrate production of lymphocytes derived from injected cells of BDF1 origin. All four mice injected with 20 pooled primary colonies induced by SF, IL-11, and IL-7 and all four mice injected with an equivalent of only 2 such colonies revealed significant number of donor-derived (H-2K^{b}-positive) surface IgM^{+} cells. An example of one such analysis is shown in Fig. 3*A*. When pooled primary colonies grown in the presence of IL-1α or IL-3 were injected, 20 primary colonies did not reconstitute spleen B cells in the *scid* mice. Examples of each case are presented in Fig. 3 *B* and *C*. These results further suggested that IL-1α and IL-3 suppress B-lymphoid development in culture.

DISCUSSION

We have demonstrated inhibitory effects of certain lymphohemopoietic cytokines on early stages of B lymphopoiesis by using a recently developed two-step methyl cellulose culture assay. IL-3 and IL-1α abrogated the B-lymphoid potential without inhibiting the myeloid potential of the primary colonies. IL-1α and IL-4 suppressed the formation of pre-B-cell colonies in secondary culture. However, none of the cytokines negatively affected the proliferation of pre-B cells in response to SF and IL-7 once the pre-B-cell colonies were established. Therefore, the inhibitory effects of these cytokines appear to be specific for relatively early stages of B lymphopoiesis.

Investigators in other laboratories have also observed inhibitory effects of IL-1α on B lymphohemopoiesis. Dorshkind (18) reported that the presence of IL-1 when normal murine marrow cells are transferred from myeloid to lymphoid long-term cultures results in a complete inhibition of B lymphopoiesis and sustained myelopoiesis. His observation may be analogous to our observation of the inhibitory effects of IL-1α on the maintenance of the B-cell potential of the primary colonies. Suda *et al.* (19) observed that addition of IL-1α to IL-7 in cultures of bone marrow cells from normal mice inhibits pre-B-cell colony formation. Their observation may be similar to our observation of the suppressive effects of IL-1α in the secondary culture. The inhibitory effects of IL-1 on B lymphopoiesis were also suggested by delayed recovery of B-lineage cells in mice treated with IL-1β after sublethal irradiation (20). Further studies are necessary to

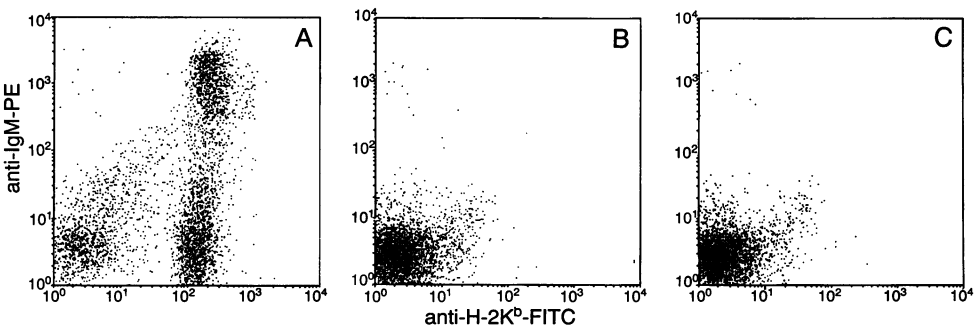

FIG. 3. Two-color flow cytometry analysis of spleen cells of *scid* mice injected with pooled primary colonies. The equivalent of 20 pooled primary colonies was injected intravenously into C.B.-17 *scid* mice. On day 114 of cell transfer, spleen cells were analyzed for H-2K^{b} and IgM. (*A*) Primary colonies were derived from cultures containing SF, IL-11, and IL-7. (*B*) Primary colonies were derived from cultures containing SF, IL-11, IL-7, and IL-1α. (*C*) Primary colonies were derived from cultures containing SF, IL-11, IL-7, and IL-3. PE, phycoerythrin; FITC, fluorescein isothiocyanate.

clarify whether IL-1 suppresses B lymphopoiesis directly or indirectly, as suggested by Dorshkind (18).

The role of IL-4 in lymphohemopoiesis appears to be complex. Peschel *et al.* (21) reported that IL-4 promotes the development of $B220^-$ immature B-lineage cells in stromal cell-dependent long-term culture and Kinashi *et al.* (22) established IL-4-dependent precursor clones that can differentiate into both B cells and myeloid cells in the presence of bone marrow stromal cells. We have observed that IL-4 plus IL-6 or IL-11 support the formation of primary colonies containing myeloid and B-lymphoid capabilities (16). Development of $B220^+$ pre-B-cell colonies in secondary culture, however, was negatively regulated by IL-4. These observations suggest complex bidirectional effects of IL-4 in lymphopoiesis that appear to be similar to the roles of IL-4 in myelopoiesis (23–25).

The most striking observation was the lineage-specific inhibition of early stages of B lymphopoiesis by IL-3. Earlier, investigators in several laboratories had established IL-3-dependent pre-B-cell (26, 27) and pro-B-cell (28–30) clones, suggesting the stimulatory roles of IL-3 in early B lymphopoiesis. In our studies, IL-3 completely abrogated the ability of the cytokine combinations containing SF to maintain the B-lymphoid potential of the primary colonies. This inhibition was not an artifact of the detection method for B-cell progenitors because the colony transplantation in *scid* mice also documented the suppression of the B-lymphoid potential of the primary colonies. Cockayne *et al.* (31) reported that transgenic mice expressing IL-3 antisense RNA develop a B-cell lymphoproliferative disorder at 3–9 months of age and that the tumor cells were $B220^+$ surface IgM^-. Thus these observations may indicate a negative regulatory role for IL-3 in the physiology of B lymphopoiesis.

The inhibition by IL-3 and IL-1α may also affect T lymphopoiesis. In the colony transfer experiments, none of four mice receiving an injection of 20 primary colonies grown in the presence of IL-3 or IL-1α had T lymphocytes in the spleen, whereas all four mice receiving only 2 primary colonies grown without IL-3 or IL-1α had T lymphocytes in the spleen. Details of these experiments will be presented elsewhere. It is possible that IL-3 and IL-1α negatively affect the development of common lymphopoietic progenitors capable of both T- and B-lymphocyte differentiation. Alternatively, these cytokines may suppress proliferation and differentiation of both committed T- and committed B-cell progenitors.

Currently, both IL-3 and IL-1α are being tested in clinical trials. Pertinent to this testing is the observation by Kimoto *et al.* (32) that perfusion of mice with IL-3 for 1 or 4 weeks causes marked diminution in the number of thymocytes. In addition, administration of IL-1β delayed recovery of thymocytes in mice treated with sublethal irradiation (20). Both cytokines are also being tested for *in vitro* expansion of stem cells and progenitors in relation to bone marrow transplantation. In preclinical studies, these cytokines are also used to stimulate cell division of cell-cycle-dormant stem cells, thereby facilitating retrovirally mediated gene transfer. Clinical use of IL-3 and IL-1α may need to be carefully monitored for preservation of lymphohemopoietic progenitors.

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