

IL-6 attenuates apoptosis, while neither IL-6 nor IL-10 affect the numbers or protease phenotype of fetal liver-derived human mast cells

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Summary

Background The combination of recombinant human stem cell factor (rhSCF), rh interleukin (IL)-6 and rhIL-10 was reported to be optimal for mast cell development from cord blood progenitors and to induce chymase expression in all such mast cells earlier in their development than tryptase.

Objective The effects of rhIL-6 and rhIL-10 in various combinations on the rhSCF-dependent development of human mast cells from fetal liver progenitors were examined in serum-free media.

Methods Dispersed fetal liver cells were cultured in serum-free AIM-V medium with rhSCF alone, or with combinations of rhIL-6 and rhIL-10. Tryptase and chymase expression, surface Kit expression, metachromasia with toluidine blue and apoptosis were measured.

Results Neither rhIL-6 nor rhIL-10 nor the two interleukins together, when included from day 0 of culture, affected the number or protease phenotype of mast cells at 1 or 3 weeks. Expression of tryptase paralleled the appearance of metachromasia and surface Kit, both of which preceded chymase expression, regardless whether a rabbit polyclonal or mouse monoclonal anti-chymase antibody preparation was used. On the other hand, rhIL-6 markedly attenuated baseline levels of apoptosis in the presence of rhSCF as well as apoptosis occurring after withdrawal of rhSCF, whereas rhIL-10 had no effect.

Conclusion RhIL-6 protected fetal liver-derived mast cells from apoptosis, particularly after withdrawal of rhSCF, but neither rhIL-6 nor rhIL-10 nor the combination of these interleukins affected the numbers or protease phenotype of these mast cells.

Keywords: mast cells, interleukin-10, stem cell factor, tryptase, interleukin-6, apoptosis, chymase

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Introduction

Mast cells play an important role as a primary effector cell in immediate-type hypersensitivity reactions. In humans, two types of mast cells have been identified based on their

protease composition [1–4]. MC_{TC} cells contain tryptase, chymase, mast cell carboxypeptidase and cathepsin G-like protease in their secretory granules, and are the predominant type of mast cell in normal skin and intestinal submucosa. MC_T cells also contain tryptase in their granules, but lack the other proteases present in MC_{TC} cells, and are the predominant type of mast cell in normal alveolar wall and small intestinal mucosa.

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Table 1. The percentages of tryptase- and chymase-positive cells

Culture Time (weeks)	Antibody	Growth Condition			
		rhSCF	rhSCF rhIL-6	rhSCF rhIL-10	rhSCF rhIL-6 rhIL-10
1	mAb anti-tryptase	45 ± 15	44 ± 9	41 ± 14	44 ± 13
	mAb anti-chymase	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	pAb anti-chymase	1 ± 2	1 ± 2	1 ± 1	2 ± 2
3	mAb anti-tryptase	95 ± 1	94 ± 1	95 ± 1	94 ± 1
	mAb anti-chymase	14 ± 8	13 ± 8	14 ± 9	12 ± 7
	pAb anti-chymase	31 ± 14	34 ± 16	36 ± 15	38 ± 14

Each value (mean ± SD) was calculated after examining 4 fields (at least 200 cells per field) in each of 3 different experiments.

Stem cell factor (SCF), which is constitutively expressed by fibroblasts and other cell types, is the ligand for Kit [5–8], and the only growth factor that by itself supports the growth and differentiation *in vitro* of human mast cells from haematopoietic precursors in bone marrow and peripheral blood placed into suspension cultures [9], cord blood [10,11] and fetal liver [12]. The MC_T phenotype appears to predominate when protease expression is examined in such *in vitro*-derived mast cells by immunocytochemistry. For cord blood-derived mast cells obtained with recombinant human (rh)SCF and rh interleukin (IL)-6, rhIL-4 enhanced the percentages of chymase-positive mast cells [13]. RhIL-4 also increased the percentage of MC_{TC}-like mast cells during the development of mast cells from fetal liver progenitors [14]. Another study reported that the combination of IL-10, IL-6 and SCF led to the development of human mast cells from cord blood that expressed chymase in all mast cells [15]. Chymase expression, detected immunocytochemically with rabbit anti-chymase polyclonal antibody (pAb), occurred earlier in mast cell development than that of tryptase.

IL-6 has diverse effects on the development of human mast cells *in vitro*. RhIL-6 has been reported to both enhance [11] and attenuate [16] mast cell numbers obtained from cultures of cord blood progenitors, and blocks rhIL-4-mediated apoptosis of cord blood-derived mast cells developed with rhSCF alone [17]. Recombinant mouse (rm)IL-10, in rodent systems, stimulates mast cell growth when added to other mast cell growth factors, including rmIL-3, rmSCF and rmIL-4 [18–20]. RmIL-10 also increases cellular levels of the β -chymases, mouse mast cell protease (mMCP)-1 and mMCP-2, principally by stabilizing the corresponding mRNA molecules [21–24]. With human cells rhIL-10 did not promote the survival of cord blood-derived mast cells [25].

The current study examined the effects of rhSCF, rhIL-6 and rhIL-10 on the development and protease phenotype of mast cells from human fetal liver. Both mouse monoclonal antibody (mAb) and rabbit pAb against chymase were used. Because fetal calf serum is a potential endogenous source of haematopoietic factors that may affect growth, serum-free media was used. RhIL-6 and rhIL-10 did not affect the protease phenotype of fetal liver-derived mast cells, which consisted mostly of MC_T cells, and had minimal impact on mast cell development. However, rhIL-6 markedly reduced apoptosis of fetal liver-derived human mast cells when SCF was withdrawn.

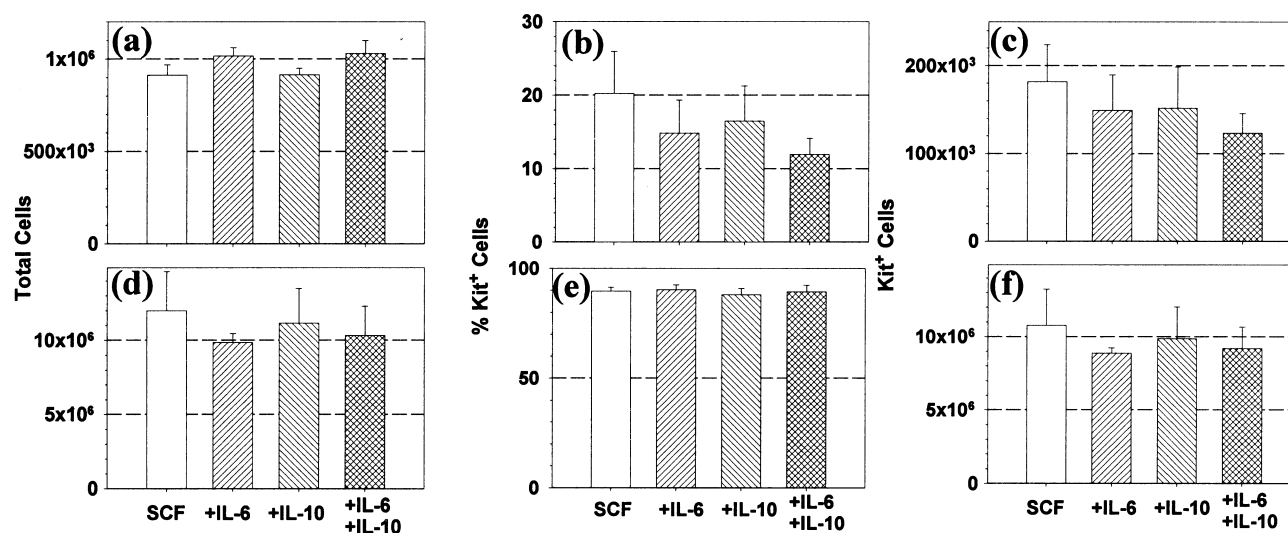
Materials and methods

Antibodies and reagents

Mouse IgG anti-Kit mAb YB5.B8 was a gift from Dr Leonie K. Ashman (Institute of Medical and Veterinary Science, Adelaide, Australia). Anti-Fc ϵ RI α mAb 22E7 [26] and protein A-purified rabbit IgG anti-chymase pAb [1,15] were used as described. Biotin-conjugated anti-chymase mAb B7 (biotin-B7) and anti-tryptase mAb G3 were obtained and prepared as described [2]. FITC-conjugated Annexin-V was purchased from R & D Systems (Minneapolis, MN, USA). RhSCF was a gift from Amgen (Thousand Oaks, CA, USA) and rhIL-6 was from Kirin Brewery Co. (Gemini Science, La Jolla, CA, USA). RhIL-10 was purchased from Endogen (Minneapolis, MN, USA).

Mast cell culture in serum-deprived condition

The serum-free human mast cell culture procedure was used [27]. Briefly, human fetal livers, 16–21 weeks of



Culture Condition

Fig. 1. Development of fetal liver-derived mast cells with different cytokine mixtures. The total numbers of viable cells (a and d); percentages of Kit-positive cells determined by flow cytometry (b and e); and numbers of Kit-positive mast cells (c and f) are shown after 1 week (a, b and c) and 3 weeks (d, e and f) of culture of fetal liver-derived mast cells in AIM-V serum-free medium containing 50 ng/mL rhSCF, 50 ng/mL of rhIL-6 and 10 ng/mL of rhIL-10 as indicated. Bar graphs display mean $\pm$ SD values from three independent experiments. One-way ANOVA tests did not reveal a significant difference between the rhIL-6, rhIL-10 and rhIL-6 plus rhIL-10 groups compared to those with rhSCF alone ($P > 0.05$).

gestation, were obtained after therapeutic abortions. The experimental protocol was approved by the Human Studies Internal Review Board at Virginia Commonwealth University. Each liver was minced and filtered over a #80 mesh stainless steel sieve. Mononuclear cells were collected after the separation with Histopaque 1077 (Sigma, St Louis, MO, USA), and suspended at 1×10^6 cells/mL in AIM-V serum-free medium (Life Technologies, Rockville, MD, USA) with 50 ng/mL rhSCF in a 6% CO₂ humidified incubator at 37 °C unless indicated otherwise. Half of the media was replaced with fresh media twice a week. After the first week non-adherent and adherent (by gentle scraping) cells were collected, combined and split with each media change. Cell numbers at 3 weeks were adjusted to account for the cells that were discarded when cultures were split. In some cases, 50 ng/mL of rhIL-6 and/or 10 ng/mL of rhIL-10 were added to the culture medium. These concentrations were based on those used previously [11,15,17].

Total cell number and viability were assessed by trypan blue staining and by flow cytometry with propidium iodide (PI). The percentage of mast cells was assessed as metachromatically stained cells with toluidine blue, and Kit-positive cells were determined by flow cytometry. Protease phenotype of cultured cells was examined by immunocytochemistry on cytocentrifuge preparations.

Flow cytometry

For detection of surface Kit and FcεRI expression, cells were first incubated in phosphate-buffered saline (PBS) containing 10% human AB serum (Sigma) for 30 min at 4 °C to block Fcγ receptor binding. After washing in PBS containing 1% fetal calf serum and 0.1% sodium azide, cells were incubated with YB5.B8, 22E7 or an isotype-matched control mouse IgG for 1 h at 4 °C, washed as above, and further incubated with FITC-conjugated goat anti-mouse immunoglobulins (BD Immunocytometry Systems, San Jose, CA, USA) for 15 min at 4 °C. Washed cells were resuspended in sheath solution and analysed with a FACScan flow cytometer (BD Immunocytometry Systems). Just before analysis, PI was added to exclude dead cells. The percentage of positive cells was calculated by subtracting the percentage of cells labelled with a negative control Ab (< 5% positive cells) from that of cells with experimental mAb. HMC-1 cells; a human mast cell leukaemia cell line, and KU812 cells; a human basophil leukaemia cell line, were used as positive controls for surface Kit and FcεRI expression, respectively.

For the detection of cells undergoing apoptosis, cells were washed in PBS and incubated in the binding buffer as provided and recommended by the manufacturer (R & D Systems) with FITC-conjugated Annexin-V, which binds to

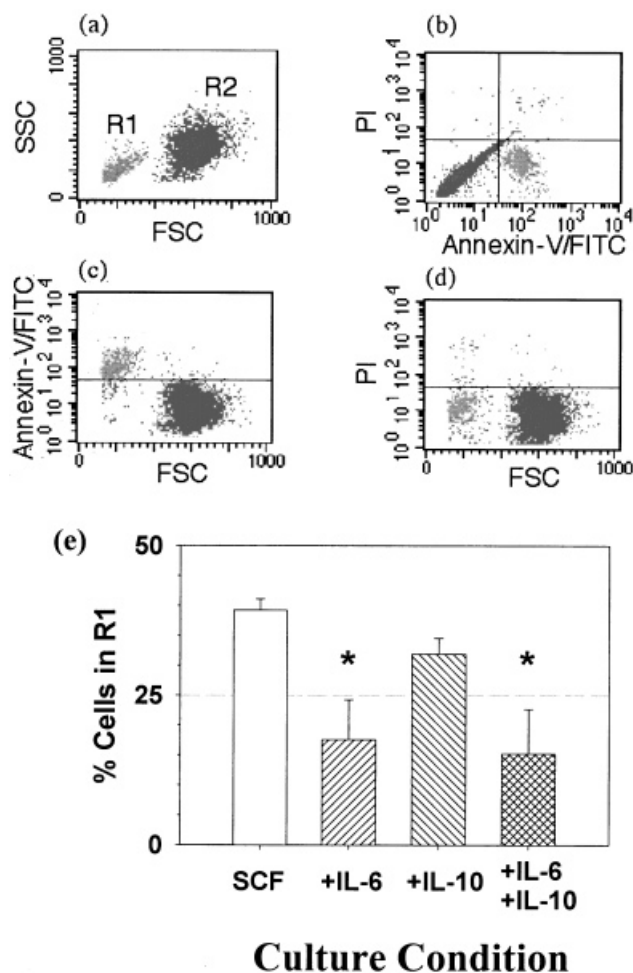


Fig. 2. The effect of rhIL-6 and rhIL-10 on spontaneous apoptosis of rhSCF-dependent fetal liver-derived cells. The percentages of the cells going to spontaneous apoptosis in the serum-free culture condition were examined by flow cytometry. (a) Forward (FSC) and side (SSC) light scatter gates were set as shown for 3-week-old fetal liver-derived cells cultured with rhSCF. There were two distinct components, indicated as R1 and R2. Only cells in R1 were surface-labelled with Annexin-V (c), while less than 3% of the cells in both R1 and R2 regions were labelled by PI (b and d). (e) Percentage of cells labelled with Annexin-V. The cultured cells consisted of 90%, 90%, 88% and 89% mast cells in the rhSCF alone, plus rhIL-6, plus rhIL-10 and plus rhIL-6 and rhIL-10 groups, respectively. Cytokine concentrations were as indicated in Fig. 1. Bar graphs display mean $\pm$ SD values from three independent experiments. A one-way ANOVA test revealed a significant difference among the four groups ($P = 0.001$); the Bonferroni *t*-test for multiple comparisons against the rhSCF alone group showed significant differences for the rhIL-6 ($P = 0.003$) and rhIL-6 plus rhIL-10 ($P = 0.002$) groups, but not for the rhIL-10 group ($P = 0.38$).

phosphatidylserine in the presence of calcium, a phospholipid that transfers from the inner to the outer side of the lipid bilayer of the plasma membrane during an early stage of apoptosis, and with PI for 15 min at 22°C to detect dead cells. Samples were directly analysed by flow cytometry.

Immunocytochemistry of cell preparation

The protease phenotype of cultured cells was assessed by indirect immunocytochemical staining as described [27]. Briefly, cytocentrifuge preparations of the cultured cells were fixed in methanol containing 0.6% H_2O_2 for 30 min at 20°C, and stored at $-20^\circ C$ until ready for use. For chymase detection, horseradish peroxidase-conjugated streptavidin (Sigma) was added to biotin-B7-labelled cells and visualized with 3-amino-9-ethylcarbazole in 0.01% H_2O_2 . For tryptase detection, alkaline phosphatase (AP)-conjugated goat-derived anti-mouse IgG (Sigma) was added to G3-labelled cells and visualized with naphthol AS-BI/new fuchsin to stain cells red.

In another experiment, slides were fixed in methanol for 30 min at 20°C, washed, then blocked with 5% skim milk and 10% human AB serum in PBS, and incubated with rabbit anti-chymase pAb or non-immune rabbit pAb overnight at 4°C. The blocking step was necessary to reduce non-specific staining. Washed cells were incubated with AP-conjugated mouse-derived anti-rabbit IgG (Sigma) for 30 min at 20°C, and visualized with naphthol AS-BI/new fuchsin to stain cells red. Non-immune rabbit pAb did not result in new fuchsin staining of fetal liver cells. Cells were lightly counterstained with haematoxylin.

Results

Neither rhIL-6 nor rhIL-10 enhance SCF-dependent development of human fetal liver-derived mast cells

Dispersed fetal liver cells ($10^6/mL$) were cultured in serum-free medium containing rhSCF and various combinations of rhIL-6 (50 ng/mL) and rhIL-10 (10 ng/mL) for up to 3 weeks, and examined after 1 and 3 weeks. As shown in Figs 1(a and d), the total concentration of cells was similar to the starting cell number after 1 week, and had increased about 10-fold by 3 weeks with rhSCF alone. Neither rhIL-6, rhIL-10 nor rhIL-6 together with rhIL-10 altered this total cell response. In terms of the percentages (Figs 1b and e) and numbers (Figs 1c and f) of Kit-positive mast cells, these increased from 20 and 0.18×10^6 at 1 week to 90 and 10.8×10^6 by 3 weeks, similar to a previous report [27]. Although there was a tendency for fewer mast cells to be detected after the interleukins were added, in no case was there a significant difference between an interleukin group and the rhSCF-alone group. At no time point or

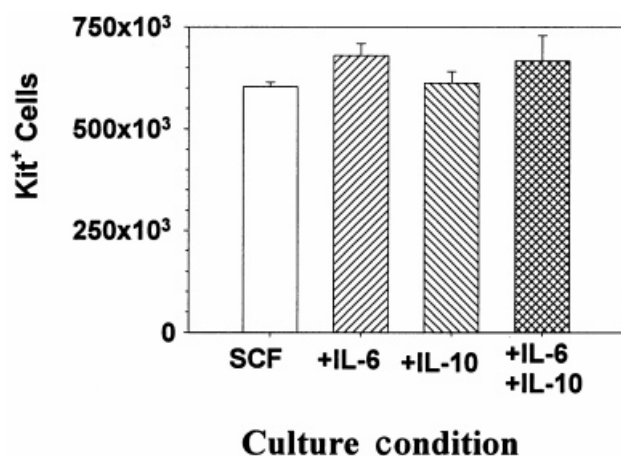


Fig. 3. Effect of rhIL-6 and rhIL-10 on the number of rhSCF-dependent fetal liver-derived mast cells. Three-week-old fetal liver-derived mast cells developed in serum-free medium containing SCF alone (90% Kit-positive mast cells) were placed into 24-well tissue-culture plates at a concentration of 5×10^5 cells/mL and incubated for another 1 week with the cytokine combination indicated at the concentrations listed in Fig. 1. Mast cell numbers were calculated from viable cell numbers and Kit-positive cell percentages. Bar graphs show the mean $\pm$ SD values for three independent experiments. A one-way ANOVA did not reveal a significant difference between the four groups ($P > 0.05$).

culture condition were Fc ϵ RI-positive cells detected (data not shown). Also, extending the culture period to 8–12 weeks with rhSCF did not yield Fc ϵ RI-positive cells. This is also consistent with previous reports [14,28]. The percentages of cells exhibiting metachromasia with toluidine blue increased in parallel to Kit-positive cells under each condition (data not shown).

RhIL-6 reduces apoptosis of fetal liver-derived human mast cells

Apoptotic cells were detected by surface labelling with FITC-conjugated Annexin-V using flow cytometry. After 3 weeks of culture 90% of the cells were mast cells. As shown in Fig. 2(a), forward and side scatter revealed two distinct subpopulations, indicated as R1 and R2. The R1 population was smaller in size and less granular than the R2 population. Only the R1 cells were surface-labelled with Annexin-V (Fig. 2c), while less than 3% of the cells in both R1 and R2 regions were labelled by PI (Figs 2b and d), regardless of the added interleukins. As summarized in Fig. 2(e), the constitutive inclusion of rhIL-6 together with rhSCF during the 3 weeks of culture from day 0, regardless as to whether rhIL-10 was included, diminished the relative portion of cells labelled with Annexin-V by about half, whereas rhIL-10 had no significant effect. Thus, rhIL-6

appears to markedly reduce spontaneous apoptosis of fetal liver-derived mast cells grown under serum-free conditions with rhSCF.

RhIL-6 and rhIL-10 were tested to see if either separately or together they could affect the number of mast cells when added after mast cells had developed. They were added to 3-week-old cultures of fetal liver cells and rhSCF, and examined after an additional week of culture with these interleukins. As shown in Fig. 3, although rhIL-10 had no effect on mast cell numbers, there was a trend for rhIL-6 to result in a slightly higher number of mast cells after 1 week, but this apparent increase did not reach statistical significance.

RhIL-6 and rhIL-10 were then tested to determine if either could protect fetal liver-derived mast cells from undergoing apoptosis when rhSCF was withdrawn. Cells were examined 3 days after withdrawal of rhSCF. As shown in Fig. 4, the percentage of apoptotic cells determined as in Fig. 2 increased from about 20 in cultures with rhSCF, to about 90 in those with media alone. Adding rhIL-10 had no effect on these percentages. In contrast, rhIL-6, both alone and together with rhIL-10, suppressed the percentage of apoptotic mast cells in rhSCF-deprived cultures to values that were not significantly different from cultures with rhSCF alone.

Protease phenotype of fetal liver-derived human mast cells cultured with rhSCF together with either rhIL-6 and/or rhIL-10

Cyocentrifuge preparations of the fetal liver-derived cells were examined after 1 and 3 weeks of culture for protease phenotype as shown in Fig. 5 and Table 1. In all culture conditions, tryptase-positive cells increased in parallel to those stained metachromatically with toluidine blue (data not shown) as reported previously [12,15,27]. After 1 week of culture, no chymase-positive cells were detected with the mAb against chymase and less than 5% of the cells were labelled with the rabbit pAb against chymase, regardless of the added cytokine mixture, while about half of the cells already were tryptase-positive and metachromatic. The percentage of tryptase-positive cells detected with rhSCF alone was not affected by rhIL-6 or rhIL-10 at this time point. By 3 weeks, tryptase-positive cells accounted for greater than 90% of the cells in all culture conditions, and these percentages were comparable to those for metachromasia with toluidine blue and Kit expression by flow cytometry. Using the mAb against chymase, about 14% of the cells were strongly positive; and the remaining cells were clearly negative. Using the pAb against chymase, about 31% of the cells were chymase-positive, the increased percentage associated with a substantial increase in more weakly stained cells. As positive controls, mast

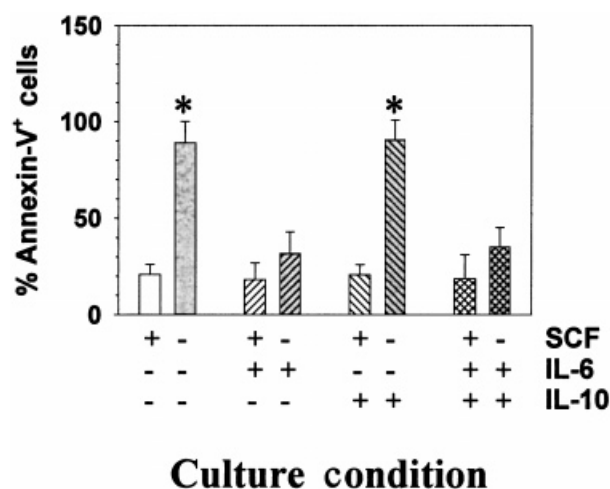


Fig. 4. Effect of rhIL-6 and rhIL-10 on apoptosis of fetal liver-derived mast cells deprived of rhSCF. Three-week-old fetal liver-derived mast cells developed in serum-free medium containing rhSCF alone were washed and placed into 24-well culture plates at a concentration of 5×10^5 cells/mL of serum-free AIM-V medium containing the cytokines indicated at the concentrations listed in Fig. 1. Cells were incubated for 3 days and then analysed by flow cytometry with FITC-conjugated Annexin-V to detect the percentage of apoptotic cells as in Fig. 2. Mean $\pm$ SD values are shown for three independent experiments. One-way ANOVA with the Bonferroni posthoc multiple comparisons test revealed significant differences only for the two groups lacking both rhSCF and rhIL-6 against the group with rhSCF alone ($P < 0.001$).

cells dispersed from adult human skin were labelled with pAb and mAb against chymase, anti-tryptase mAb, and toluidine blue and showed identical percentages of positive cells (data not shown).

Discussion

The current study found that rhIL-6 reduced the number of fetal liver-derived mast cells undergoing spontaneous apoptosis in the presence of rhSCF as well as those undergoing apoptosis upon withdrawal of rhSCF under serum-free culture conditions. RhIL-10 by itself or in combination with rhIL-6 had no effect on these apoptotic outcomes. This result extends those of a previous report that rhIL-6, but not rhIL-10, diminished the decrement in numbers of cord blood-derived mast cells after withdrawal of rhSCF [25]. Consistent with this protective role was the observation that rhIL-6 protects cord blood-derived mast cells from rhIL-4-mediated apoptosis [17].

Neither rhIL-6, rhIL-10 nor the combination of these cytokines stimulated the growth of fetal liver-derived mast cells in serum-free media containing rhSCF. This contrasts

with a report that rhIL-6 stimulated the growth of cord blood-derived mast cells in serum-containing media [11]. Possibly, the growth enhancing effect of rhIL-6 requires serum. Another study employing serum-free media found that rhIL-6 diminished the numbers of total mast cells derived from cord blood in the presence of rhSCF, and increased the percentages of chymase-positive cells without a substantial change in their numbers [16]. This previous study utilized only 10 ng/mL (10-fold lower than the current study) of rhSCF and an atmosphere consisting of 5% CO₂, 5% O₂ and 90% N₂ (~25% of the O₂ content used in the current study) to develop mast cells, possibly accounting for the different outcomes. A third study claimed that a combination of rhSCF, rhIL-10 and rhIL-6 was optimal for the growth of cord blood-derived mast cells [15]; but comparisons were not made with isolated or paired combinations of these potential growth factors. The current study directly assessed whether addition of these cytokines to rhSCF would affect the number of mast cells obtained from fetal liver cells, and found no statistically significant increase or decrease.

In the current study using fetal liver cells, tryptase expression, assessed by immunocytochemistry, preceded that of chymase, and was already detected on almost half of

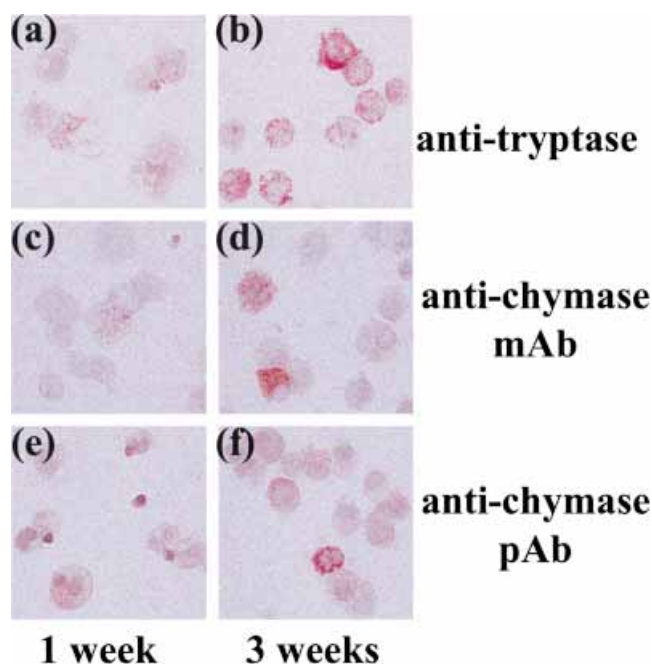


Fig. 5. Fetal liver-derived mast cells labelled with anti-tryptase and anti-chymase Abs. Fetal liver cells cultured for 1 week (a, c and e) and 3 weeks (b, d and f) in rhSCF were labelled with mouse anti-tryptase mAb (a and b), mouse anti-chymase mAb (c and d) and rabbit anti-chymase pAb (e and f) as described. The sections are representative of three separate experiments, the results for which are summarized in Table 1.

the cells by 1 week, and nearly all of the cells by 3 weeks, regardless of the cytokine mixture used. With both a mouse mAb and protein A-purified rabbit pAb against chymase, expression of this protease was minimal (< 5%) in all cases at 1 week. By 3 weeks, chymase was detected in about 14% of the cells labelled with the mAb, and about 31% of the cells labelled with the pAb. The pAb required more stringent blocking conditions to diminish non-specific staining, and appeared to yield more weakly stained cells. Thus, whether the additional cells labelled with the pAb contained small amounts of chymase that were missed with the mAb, or whether they reflected non-specific labelling was not resolved. Nevertheless, these results conflict with reports in mouse [21–23] and human [15] systems that IL-10 induces chymase expression. However, human chymase is a single gene product that is classified as an α -chymase, related most closely to mMCP-5 [29]. Mice express at least eight chymases (mMCP-1–5, 8–10) [30–32], only mMCP-5 being of the α -chymase subclass, which has not yet been examined for IL-10 responsiveness.

In contrast to the current study, human cord blood-derived mast cells cultured with rhSCF, rhIL-10 and rhIL-6 were reported to express chymase before tryptase [15], such that by 4 weeks of culture 85% of the cells were labelled with rabbit anti-chymase pAb, whereas only about half of these chymase-positive cells were labelled with anti-tryptase mAb and stained metachromatically with toluidine blue. This led to the apparent production of chymase-positive, tryptase-negative mast cells that did not exhibit metachromasia. A similar protease phenotype (MC_C) was reported when cord blood cells were cultured with SCF together with conditioned media from a mastocytosis cell line; by 10 days of culture 10–15% of the cells were mast cells and all mast cells were chymase-positive and tryptase-negative [33]. These *in vitro* observations are consistent with a report of such a phenotype occurring *in vivo* [34], in which 17% of the mast cells in bowel submucosa and 7% of those in the mucosa stained positively for chymase but not for tryptase. However, in other studies this phenotype was not detected in the intestine or other tissue sites by immunocytochemistry [1,2,35], enzyme histochemistry [36] or immunogold electron microscopy [3,37]. In a study of synovial tissue, the MC_C phenotype was related to insufficient staining sensitivity for tryptase, i.e. all mast cells were found to be tryptase-positive with adequate staining sensitivity [38]. Thus, there are conflicting reports as to whether all mast cells express tryptase and whether chymase might be expressed before tryptase.

Technical differences and similarities between the current study and the one by Ochi *et al.* [15] should be considered. For example, the rabbit anti-chymase pAb used in each study was from the same lot, and an identical anti-tryptase mAb was used. Two technical differences are of

potential import. First, the apparent use of trypsin to enhance labelling in the former study, in our hands increases non-specific immunoreactivity. Second, amplification using biotinylated goat anti-rabbit pAb and alkaline phosphatase-avidin-biotin complex was not required in the current study to obtain strong chymase labelling. Finally, the source of mast cells were different between the two studies. Indeed, cord blood and fetal liver-derived mast cells, each developed with rhSCF, are known to behave differently. Cord blood-derived mast cells express functional Fc ϵ RI on their surface and undergo IL-4-mediated apoptosis [17]; whereas fetal liver-derived mast cells do not express surface Fc ϵ RI [14,28] and are resistant to IL-4-mediated apoptosis [17]. Infant vs. fetal progenitor cells may respond differently to the growth factors used, and the associated accessory cells, which also differ, may affect the development of these mast cells. When cocultured with mouse 3T3 fibroblasts, fetal liver cells develop mostly into mast cells expressing tryptase but not chymase [39], while cord blood cells develop mostly into mast cells expressing both of these proteases [40]. Thus, to resolve whether human mast cells, either during development or after maturation, produce chymase in the absence of tryptase will require further study.

Acknowledgements

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