Interleukin-4 Inhibits the Expression of Kit and Tryptase During Stem Cell Factor-Dependent Development of Human Mast Cells From Fetal Liver Cells

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Although interleukin-4 (IL-4) in mice is known to augment the proliferation of mast cells and to modulate the expression of certain mast cell protease transcripts, its effect on human mast cells is less well understood. The current study examined the effects of recombinant human IL-4 (rhu-4) on stem cell factor (SCF)-dependent fetal liver-derived human mast cells in liquid culture. In no case did rhu-4 augment proliferation of mast cells. rhu-4 selectively inhibited certain aspects of the development of mast cells in cultures of fetal liver cells with rhuSCF. These include lower numbers of mast cells and basophils, show ultrastructural similarities for progenitor cells exposed to SCF.

STEM CELL FACTOR (SCF), the ligand for Kit (product of the c-kit proto-oncogene), is a major growth and differentiation factor for human mast cells derived from fetal liver, cord blood mononuclear cell progenitors, and bone marrow. Mast cells and basophils have been shown to be derived from CD34+ cells in human bone marrow. SCF is produced in part by stromal cells and fibroblasts, either as a membrane-bound form or as a soluble form, shown in rodents to be determined by alternative splicing. Both forms of SCF induce the differentiation of mast cells. SCF also acts to enhance IgE-mediated degranulation of human and rodent mast cells, to enhance survival of rodent mast cells, and as a chemooattractant for rodent mast cells. SCF is a survival factor for rodent mast cells, analogous to its effect on erythroid progenitors and primordial germ cells to suppress apoptosis. Membrane-bound SCF also may act as an adhesion molecule for mast cells, which express Kit throughout their development. In contrast, lymphocytes and other granulocytes express Kit predominantly during their early progenitor stages, but not as they mature further. In mouse bone marrow-derived mast cells, SCF induces the expression of MMCP-4 and MMCP-6 transcripts and shows procedures of the biogenic amine, serotonin.

In humans, SCF enhances the effects of specific colony-stimulating factors, such as interleukin-3 (IL-3), granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage-CSF (GM-CSF), on hematopoietic cells in semisolid media, but exhibits negligible activity by itself. In contrast, SCF alone in liquid cultures selectively promotes the growth and differentiation of mast cells from hematopoietic progenitor cells; although a few adherent stromal cells persist, leukocytes do not mature and survive under these conditions. Thus, the mast cell lineage may be a default pathway for progenitor cells exposed to SCF.

Fetal liver-derived mast cells are toluidine blue-positive, contain histamine and tryptase, show ultrastructural similarities to tissue mast cells, and express high levels of Kit on their surface. However, they express little, if any, high-affinity receptor for IgE (FceRI) and appear morphologically immature by ultrastructure, and only in a small portion of these cells is chymase weakly expressed. Additional growth factors or conditions must be involved in mast cell development. In contrast to such mast cells developed from fetal liver cells, those developed after 10 weeks of culture of cord blood mononuclear cells with recombinant human SCF (rhSCF) express 1.5 to 4.2 x 10^6 FceRI surface molecules per cell determined by direct binding using 125I-labeled human IgE. Furthermore, these cord blood-derived mast cells sensitized with IgE release histamine, leukotriene C4, and prostaglandin D2 on challenge with anti-IgE antibody.

Putative factors that inhibit mast cell differentiation also need to be considered. For example, GM-CSF, in vitro, inhibits the differentiation of murine mast cells. The hematopoietic environment may be relatively nonpermissive for complete mast cell maturation to occur because of cytokines that inhibit mast cell differentiation.

IL-3 is a major growth factor for rodent, but not for human, mast cells. Instead, IL-3 promotes the differentiation of human basophils and other hematopoietic progenitors. IL-4, a minor growth factor for rodent mast cells, also has pleiotropic effects on several hematopoietic cell types. IL-4 is produced mainly by T helper 2 lymphocytes on activation, but also may be produced by human mast cells and basophils. In mouse SCF-dependent bone marrow-derived mast cells, IL-4 attenuates the expression of mouse mast cell protease (MMCP)-1 and MMCP-2 mRNA induced by both IL-10 and IL-9, and of MMCP-4 mRNA induced by SCF alone. Human mast cells likely have receptors for IL-4, because, on exposure to IL-4, CD54 expression is increased on human lung mast cells, and expression of Kit is downregulated on a human mast cell leukemia line, HMC-1. The current study examines the effects of rhuIL-4 on the development of mast cells from fetal liver-derived progenitor cells and on mast cells already developed under the influence of rhuSCF. Data are presented...
to show that rhuIL-4 inhibits the production of tryptase and histamine in fetal liver cells cultured with SCF, an effect associated with suppression of the production of Kit on the cell surface.

MATERIALS AND METHODS

Cell cultures. Fetal livers were obtained at the time of therapeutic abortions. The protocol was reviewed and approved by the Human Studies Committee at Virginia Commonwealth University. Fetal liver cultures were dispersed and separated on Ficoll-Paque (Pharmacia AB, Uppsala, Sweden). Low-density cells were collected from the interphase, washed three times with phosphate-buffered saline (PBS), and resuspended in RPMI-1640 media supplemented with 10% fetal calf serum, L-glutamine (2 mmol/L), HEPES (10 mmol/L), nonessential amino acids (0.1 mmol/L), 2-mercaptoethanol (50 μmol/L), penicillin (100 U/mL), and streptomycin (50 μg/mL) (all purchased from Sigma Chemicals, St Louis, MO). Cells were plated at 1 × 10^6 cells in 1 mL in a 24-well culture plate (Costar, Cambridge, MA), in the presence of rhuSCF (Amgen, Thousand Oaks, CA) at 50 ng/mL, of rhu-L4 (DNAX Research Institute, Palo Alto, CA) at 50 μU/mL, or of a combination of the two cytokines. The concentration of rhu-L4 was chosen according to previous reports on the effects of IL-4 on human mast cells.\(^6,7\) Half of the culture medium was replaced weekly. Cell growth was determined by counting of viable cells with the use of a hemacytometer (Reichert Scientific Instruments, Buffalo, NY) and trypan blue exclusion.

Cells cultured in the presence of rhuSCF for 3 to 10 weeks were washed and replated in the presence of rhuSCF, rhuIL-4, rhuSCF + rhuIL-4, or media control (cytokine concentrations as above). These cultures were set up at 5 × 10^3 cells in 500 μL in a 48-well culture plate (Costar). Proliferation assays were performed by plating these cells in microwell: wells at 50 × 10^3 cells per well. \(^7\)Th-Thymidine (1 μCi; Du Pont, Boston, MA) was added to each well and plates were incubated for an additional 6 hours. Cells were harvested onto glass fiber strips and the incorporation of \(^3\)H-thymidine was measured in a Beckman scintillation counter (Fullerton, CA).

Immunohistochemistry and flow cytometry. Cultured cells were dispersed from plastic surfaces and recovered by vigorous pipetting, counted, and subjected to cytocentrifugation (Shandon, Pittsburgh, PA) or flow cytometry (FACScan; Becton Dickinson, Mountain View, CA). Cells containing metachromatic granules were identified on cytospins by staining with toluidine blue, 0.5% in 0.5 mom HC1 (pH <1). Immunoperoxidase staining for tryptase was performed using the YB5.B8 monoclonal antibody as described previously.\(^8,9\) Analyses of Kit expression were performed by flow cytometry using the YB5.B8 monoclonal antibody,\(^8,9\) as described elsewhere.\(^1\) Briefly, cells to be labeled were preincubated in RPMI containing 10% human serum for 1 hour to block nonspecific staining, and then washed three times with PBS containing 1% bovine serum albumin and 0.1% sodium azide (washing buffer) before the YB5.B8 monoclonal antibody in ascites was added at 1:500 dilution in wash buffer. After 30 minutes of incubation on ice, the cells were washed and fluorescein isothiocyanate-labeled F(ab')\(^2\) rabbit-antimouse Ig (DAKO Corp, Carpinteria, CA) was added. To assess the expression of surface Kit, cells were stained with YB5.B8 as described previously,\(^1\) and the percentage of total cells staining positive was measured by flow cytometry. Antibody against a basophil surface antigen (Bsp-1), used for flow cytometry as previously described,\(^9\) was a gift from M.P. Bodger (Christchurch Hospital, Christchurch, New Zealand).

Mediator assays. Analysis of histamine and tryptase content was performed in the cell-free culture supernatant and in the cell pellet. Frozen cell pellets were thawed and resuspended in 0.01 mol/L 2-(N-morpholino) ethane sulfonic acid buffer containing 2 mol/L NaCl at pH 6.0. Cell extracts were prepared by sonication of the cell pellets with a Model W-225R sonicator and microtip (Heat Systems-Ultrasonics, Inc, Plainview, NY). Supernatants from the cell extracts containing solubilized material were obtained by centrifugation of the sonicate at 12,000 rpm for 45 minutes at 4°C in a microcentrifuge. Histamine determinations were performed by radioimmunoassay (AMAC Inc, Westbrook, ME). Tryptase assays were performed by a specific sandwich enzyme-linked immunosorbent assay (ELISA) described previously.\(^10\)

Statistical analysis. An analysis of variance (ANOVA) was used. Values presented are the means ± SEM, unless otherwise indicated.

RESULTS

Time course of the effect of rhuIL-4 on the development of SCF-dependent fetal liver mast cells. Culturing human fetal liver cells in the presence of rhuSCF induces the development of tryptase-positive, Kit-positive cells.\(^1\) To determine whether other growth factors affect rhuSCF-mediated differentiation of fetal liver-derived mast cells, fetal liver cells were cultured with rhuSCF (50 ng/mL), rhuIL-4 (50 U/mL), or a combination of rhuSCF and rhuIL-4. Cells were analyzed after 2, 3, or 4 weeks of culture. The decline in total cell numbers between 3 and 4 weeks for cultures with rhuSCF and rhuSCF plus rhuIL-4 (Fig 1A) presumably reflects the failure of cells from most hematopoietic lineages to mature and survive under these conditions. Furthermore, the addition of rhuIL-4 to rhuSCF-supplemented cultures resulted in significantly lower total cell counts at the 3- and 4-week time points compared with cultures with rhuSCF alone. Cultures supplemented with rhuIL-4 alone showed a steady decline in cell numbers. As expected, rhuSCF induced the development of cells that expressed tryptase and were metachromic. In cultures supplemented with both rhuSCF and rhuIL-4 compared with rhuSCF alone, a significantly lower percentage of tryptase-positive cells were found at 4 weeks (Fig 1B), although staining intensity for tryptase was notably lower in cultures with rhuIL-4. rhuIL-4 alone did not induce the development of any tryptase-positive cells. In contrast, the addition of rhuIL-4 to rhuSCF-supplemented cultures did not result in a significant decrease in the percentage of metachromatic cells at any time point as assessed by staining with toluidine blue, but no metachromatic cells were seen in cultures supplemented with rhuIL-4 alone (Fig 1C).

Visually, the intensity of metachromasia was stronger in cultures supplemented with rhuSCF plus rhuIL-4 (Fig 2A) as well as increased amounts of histamine (Fig 2D) and, to a much lesser degree, tryptase (Fig 2C) in the culture medium. The levels of tryptase and histamine in cell extracts were lower in cultures supplemented with both rhuSCF and rhuIL-4 compared with those supplemented with rhuSCF alone, and were nearly undetectable in cultures supplemented with rhuIL-4 alone. These
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![Graph A](https://example.com/graph_a.png)

![Graph B](https://example.com/graph_b.png)

![Graph C](https://example.com/graph_c.png)

Fig 1. Differential effects on fetal liver cells cultured in the presence of rhuSCF (●), rhuIL-4 (▲), or rhuSCF + rhuIL-4 (▼). (A) Number of fetal liver cells; (B) percentage of cells staining positive for tryptase; and (C) percentage of cells stained with toluidine blue. Means and standard errors are depicted from four separate experiments.

results correspond to the immunohistochemical data presented in Fig 1. Amounts of tryptase in the media of cultures with rhuSCF alone and with rhuSCF and rhuIL-4 by 4 weeks were substantially lower than levels from the corresponding cells. In the media of cells cultured with rhuIL-4 alone, tryptase was not detectable, and histamine was present at very low levels.

Flow cytometric analysis. As described before, fetal liver cells cultured in the presence of rhuSCF increase in size (forward light scatter), and express greater amounts of Kit as they develop into mast cells. To examine the effect of rhuSCF and rhuIL-4 on these parameters, fetal liver cells exposed to rhuSCF, rhuIL-4, or rhuSCF together with rhuIL-4 were analyzed by flow cytometry. At 3 weeks, the size of fetal liver cells treated with rhuSCF was substantially larger than those treated with rhuIL-4 or with both cytokines together, as shown in Fig 3A. The percentage of cells expressing Kit, the receptor for SCF, was assessed by flow cytometry with the monoclonal antibody YB5.B8 at 0 to 4 weeks. In the presence of rhuSCF, 50% of the cells were Kit-positive by 4 weeks (Fig 3B), and these cells exhibited higher forward light scatter values than Kit-negative cells, as described previously. In the presence of rhuIL-4, or of rhuIL-4 and rhuSCF, substantially lower percentages of the cells expressed Kit. Furthermore, the mean intensity of fluorescence (MIF) (Fig 3C) and forward light scatter values (data not shown) only increased for cells grown with rhuSCF alone that were Kit-positive. At 4 weeks, cells cultured with rhuSCF alone and those cultured with rhuIL-4 and rhuSCF were negative for Bsp-1 by flow cytometry, making it unlikely that basophils were present. Thus, Kit expression on mast cells stimulated by rhuSCF is largely suppressed by the additional presence of rhuIL-4.

Effect of rhuIL-4 on SCF-dependent fetal liver mast cells. The effects of rhuIL-4 on fetal liver cells that had differentiated into mast cells in the presence of rhuSCF in vitro were examined next. Fetal liver cells were cultured with rhuSCF for 5 to 6 weeks, by which time more than 80% of the cells were tryptase-positive. These cells were washed and then cultured in the presence of rhuSCF, rhuIL-4, or rhuSCF together with rhuIL-4 or in media without exogenous cytokines. DNA synthesis was assessed by thymidine incorporation as a guide to cell proliferation. Cells cultured in the presence of rhuSCF as well as those cultured with a combination rhuSCF and rhuIL-4 showed a markedly higher uptake of \(^{3}H\)-thymidine than those cultured with media alone and with rhuIL-4 alone at 3- and 6-day time points (Fig 4A). Cell numbers were evaluated over a 10-day period (Fig 4B). For cells cultured with rhuSCF together with rhuIL-4 and those with rhuSCF alone, cell numbers did not differ from one another or from the starting numbers over a 10-day culture period. By contrast, for cells cultured with rhuIL-4 alone, cell numbers decreased to approximately 30% of the starting number by 10 days, suggesting that rhuSCF is a survival factor for fetal liver-derived human mast cells.

The decreased thymidine incorporation and cell growth of fetal liver-derived mast cells when rhuSCF was withdrawn was paralleled by similar effects on the expression of tryptase and Kit. The percentages of tryptase-positive cells when cultures were continued with rhuSCF alone and with rhuSCF and rhuIL-4 for 10 days were not substantially different from one another or with the starting population of cells (Fig 4C). However, when rhuIL-4 was substituted for rhuSCF, the percentage of tryptase-positive cells decreased approximately twofold by 10 days, and the total number of tryptase-positive cells decreased about sixfold.

When fetal liver-derived mast cells were exposed to varying combinations of cytokines, the cellular content of both tryptase and histamine decreased markedly in cultures sup-
plemented with rhuIL-4 alone. In contrast, in cultures treated with rhuSCF, levels were not significantly different in the presence and absence of rhuIL-4 (Fig 5A and B), although tryptase levels tended to be somewhat lower when the two cytokines were combined. In the media, small amounts of tryptase were detected (Fig 5C), whereas histamine levels substantially increased over time in all cultures, particularly when rhuIL-4 alone was present (Fig 5D), perhaps reflecting cell death and release of granule histamine.

To examine the effect of cytokines on ongoing Kit expression, fetal liver-derived mast cells (80% purity) exposed to rhuSCF, rhuSCF and rhuIL-4, rhuIL-4, or media alone for 9 days were examined by flow cytometry with the YB5.B8 antibody (Fig 6). The MIF decreased to background in the absence of exogenous rhuSCF. Addition of rhuIL-4 to rhuSCF shifted the MIF of Kit-positive cells to lower values, but did not appreciably alter the percentage of cells remaining positive for Kit. Thus, maintenance of mast cells under the culture conditions used was dependent on the continued presence of rhuSCF. Addition of rhuIL-4 to rhuSCF resulted only in modest decrements in Kit expression on cell surfaces after 9 days.

### DISCUSSION

SCF was the first growth factor identified for human mast cells. Other factors are likely to be involved both as inhibitors and potentiators of various aspects of mast cell differentiation. For example, an inhibitor of mast cell maturation at hematopoietic sites could be one explanation for the relative lack of mast cell maturation that normally occurs at such sites. On the other hand, factors necessary for the full expression of high-affinity IgE receptors on the cell surface and of chymase in secretory granules, and for the selective differentiation of MCs and MCt types of mast cells remain to be clarified. In rodent mast cells, SCF, IL-3, IL-4, IL-9, and IL-10 each exert characteristic effects on the expression of...
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The current study examined the effects of rhuIL-4 on the development of human mast cells from fetal liver cells, and suggests that IL-4 does not augment proliferation of mast cells as judged by thymidine incorporation or cell number, but instead is a potential inhibitor of mast cell differentiation based on suppression of Kit production. Consequently, production of tryptase and histamine is suppressed. These results are in contrast to those in mice, in which IL-4 enhances the proliferation of mast cells, but may be analogous to the suppression by IL-4 of MMCP-1 and MMCP-2 transcripts in SCF-dependent bone marrow-derived mast cells exposed to IL-10 or IL-9. Whether Kit expression on the surface of these cells is suppressed by IL-4 was not examined. Results of the current study are consistent with the hypothesis that IL-4 inhibits the development of mast cells from fetal liver cells.

Fig 3. Flow cytometric analysis of fetal liver cells grown in the presence of rhuSCF, rhuIL-4, or rhuSCF + rhuIL-4. (A) Forward light scatter (FSC) of cells cultured for 3 weeks. (B) Percentage of cells expressing Kit. Cells cultured in the presence of rhuSCF (●), rhuIL-4 (△), and rhuSCF + rhuIL-4 (▼). (C) Mean intensity of fluorescence after staining with YB5.B6 monoclonal antibody directed against Kit. Background MIF with an irrelevant monoclonal antibody = 11. Means and standard errors are depicted from four separate experiments.

Fig 4. Effect of rhuIL-4 on rhuSCF-dependent fetal liver-derived mast cells. (A) Incorporation of 3H-thymidine into fetal liver mast cells. Cells (50 x 10^5) were incubated with rhuSCF (●), rhuIL-4 and rhuSCF (△), rhuIL-4 (▼), or media alone (□). Representative data from one of two experiments is shown. (B) Effect on cell number. Cultures containing 5 x 10^5 cells were cultured for 10 days in the presence of rhuSCF (●), rhuIL-4 + rhuSCF (△), rhuIL-4 (▼), and media alone (□). (C) Percentages of cells staining positive for tryptase. Mean and range values (B and C) are depicted for two separate experiments, one in which fetal liver mast cells of 95% purity taken at day 38 were used and another in which fetal liver mast cells of 82% purity taken at day 36 were used.
tent with a previous study of human cord blood mononuclear cells, in which IL-4 did not enhance the effects of rhuSCF on mast cell development as assessed by metachromatic staining.

Inhibition by rhuIL-4 of mast cell development from fetal liver cells exposed to rhuSCF was seen as lower numbers and percentages of cells expressing tryptase and Kit, lower amounts of tryptase and histamine, smaller cells, and markedly lower amounts of surface Kit in cultures with rhuIL-4 and rhuSCF compared with rhuSCF alone (Figs 1 through 3). The downregulation of Kit is probably a critical factor, because stimulation through Kit affects both mast cell survival and production of tryptase and histamine. Whether IL-4 exerts this affect directly on mast cell progenitors, or indirectly on accessory cells present in these complex cultures, is not completely resolved by this study. Variable numbers of adherent stromal cells as well as progenitors of various cell lineages are present among dispersed fetal liver cells. IL-4 is known to affect a variety of cell types, including downregulation of extracellular matrix production by human fibroblasts and induction of eosinophil-dependent killing of tumor cells in mice. However, a direct affect on mast cells also is likely because a similar downregulatory effect of IL-4 (50 U/mL) on the expression of Kit has been described for a human mast cell-like cell line, HMC-1. Compared with tryptase and Kit, less dramatic effects were observed on the percentage of toluidine blue-positive cells in the current study. However, total cell numbers increased with the combination of both cytokines despite the lack of tryptase-positive cells. These results suggest either that the differentiation and growth of cells from other lineages were stimulated by adding rhuIL-4 to rhuSCF, or that mast cells could not fully develop. By light microscopy, comparing the metachromatic cells that developed with both cytokines to...
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those that developed with rhuSCF alone showed no difference in nuclear morphology, and no surface expression of the basophil-specific antigen, Bsp-1, in either case (data not shown). By flow cytometry, cells grown in the presence of rhuIL-4 and rhuSCF remained small. These observations lead us to conclude that the Kit-negative, tryptase-deficient cells that develop when rhuIL-4 is added to rhuSCF are mast cells that have certain developmental features of their phenotype suppressed and show decreased survival.

Compared with developing mast cells, the effects of rhuIL-4 on mast cells already developed from fetal liver cells were not as pronounced. By 6 days with rhuIL-4 and rhuSCF compared with rhuSCF alone, there were no appreciable differences in thymidine incorporation, and by 10 days no differences in cell number and in the percentage of tryptase-positive mast cells were appreciated. By contrast, replacement of rhuSCF with just media or with rhuIL-4 alone produced a dramatic decrease in both cell number and the percentage of tryptase-positive, Kit-positive mast cells, indicating that SCF is an important factor for mast cell survival as well as development. That SCF protects mast cells from apoptosis was suggested previously in a study on the effects of rhuSCF in monkeys that showed a rapid regression of mast cells that have certain developmental features of their phenotype suppressed and show decreased survival.

In humans, IL-4 is produced by T cells and also by mature mast cells, mature basophils, and bone marrow-derived mast cell/basophil progenitors expressing high-affinity receptors for IgE. The role of the IL-4 produced by mast cells or basophils is not known, but the current findings suggest a possible autocrine or paracrine inhibitory role for IL-4 in the differentiation of mast cells. In conclusion, rhuIL-4 inhibits certain aspects of the differentiation of human mast cells from fetal liver progenitors in vitro, and may play a similar role in vivo to regulate mast cell growth and differentiation.

REFERENCES


6. Flanagan JG, Chan DC, Loder P: Transmembrane form of the Kit ligand growth factor is determined by alternative splicing and is missing in the Sld mutant. Cell 64:1025, 1991


12. Pesce M, Farrace MG, Piacentini M, Dolci S, De Felici M:
Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death (apoptosis). Development 118:1089, 1993


34. Ekland KK, Ghildyal N, Asten CF, Stevens RL: Induction by IL-9 and suppression by IL-3 and IL-4 of the levels of chromosome 14-derived transcripts that encode late-expressed mouse mast cell proteases. J Immunol 151:4266, 1993


43. Ghildyal N, McNiel HP, Stechschulte S, Asten CF, Sil-


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G Nilsson, U Miettinen, T Ishizaka, LK Ashman, AM Irani and LB Schwartz