Bone marrow stromal cells appear to be key regulatory elements in hematopoiesis and lymphopoiesis. These stromal cells respond to cytokine exposure and alter their pattern of hematopoietic growth factor production, suggesting a degree of functional plasticity. We examined the effect of two cytokines, interleukin-1 (IL-1) and IL-4, on stromal cell regulation of pre-B cell generation using the bone marrow stromal cell line, S17. Neither lymphokine potentiated pre-B cell generation in the absence of stromal cells. However, addition of either 10 U/mL rIL-1 or 50 U/mL rIL-4 to cultures of bone marrow cells containing S17 cells dramatically suppressed subsequent pre-B cell formation. Preculture of S17 stromal cells with either rIL-1 or rIL-4 completely abrogated their ability to support pre-B cell generation in subsequent coculture with freshly explanted bone marrow cells. Conditioned medium from IL-1- or IL-4-treated S17 cells also suppressed pre-B cell generation in culture. Although it is not yet known which induced stromal cell factors are responsible for failure of pre-B cell generation in treated cultures, these data do clearly demonstrate that local levels of IL-1 and IL-4 in the hematopoietic microenvironment may play a significant role in regulation of bone marrow stromal cell function. These data also demonstrate that fibroblastic stromal cells are primary target cells that respond to cytokine concentration and affect lymphopoietic cell development.

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fluorescein isothiocyanate (FITC)-conjugated affinity purified goat-anti-mouse Ig. FITC-conjugated affinity purified goat-anti-mouse \( \lambda \) and goat-anti-mouse \( \kappa \) and tetramethylrhodamine isothiocyanate (TRITC)-conjugated affinity purified goat-anti-mouse \( \mu \) heavy chain antibodies were purchased from Southern Biotechnology Associates (Birmingham, AL). Monoclonal rat-anti-mouse IL-4 (hybridoma clone 11B11) was a generous gift from Dr William Paul (Laboratories of Immunology, National Institutes of Health, Bethesda, MD). All antibodies were used at saturating concentrations in antibody binding studies.

Preparation of cell suspensions. Mice were killed by cervical dislocation. Femora and tibiae were removed and the marrow plug flushed from bones with RPMI 1640 (GIBCO) containing 5% heat-inactivated fetal calf serum (HIFCS, Hyclone Laboratories, Logan, UT) using a syringe fitted with a 23-gauge needle. Bone spicules and debris were allowed to settle into an underlayer of HIFCS and cells were transferred to a second tube. Cell viability and cell counts were determined in a hemocytometer.

Recombinant growth factors. Recombinant human IL-1\( \alpha \) (10^6 U/mg) and recombinant murine IL-4 (10^6 U/mg) were obtained from Genzyme (Boston, MA).

Cell depletion. Adherent cells were depleted from bone marrow cell suspensions using G10 Sephadex (Pharmacia, Piscataway, NJ). Sterile G10 columns were prepared exactly as previously described. Briefly, sterile 10-mL syringe barrels were plugged with glass wool, autoclaved, and filled with 8 mL of sterile preswollen G10 Sephadex. Columns were washed with sterile phosphate buffered saline (PBS) and equilibrated with 20 mL warm (37\(^\circ\)C) RPMI 1640 containing 5% HIFCS. Two milliliters of medium containing 10\(^6\) bone marrow cells were applied to each column. Columns were incubated for 30 minutes at 37\(^\circ\)C and cells washed from columns with 30 to 35 mL of warm RPMI 1640 containing 5% HIFCS.

Monoclonal antibody (MoAb) 14.8 binds to a determinant on the Ly-S\( \text{\mu} \) (B220) cell surface molecule of lymphoid cells. Depletion of cells with this cell surface antigen results in removal of B-lineage cells, which express either sIg (B cells) or cytoplasmic \( \mu \)-heavy chains of IgM (pre-B cells). In our studies, 14.8\(^+\) cells were depleted from cell suspensions on antibody-coated polystyrene Petri dishes (Falcon 1001, Becton-Dickinson, Oxnard, CA) exactly as previously described. Briefly, sterile 10-mL syringe barrels were plugged with glass wool, autoclaved, and filled with 8 mL of sterile preswollen G10 Sephadex. Petri dishes were first coated with affinity-purified 14.8 antibody in 3 mL of warm (37\(^\circ\)C) RPMI 1640 containing 5% HIFCS. Two milliliters of medium containing 10\(^6\) bone marrow cells were applied to each column. Columns were incubated for 30 minutes at 37\(^\circ\)C and cells washed from columns with 30 to 35 mL of warm RPMI 1640 containing 5% HIFCS, and held at 4\(^\circ\)C until use. All plates were used the same day they were prepared.

In most experiments, bone marrow cells were first depleted of adherent cells on G10 Sephadex. Remaining cells were then transferred to antibody-coated plates (2.5 \( \times \) 10\(^6\) cells/plate) and incubated at 4\(^\circ\)C for 70 minutes. In all experiments, cells were depleted on three sequential plates to stringently remove appropriate cells. Nonadherent cells from this series of depletions were collected as 14.8\(^+\) (B-cell and pre-B-cell-depleted) bone marrow cells for use in experiments. Antibody depletions were checked by staining with fluorescent-labeled 14.8 antibody. Depletions were further monitored for efficiency of pre-B cell removal by staining depleted cells for expression of Ig light chain and \( \mu \)-heavy chain expression as described below.

Stromal cell cultures and conditioned medium. Murine bone marrow stromal cell line S17 was a generous gift from Dr Kenneth Dorskikh (University of California-Riverside), and its isolation and biologic characteristics have been previously described in detail. S17 cells were grown to confluence in 75-cm\(^2\) flasks in a minimal essential medium (MEM) (GIBCO) containing 15% FCS. Stromal cells were passaged weekly by 0.25% trypsin (GIBCO) treatment of adherent cell layers. For coculture experiments, cells were transferred to 24-well plates and used as confluent adherent layers of supporting cells. S17 underlayers were separated from target cells by a cellulose 0.45-\(\mu\)m microporous membrane (Millipore-HA, Millipore, Bedford, MA).

S17 conditioned medium was prepared by growing cells to confluent monolayers in aMEM supplemented with 5% FCS. Fresh medium was added to flasks and conditioned medium collected at 24 hours of culture. Conditioned medium was concentrated 10-fold on Amicon YM-5 membranes (Amicon), dialyzed, and stored in small aliquots at -85\(^\circ\)C.

In some experiments, conditioned medium was collected from either IL-1- or IL-4-treated S17 cells. Growth medium on confluent S17 cells was replaced with fresh RPMI 1640 containing 5% FCS and either 50 U/mL rIL-4 or 10 U/mL rIL-1\( \alpha \). At 24 hours, medium including factors was removed, cells were washed with sterile PBS, and medium replaced with fresh RPMI 1640 containing 5% HIFCS. Conditioned medium was collected from treated S17 cells after 24 hours of culture and stored at -85\(^\circ\)C.

Antibody labeling of cells and fluorescence microscopy. B and pre-B cells were identified by immunofluorescent staining of Ig light and heavy chains on fixed cytocentrifuged preparations. For direct immunofluorescence, 10\(^6\) cells were pelleted and resuspended in 5 to 10 \( \mu \)L labeled affinity-purified antibody (100 \mu g/mL). After a 20-minute incubation on ice, cells were washed with cold RPMI containing 5% FCS, pelleted at 400 \( \times \) g at 4\(^\circ\)C, and fluorescently labeled cells enumerated under epi-fluorescence using a Zeiss Universal microscope (Carl Zeiss, Inc, Thornwood, NY). Indirect labeling with 14.8 antibody was done in essentially the same way except that 10\(^6\) cells were first incubated with 20 \mu g of the monoclonal 14.8 antibody. Cells were then washed, pelleted, and incubated with 10 \mu g FITC goat-anti-rat Ig at 4\(^\circ\)C to develop the antibody staining.

For cytoplasmic Ig staining, 10\(^6\) cells were cytocentrifuged onto glass slides, air dried, and fixed in absolute ethanol containing 5% glacial acetic acid at -20\(^\circ\)C for 15 minutes. Fixed preparations were rehydrated in 3 volumes of PBS for 15 minutes each, and incubated with FITC goat-anti-mouse \( \lambda \) light chain (50 \mu g/mL) and FITC goat-anti-mouse \( \kappa \) light chain (50 \mu g/mL) containing 0.1% NaN\(_3\), All incubations were done in a humidified chamber at room temperature for 30 minutes. Slides were rinsed with PBS and incubated with TRITC-goat-anti-mouse \( \mu \)-heavy chain (50 \mu g/mL) for 30 minutes in a humidified chamber at room temperature. Slides were then washed in PBS, with the last wash left overnight at 4\(^\circ\)C. Washed slides were mounted in Elvanol (Fluorount-G, Southern Biotechnology Associates) and examined under epillumination on a Zeiss Universal microscope. Cells with detectable green fluorescence (light chain\( ^-\)) were considered B cells. Only cells with detectable \( \mu \)-heavy chain of Ig but not light chain were considered pre-B cells in these studies.

Bone marrow cell cultures. Bone marrow cells were depleted of adherent cells on G-10 Sephadex as described and then depleted of pre-B cells by adherence to 14.8-coated Petri dishes as described above. Nonadherent and 14.8\(^+\) cells were cultured at 10\(^6\) cells/mL in RPMI 1640 containing 5% FCS, 5 \( \times \) 10\(^{-5}\) mol/L 2-mercaptoethanol, essential and nonessential amino acids (GIBCO), 2 mmol/L L-glutamine, MEM vitamins (GIBCO), 100 U/mL penicillin G, and 100 \mu g/mL streptomycin. In experiments that required an underlayer of stromal cells, bone marrow cells were cultured in diffusion chamber culture inserts (Millipore-HA, Millipore) to prevent cell contact between bone marrow cells and stromal cells.

Thymocyte proliferation assays. C3He/1 thymocytes were cultured 72 hours at 5 \( \times \) 10\(^3\) cells/200 \mu L in RPMI 1640 containing
10% FCS, $5 \times 10^{-7}$ mol/L 2ME, and 2 µg/mL purified phytohemagglutinin (Wellcome Diagnostics, Dartford, England) in 96-well culture plates as previously described. Serial two-fold dilutions of rIL-1α, S17 CM, or lymphokine-treated S17 CM were added to cultures as indicated. Triplicate cultures were pulsed for the final 18 hours of culture with 1.0 µCi/well $1^H$-TdR. Thymidine uptake was measured at the termination of culture by automated harvesting of cells onto glass fiber filters using a PHD cell harvester (Cambridge Scientific, Boston, MA) and radioactivity quantitated by liquid scintillation (Packard Instrument Co, Downers Grove, IL).

Presentation of data. In all experiments, the absolute number of pre-B cells generated during the period of culture is presented. This calculation of actual pre-B cells lost or gained in cultures prevents misinterpretation of data due to secondary frequency changes caused by net losses or gains in another cell population in the bone marrow inoculum. In any experiment, cell viability for treated and control cultures did not vary more than 10%.

RESULTS

Pre-B cell generation is inhibited by rIL-1α. As illustrated in Fig 1, addition of 5% S17 conditioned medium (CM) preparation to cultures of G-10 Sephadex nonadherent, 14.8 antibody-depleted bone marrow cells consistently resulted in generation of increased numbers of pre-B (cμ', lκ') cells during the initial 48 hours of culture. Pre-B cell numbers declined in cultures after 72 hours (data not presented), presumably due to deterioration of culture conditions. Addition of recombinant human IL-1α to cultures containing 5% S17 CM resulted in a dose-responsive inhibition of pre-B cell generation (Fig 1). Addition of 10 U/mL rIL-1α to S17 potentiated cultures resulted in more than 90% inhibition of pre-B cell generation in four virtually identical experiments. In accord with previously reported data, addition of 0.5 to 50 U/mL IL-1α in the absence of S17 CM did not potentiate pre-B cell generation above that observed in medium controls. Polymyxin B addition has been previously used to detect possible effects of endotoxin in recombinant preparations. Addition of polymyxin B (10 µg/mL) to cultures did not alter the suppressive effect of rIL-1 in these studies (data not presented).

IL-1 suppresses stromal cell support of pre-B cell formation. Although the bone marrow target cells used in these experiments were depleted of adherent cells on G-10 Sephadex, we could not rule out the possibility that regulatory cells remained and contributed to observed suppressive effects of IL-1α. We therefore directly addressed the possibility that the observed suppressive effect of IL-1α on pre-B cell formation might be mediated by altered stromal cell function. Confluent S17 stromal cell layers were pretreated with 0.5 to 50 U/mL rIL-1α, washed into fresh medium, and then cocultured with fresh G10-depleted, 14.8-depleted bone marrow cells. In all coculture experiments, stromal cell layers and target cells were physically separated by placing target bone marrow cells into tissue culture diffusion chamber inserts fitted with a 0.45-µm pore cellulose membrane (Millipore). Pre-B cell generation was stimulated in these cultures when a confluent S17 layer was present, but failed in cultures lacking a stromal cell layer (Fig 2). Addition of 1 U/mL rIL-1α dramatically reduced the ability of S17 to potentiate pre-B cell generation from fresh bone marrow targets (Fig 2), and addition of 5 U rIL-1α/mL to S17 was sufficient to virtually eliminate their ability to sustain pre-B cell formation in culture in seven virtually identical experiments. The suppressive effect of IL-1 on S17 cells was not altered by addition of polymyxin B (10 µg/mL) to stromal cell cultures at the time of IL-1 treatment (data not shown).

IL-4 inhibits pre-B cell generation. Our previous experiments suggested that IL-1 stimulation of some bone marrow stromal cell lines resulted in elevated IL-4 levels in cultures, and we investigated whether IL-4 might play a role in the suppression of pre-B cell generation in S17 potentiated cultures. Sephadex G10 nonadherent, 14.8-depleted bone marrow cells were cultured in the presence of increasing concentrations of rIL-4 with or without addition of 5% of our standard preparation of S17 CM (Fig 3). When added alone, IL-4 did not potentiate the generation of pre-B cells at any concentration tested. However, when IL-4 was added to S17 CM potentiated cultures, pre-B cell generation was suppressed, with complete failure of pre-B cell generation in cultures containing 50 U/mL rIL-4. This level of suppression was consistent in two identical experiments.

IL-4 suppresses stromal cell potentiation of pre-B cell formation. To determine whether IL-4 also affected stromal cell function, confluent S17 cells were first cultured for 18 hours in the presence of 2.5 to 200 U/mL rIL-4, washed several times to remove potentiation, and cocultured with fresh G10-depleted, 14.8-depleted cells as previously described. As shown in Fig 4, addition of 25 to 200 U/mL rIL-4

![Graph](image-url)
abrogated pre-B cell formation in culture. This functional suppression was consistently found in six similar experiments and was completely inhibited when 25 U of rIL-4 was incubated with 12.5 µg MoAb 11B11 (monoclonal anti-murine IL-4) before factor addition to cultures (Fig 5). Addition of polymyxin B (10 µg/mL) to IL-4-treated cultures did not alter these results (Fig 4).

**Supernatants from IL-1 or IL-4-treated S17 cells suppress pre-B cell formation.** The finding that IL-1- or IL-4-treated stromal cells did not support pre-B cell formation in culture could result either from a failure to produce pre-B cell growth and differentiation factors or the release of inhibitory substances in our cultures. To address these possibilities, CM was collected from IL-1- or IL-4-treated S17 cells 24 hours after removal of lymphokine and added to untreated S17 CM potentiated cultures of G10 Sephadex-nonadherent, 14.8-depleted bone marrow cells. As shown in
Fig 5. Generation of pre-B cells from G10-depleted, 14.8-negative bone marrow cells in cultures containing no stromal cell layer (□); S17 cells (○); or S17 cells pretreated with 10 U/ml rIL-1α (△), 60 U/ml rIL-4 (△), or 50 U/ml rIL-4 and 25 μg/ml MoAb 11B11 (△). Data are presented as absolute numbers of cytoplasmic μ⁺, light chain⁻ cells/10⁶ cells cultured and are representative of four identical experiments.

Fig 6. Supernatants from either IL-1- or IL-4-treated stromal cells suppressed S17 potentiated pre-B cell formation. This suppressive effect of cytokine-treated S17 CM was dose-responsive, and maximal inhibition of pre-B cell formation was achieved with 50% CM from either IL-1- or IL-4-treated S17 cells in four identical experiments. Addition of 12.5 μg/ml 11B11 antibody to stromal cell cultures at the time of IL-4 addition (ie, during the preculture period) negated the effect of IL-4 (Fig 6); however, IL-4-induced suppression was not altered by the presence of 12.5 μg/ml 11B11 during the coculture phase of the experiment.

rIL-1 or rIL-4 do not appear to be trapped and released from S17 stromal cell layers. Several recent studies reported growth factor binding by extracellular matrix components and, for that reason, we questioned whether S17 cell layers were capable of binding and subsequently releasing IL-1 or IL-4 in our experimental protocol. Supernatants used in experiments that demonstrated release of suppressive factors were tested for IL-1 activity by bioassay on phytohemagglutinin (PHA)-stimulated thymocytes. rIL-1α co-stimulated thymocyte proliferation in PHA-stimulated cultures, with optimal activity at 2.5 U/ml (Fig 7). Stimulation of thymocyte proliferation was not detected in cultures containing serial dilutions of S17 CM or CM from S17 cells precultured with either 10 U/ml rIL-1 or 50 U/ml rIL-4.

As noted above, addition of 11B11 antibody to cultures containing IL-4-treated S17 cells did not alter the suppressive effect of IL-4 pretreatment (Fig 6), and this indicated that IL-4 was not released from treated stromal cell layers and responsible for the observed suppressive effects on pre-B cell formation.

IL-1-induced suppression of S17 function is mediated, in part, by IL-4. Previous experiments suggested that IL-1-induced stromal cells produce IL-4 or an IL-4-like molecule. Therefore, we addressed the possibility that suppression of pre-B cell formation in cultures containing rIL-1α-treated stromal cells was mediated by IL-4. As shown in Fig 6, the suppressive effect of IL-1 could be ameliorated if monoclonal 11B11 antibody was added to cultures potentiated with

Fig 7. Proliferation of C3He/J thymocytes cultured in the presence of 2 μg/ml PHA (○) and serial dilution of 10 U/ml rHu-IL-1α (△), S17 CM (△), or CM from S17 cells pretreated with either 10 U/ml rIL-1α (□) or 50 U/ml rIL-4 (□).
rIL-1α-treated S17 CM. This reversal of lymphopoietic suppression was partial, and equivalent reversal of the suppressive effect was also consistently observed when IL11 was added to rIL-1α-treated S17 stromal layers in coculture experiments (data not presented).

Although incubation of 12.5 μg/mL IL11 with rIL-4 efficiently abrogated its suppressive effects on S17 stromal layers (Fig 5), addition of IL11 to rIL-4-treated stromal cell layers after 24-hour pretreatment with rIL-4 did not alter the suppressive effect of this lymphokine.

**DISCUSSION**

The information that single-cloned stromal cell lines could support either lymphoid or myeloid development2,9,10 and that these regulatory potentials were expressed under different conditions in vitro, raises important questions about regulation of stromal cell function. A number of cytokines originally described as growth factors for mature lymphoid cells are now known to alter the progression of hematopoiesis in vitro6,17,27,34 and in vivo7,25,36 and it will be essential to delineate which of these factors act directly on hematopoietic progenitor cells and whether some of them influence cell development indirectly by regulating changes in stromal cell function. In this study, we examined the effects of rIL-1α and rIL-4 on the generation of pre-B cells in vitro. Neither of these cytokines directly potentiated the generation of pre-B cells in our studies. However, pre-B cells were generated in cultures containing either S17 stromal cells or CM from this stromal cell line. Addition of either rIL-1α or rIL-4 to S17 potentiated cultures efficiently suppressed pre-B cell generation. Pre-B cell formation was equally suppressed when S17 cells were pretreated with either rIL-1α or rIL-4 and subsequently cocultured with fresh bone marrow cells in the absence of detectable cytokine. These data suggest that suppression of pre-B cell development by either rIL-1α or rIL-4 may be due to both direct effects on progenitor cells and to changes in the overall pattern of growth factor production by bone marrow stromal elements after exposure to either interleukin.

Both IL-1 and IL-4 have been previously shown to function as hematopoietic regulatory molecules. Although IL-1 was first described as a product of activated macrophages, it is now known to be produced by a variety of cell types, including mature B cells.37 IL-1 production is inducible and its release results in a cascade of biologic sequelae, including fever, prostaglandin E2 production, and proliferation of fibroblasts, T lymphocytes, and B lymphocytes.37 Importantly, IL-1 synergizes with G-CSF,38 GM-CSF,29 and M-CSF,28,29 and results in increased numbers of myeloid colony-forming cells when administered in vitro37,29 or in vivo.27,35 In B lymphopoiesis, IL-1 acts as a cofactor in B-cell growth and differentiation,35 and potentiates cell surface Ig expression on maturing pre-B cells.25,34 Recent studies in our laboratory suggest that this latter effect of IL-1 may be indirect, resulting, at least in part, from increased production of IL-4 (or an IL-4-like molecule) by bone marrow stromal cells after IL-1α exposure.25

In this study, IL-1α proved to be a potent inhibitor of pre-B cell generation in short-term cultures of G10 passed bone marrow cells. This was true when rIL-1α was added to cultures of nonadherent bone marrow cells potentiated by S17 CM or when S17 cells were exposed to IL-1α, washed to remove the mediator, and then cocultured with fresh nonadherent bone marrow cells. This effect of IL-1α on S17 cells did not appear to result from IL-1 binding to either cells or the extracellular matrix because bone marrow target cells were physically separated from the stromal cell layer by containment in a culture well diffusion chamber, and IL-1 could not be detected in supernatants from IL-1α-treated S17 cells (Fig 6).

However, IL-1α treatment of S17 cells did result in release of detectable inhibitory factors, which were sufficient to suppress pre-B cell formation. IL-1α-treated bone marrow stromal cells are known to release increased levels of G-CSF,17 GM-CSF,13,37 and IL-4,29 and it is possible that one or a combination of these regulatory molecules may be responsible for actively suppressing pre-B cell generation. Dorshkind15 reported that the addition of 50 U/mL r-hu-IL-1α to murine long-term bone marrow cultures at the time they are switched from myeloid permissive (or Dexter) to lymphoid permissive (or Whitlock-Witte) culture conditions resulted in continued myeloid cell production and a failure of initiation of lymphopoiesis. Increased stromal cell CSF production was demonstrated under these conditions and GM-CSF, G-CSF, and M-CSF were all shown to be sufficient to prevent lymphoid outgrowth from lymphoid progenitors that survive in these cultures. Recent experiments in our laboratory also support the idea that GM-CSF, G-CSF, and M-CSF suppress pre-B cell formation in vitro, but our data suggest that in all of these cases, this is an indirect effect mediated, in part, by alterations of stromal cell function (Billips L, Petitte D, Landreth KS, unpublished results, 1989).

Because IL-4 has been previously shown to both suppress lymphoid cell growth in long-term bone marrow cultures13 and because the suppressive effect of CM from IL-1α-treated S17 cells in the experiments presented here was ameliorated when anti-IL-4 antibody (11B11) was added to cultures (Fig 6), we questioned whether this lymphokine might be involved in suppression of pre-B cell generation. Addition of 25 to 50 U/mL rIL-4 abrogated pre-B cell formation in short-term bone marrow cultures containing either supernatants from untreated S17 bone marrow stromal cells or a confluent underlayer of stromal cells. Pre-B cell formation was equally suppressed when S17 stromal cells were pretreated with rIL-4, and this alteration of stromal cell function persisted for at least 3 days after treatment (Fig 5). Although several hematopoietic factors have been shown to bind to extracellular matrix components,26,27 the possibility of capture and subsequent release of IL-4 in these cultures was ruled out by the addition of 11B11 antibody along with fresh bone marrow cells in the second phase of these experiments (Fig 6).

Although IL-4 was originally described as a B-cell stimulatory factor derived from T12 cells,40,41 our data25 and unpublished studies (Rennick D, personal communication, 1988) suggest that IL-4 may also be inducibly produced by bone marrow stromal cells. IL-4 plays a role in both lymphoid and myeloid cell development in addition to its well-described role as a cofactor in the proliferation of B and T lymphocytes.42 Several studies have shown that IL-4 does not stimulate
myeloid colony formation directly, but synergizes with GM-CSF, G-CSF, erythropoietin, and IL-3 to potentiate increased myeloid proliferation in vitro. IL-4 has multiple effects on myeloid cells and has demonstrated suppressive effects on proliferation of early IL-3–dependent myeloid progenitor cells in vitro.

IL-4 has multiple effects on developing B-lineage cells as well. IL-4 added to established lymphoid long-term bone marrow cultures suppressed the appearance of lymphoid cells and the growth of a stromal-cell–dependent pre-B cell line. This effect was achieved with 200 U/mL r-murine-IL-4 and was considered to be at the level of responding B-lineage cells. Addition of 300 U/mL to long-term bone marrow cultures also resulted in failure of lymphoid long-term bone marrow culture cells to progress to Ly-5 or Ig expression. Our data are in agreement with those previously reported in that IL-4 suppressed pre-B cell generation in our stromal-cell–dependent cultures; however, maximal suppression of pre-B cell formation in our experiments required only 25 to 50 U/mL rIL-4. Our study also differs in that it clearly identifies the fibroblastic stromal cell as a regulatory element that has the capacity to respond to IL-4 and affect B-cell production in the marrow. Bone marrow stromal cell lines have not been previously reported to functionally respond to IL-4; however, they are known to possess high affinity receptors for this cytokine and functional responses to IL-4 exposure were not unexpected.

These studies suggest that both IL-1α and IL-4 have direct effects on bone marrow stromal cells. Exposure of the bone marrow stromal cell line S17 to either lymphokine altered its hematopoietic support capacity, even after extensive washing to remove added lymphokine. However, the possibility of substantial cell or cytokine interaction in the target cell population is not ruled out. Although G-10 depletion appears to efficiently remove adherent stromal elements from the bone marrow cell suspensions used for these studies, it is possible that functional hematopoietic regulatory cells (fibroblastic, myeloid, or lymphoid) remain and respond to changes in soluble mediator release from the stromal cell line used. This possibility has led us to look for regulatory cascades that may be initiated by altered stromal cell function as described here. Initial observations suggest that, at least, T cells will be involved in this regulatory circuit (unpublished observations, 1989).

Induction of suppressive factors that affect myeloid cell growth have been observed when heterogeneous stromal cell layers were treated with high levels of IL-4 (300 U/mL) but not IL-1, and this effect was attributed to macrophages in the stromal layer used. Because our studies used a bone marrow stromal cell line as the target for lymphokine treatment, the possibility that this effect was due to contaminating macrophages was ruled out. Suppression of B-cell formation by TGF-β and PGE₂ has also been described, and the question of whether either of these molecules is inducibly produced by S17 cells is currently under study.

Several soluble stromal-cell–derived molecules are now known that play a role in the proliferation and differentiation of B-lineage cells, and exposure to IL-1 or IL-4 may also alter specific messenger RNA (mRNA) transcription for these lymphopoietic factors. We described a stromal-derived lymphopoietic factor (SDLF) that potentiates the differentiation of pre-B cells from Ig⁺ progenitor cells, and this SDLF has been cloned by direct expression vector cloning (Stenberg R, Dorshkind D, Landreth KS, unpublished results, 1989). Namen et al described a lymphopoietic factor (IL-7) that supports the proliferation of lymphoid cells from long-term cultures, but lacks detectable differentiation activity. This latter factor has been cloned and sequenced. The availability of cDNAs for the aforementioned lymphopoietic factors will make it possible to directly address whether changes in mRNA levels for lymphopoietic factors in stromal cells also occurs after IL-1α or IL-4 treatment.

Sustained and efficient production of hematopoietic and lymphopoietic cells depends on a hierarchy of essential regulatory events. Taken together, results presented here suggest that some bone marrow stromal cell populations provide regulatory signals for both myeloid and lymphoid differentiation, that these regulatory potentials are inversely related, and that expression of stromal cell function is regulated, in part, by local levels of IL-1 or IL-4. Several studies have suggested a stimulatory role for macrophage products in B-cell formation. Our data also suggest that macrophages may play a key role in regulating stromal cell function. That is, increased levels of IL-1 secretion by macrophages in the marrow microenvironment would result in local shifts to increased myeloid and B-lymphoid end cell production and transient decreases in the generation of new pre-B cells from early B-lymphoid progenitors. Accelerated maturation of B cells and mature myeloid elements at the expense of early progenitors for these lineages may have a distinct selective advantage when the immune system is stressed by increased levels of antigenic challenge.

Less is known about the role of T cells in primary B-cell formation. Although we reported that IL-4 appears to be inducibly produced by some bone marrow stromal cell lines, it also seems likely that T cells resident in the marrow would contribute to local IL-4 concentrations as they participate in antibody formation in that tissue. Certainly, these data could be interpreted to suggest a role for soluble mediators released during antigenic stimulation in the regulation of stromal cell function and, ultimately, in the differential production of hematopoietic end cells, including B lymphocytes.

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