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Human Intestinal Mast Cells Are Capable of Producing Different Cytokine Profiles: Role of IgE Receptor Cross-Linking and IL-4[1]

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Mast cells are recognized as a new type of immunoregulatory cells capable of producing different cytokines. So far, little is known about the cytokine profile of mature human mast cells isolated from intestinal tissue and cultured in the presence of stem cell factor (SCF). We observed that these cells express the proinflammatory cytokines TNF-α, IL-1β, IL-6, IL-8, IL-16, and IL-18 without further stimulation. Both IgE-dependent and IgE-independent agonists (e.g., Gram-negative bacteria) enhanced expression of TNF-α. Another set of cytokines consisting of IL-3, IL-5, IL-9, and IL-13 was expressed following activation by IgE receptor cross-linking. If mast cells were cultured in the presence of IL-4 and SCF, the production and release of IL-3, IL-5, and IL-13 was increased up to 4-fold compared with mast cells cultured with SCF alone. By contrast, IL-6 expression was completely blocked in response to culture with IL-4. In summary, our data show that mature human mast cells produce proinflammatory cytokines that may be up-regulated following triggering with IgE-independent agonists such as bacteria, whereas activation by IgE receptor cross-linking results in the expression of Th2-type cytokines. IL-4 enhances the expression of Th2-type cytokines but does not affect or even down-regulates proinflammatory cytokines. ***The Journal of Immunology,*** **2000, 164: 43–48.**

Tissue mast cells are key effector cells of immediate-type allergic reactions. They exert their biological effects by releasing preformed and de novo-synthesized mediators such as histamine, proteases, leukotrienes, PGs, and cytokines upon cell activation by Ag-induced IgE receptor cross-linking. In addition, there is increasing evidence that mast cells are also involved in IgE-independent inflammatory and repair processes (1). The recognition of mast cells as cytokine-producing cells has extended their potential functions from proinflammatory effector cells to regulatory components of the immune system (2). In previous studies, it was reported that murine mast cells produce a variety of cytokines including TNF-α, IL-1, IL-3, IL-4, IL-6, and IL-13 (3, 4). Because mast cells are known to exhibit marked species differences (1), it has to be examined whether those data can be transferred to the human system. Little is known about the cytokine profile of mucosa-derived human mast cells, because the cells can be obtained only in limited quantities and they are difficult to purify. The few data on cytokine production by human mast cells are mainly derived from immunohistochemical studies (5–7), or studies using immature bone marrow or cord blood-derived mast cells (8–10), or the immature leukemic mast cell line HMC-1 (11–14). Human lung mast cells were found to express mRNA for IL-4, IL-5, and GM-CSF and to release the products by IgE-dependent activation (15–17). Using recently developed methods for isolation and culture of human intestinal mast cells (18, 19), we could show that these cells are an important source of TNF-α and IL-5 in human intestinal tissue in vitro as well as in vivo (20, 21). IL-5 production was only observed if mast cells were triggered by IgE receptor cross-linking, whereas TNF-α production occurred constitutively but could be enhanced by IgE receptor-dependent activation (20, 21). Furthermore, we found recently that IL-4, known to play an important role in B and T lymphocyte differentiation, strongly enhances the proliferation and mediator release by human intestinal mast cells and thus may be involved, apart from stem cell factor (SCF),[3] in human mast cell regulation (22). In this study, we examined the expression of a number of different cytokines by purified human intestinal mast cells with particular respect to the modulatory effect of IgE receptor cross-linking and IL-4 on the cytokine profile produced by this cell type.

Materials and Methods

Isolation and culture of human intestinal mast cells

Human intestinal mast cells were isolated from surgery tissue specimens (macroscopically normal border sections, free of tumor cells as determined by histological examination of the tissue) derived from 18 patients (40–77 years of age, 9 men, 9 women), who underwent bowel resection because of cancer. Permission to conduct the study was obtained from the local ethical committee of the Medical School of Hannover. The methods of mechanical and enzymatic tissue dispersion yielding single-cell preparations containing 4 $\pm$ 2% (mean $\pm$ SD) mast cells has been described in detail elsewhere (18). After overnight incubation in culture medium (RPMI 1640 supplemented with 10% heat-inactivated FCS, 25 mM HEPES, 2 mM glutamine, 100 μg/ml streptomycin, 100 μg/ml gentamicin, 100 U/ml penicillin, and 0.5 μg/ml amphotericin; all cell culture reagents were obtained from Life Technologies, Paisley, U.K.), mast cells were enriched by positive selection of c-*kit* expression using magnetic cell separation (MACS system; Miltenyi Biotech, Bergisch-Gladbach, Germany) and the mAb YB5.B8 (PharMingen, Hamburg, Germany) as described (19–21). The fraction containing the c-*kit*-positive cells (mast cell purity 60 $\pm$ 25%) was cultured at a density of 2×10^5 MC per ml for up to 21 days in medium supplemented with 25 ng/ml of recombinant human SCF (PeproTech, Rocky Hill, NJ) alone or with 10 ng/ml IL-4 (Novartis, Vienna, Austria) and SCF. Cultured mast cells were fed once a week by exchanging half of the culture

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[3] Abbreviation used in this paper: SCF, stem cell factor.

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medium supplemented with SCF or with SCF and IL-4. During culture for 14–21 days, mast cell purity increased to 98 ± 2%. The endotoxin content of the culture medium was 3 pg endotoxin/ml culture medium measured by LAL COATEST Endotoxin (Chromogenix, Mölntal, Sweden).

Stimulation of mast cells and coculture experiments

Mast cells were stimulated by IgE receptor cross-linking using the purified mAb 29C6 (provided by Hoffmann-La Roche, Nutley, NJ) directed against a non-IgE binding epitope of the high-affinity IgE receptor α-chain. To distinguish between existing and newly transcribed mRNA, mast cells were incubated with 400 ng/ml actinomycin D (Sigma, St. Louis, MO) before stimulation for 14 h as described (21). For coculture experiments, eosinophils (purity 89–96%, $n = 3$) were isolated from venous blood of informed healthy volunteers as described previously (23) and cultured directly after isolation with purified mast cells in 24-well plates separated by a transwell membrane ($\varphi = 0.4$ μm, Nunc, Roskilde, Denmark) using the medium described for mast cell culture. As a positive control, IL-5 (PeproTech) instead of mast cells was added to the medium at a final concentration of 2 ng/ml.

RNA preparation and semiquantitative RT-PCR

Total RNA was prepared from human intestinal cell preparations containing 98–100% mast cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany). For RT-PCR, 200 ng of total RNA was treated for 15 min at 37°C with 10 U RNase-free DNase (Promega, Madison, WI) to remove genomic DNA. After denaturation for 10 min at 70°C, cDNA was synthesized for 1 h at 37°C by adding Superscript reverse transcriptase (Life Technologies, Eggenstein, Germany) and 20 pmol oligo(dT) primers (Pharmacia, Uppsala, Sweden). A one-tenth volume of the cDNA was used for one PCR. Thirty-five cycles (60 s at 94°C, 80 s at 60°C, 70 s at 72°C) were performed with 2.5 U *Taq* DNA polymerase (Life Technologies) and 20 pmol of the specific sense and antisense primers for the cDNAs of IL-1β (5′-CGATCACTGAACTG CACGCTCCG-3′; 5′-GGTGAAGTCAGTTATATCCTGGCCG-3′), IL-2 (5′-CAAGAATCCCAAACTCACCAGG-3′; 5′-CAATGGTTGCTGTCTC ATCAGC-3′), IL-3 (5′-CCTTGAAGACAAGCTGGGTT-3′; 5′-ATTCATT CCAGTCACCGTCC-3′), IL-4 (5′-CGGACACAAGTGCGATATCACC-3′; 5′-CCAACGTACTCTGGTTGGCTTCC-3′), IL-5 (5′-CGAACTCTGCT GATAGCCAATG-3′; 5′-CCACTCGGTGTTCATTACACCAAG-3′), IL-6 (5′-CACACAGACAGCCACTCACCTC-3′; 5′-CTCAGGCTGGACTG CAGGAAC-3′), IL-7 (5′-GGACTTCCTCCCCTGATCCTTG-3′; 5′-CT TTGTTGGTTGGGCTTCACCCAG-3′), IL-8 (5′-GCAGCTCTGTGTGA AGGTGCAG-3′; 5′-GCATCTGGCAACCCTACAACAG-3′), IL-9 (5′-GAT CCTGGACATCAACTTCCTC-3′; 5′-CTTGCCTCTCATCCCTCTCATC-3′), IL-10 (5′-CCGTGGAGCAGGTGAAGAATGC-3′; 5′-CAGCTAG AAAGCGTGGTCAGGC-3′), IL-11 (5′-ATGAACTGTGTTTGCCGC CTGGTC-3′; 5′-CGTCAGCTGGGAATTTGTCCTTC-3′, IL-12 (5′-CCAAGAACTTGCAGCTGAAG-3′; 5′-TGGGTCTATTCCGTTGTGTC-3′, IL-13 (5′-ATGCATCCGCTCCTCAATCC-3′; 5′-TTCAGTTGAAC CGTCCCTCG-3′), IL-15 (5′-GTATTGTAGGAGGCATCGTGG-3′; 5′-GTTCATCTGATCCAAGGTCTG-3′), IL-16 (5′-GCCCGACCTCA ACTCCTCCACT-3′; 5′-GTCTCCAGCAGCTGTGGTTTCC-3′), IL-17 (5′-GGACTGTGATGGTCAACCTG-3′; 5′-CGGACACCAGTATCTTCTCC-3′), IL-18 (5′-GCTGCTGAACCAGTAGAAGA-3′; 5′-GTTCTCACA GGAGAGAGTTG-3′), TNF-α (5′-GAGCTGAGAGATAACCAGCTGG TG-3′; 5′-CAGATAGATGGGCTCATACCAGGG-3′), GAPDH (5′-ACCACAGTCCATGCCATCAC-3′; 5′-TCCACCACCCTGTTGCTGTA-3′), c-*kit* (5′-GGAGATCTGTGAGAATAGGCTC-3′; 5′-CCCATAGGAC CAGACGTCACTTTC-3′), CD3 (5′-AGTTGGCGTTTGGGGGCAAGAT GGTAATGAAGAAA-3′; 5′-CCCAGGAAACAGGGAGTCGCAGGGG GACTGGAGAG-3′ (synthesized by Life Technologies). A one-fifth volume of the PCR products was separated on 1% agarose gel containing ethidium bromide (500 ng/ml) and photographed. To exclude false positive results by contaminating lymphocytes, negative controls were conducted using the specific primer pair for CD3. Positive controls for mast cell mRNA were performed using the primer pair for c-*kit*. To observe relative changes in mRNA expression, duplex PCR according to the "primer-dropping" method was performed as described (20). Duplex PCRs were started with the primer pair for the cytokine of interest, and after a determined number of cycles the primer set of GAPDH was added. In each case three samples of the reactions were taken in the course of exponential DNA increase, separated on 1% agarose gels containing ethidium bromide and analyzed using an automated bioimaging analyzer (Fuji BAS-1000; Raytest, Germany). To ensure that specific cDNAs were amplified, PCR fragments were sequenced by the dideoxy method using the T7 SequencingTM kit (Pharmacia).

Immunocytochemistry

Cultured cells were harvested, cytocentrifugated on slides and fixed in acetone for 10 min. Immunostaining was performed using the primary Abs mouse anti-human IL-1β mAb (R&D Systems, Wiesbaden, Germany), rat anti-human IL-3 Ab (PharMingen, San Diego, CA) at 50 μg/ml, rat anti-human IL-4 Ab (PharMingen) at 50 μg/ml, rat anti-human IL-5 Ab (PharMingen) at 50 μg/ml, rat anti-human IL-6 Ab (PharMingen) at 50 μg/ml, mouse anti-human IL-8 mAb (LeukoSite, Cambridge, MA) at 10 μg/ml, goat anti-human IL-9 Ab (Genzyme, Cambridge, MA) at 20 μg/ml, rat anti-human IL-13 Ab (PharMingen) at 50 μg/ml, mouse anti-human IL-16 mAb (ImmunoDiagnostics, Bedford, MA) at 10 μg/ml, rabbit anti-human IL-18 Ab (BioSource, Camarillo, CA) at 1 μg/ml, and rabbit anti-human TNF-α Ab (Genzyme) at 0.28 μg/ml. Slides were incubated with the primary Abs for 16 h at 4°C. The primary Abs were linked by a biotinylated secondary Ab to a streptavidin-peroxidase conjugate, and the substrate/chromogen (hydrogen peroxide/AEC) was converted to a red deposit (LAB-SA System; Zymed, South San Francisco, CA). Controls were conducted with appropriate nonimmune control sera diluted corresponding to the primary Ab concentration (Immunotech, Marseille, France). Slides were counterstained with Mayer's hemalam (Merck, Darmstadt, Germany).

Measurement of histamine and cytokines in supernatants

Histamine was measured by RIA (Coulter-Immunotech, Krefeld, Germany). Cytokines were quantified by ELISA according to the manufacturer's instructions. The kits from R&D Systems were used to measure TNF-α, IL-3, IL-4, IL-5, and IL-6, and the kits from BioSource were used to measure IL-10 and IL-13. The detection limits were 5 pg/10^6 cells (TNF-α), 31.2 pg/10^6 cells (IL-3), 23.4 pg/10^6 cells (IL-4), 7.8 pg/10^6 cells (IL-5, IL-6), 1 pg/10^6 cells (IL-10), or 19.5 pg/10^6 cells (IL-13), respectively.

Results

Cytokine mRNA expression by isolated human intestinal mast cells

Mature human mast cells were isolated from intestinal tissue and cultured in the presence of SCF. As demonstrated in Fig. 1, the mRNA for IL-1β IL-6, IL-8, IL-16, and IL-18 was clearly detectable in mast cells that were not further stimulated. As shown recently (21), IL-5 mRNA was hardly detectable under such conditions but clearly present after stimulation of the cells by IgE receptor cross-linking with 100 ng/ml mAb 29C6 for 6 h. The mRNA for IL-3, IL-9, and IL-13 was not expressed in SCF-cultured cells without further stimulation but was detected following stimulation for 6 h. Inconsistently, in four of eight experiments IL-10 mRNA was expressed with further induction following IgE receptor cross-linking. IL-4 mRNA was not ($n = 5$) or only hardly detectable ($n = 3$) after stimulation with mAb 29C6. The mRNA expression for IL-16 and IL-18 was moderately down-regulated following IgE receptor cross-linking. Neither in resting nor in stimulated mast cells was mRNA found for IL-2, IL-7, IL-11, IL-12, IL-15, and IL-17. To distinguish between stabilized existing and newly transcribed mRNA in response to IgE receptor cross-linking, mast cells were incubated for 14 h with actinomycin D, known to inhibit mRNA transcription, before stimulation (21). After pretreatment with actinomycin D and subsequent mast cell stimulation, mRNA levels for IL-6, IL-8, IL-16, and IL-18 remained unaffected. In contrast, mRNA expression for IL-3, IL-5, IL-9, IL-10, and IL-13 was absent or only hardly detectable, indicating a regulation of cytokine expression in response to IgE-receptor cross-linking by induction of transcription ($n = 3$).

Fig. 2*A* shows the time kinetics of mRNA expression by mast cells in response to stimulation with high-affinity IgE receptor cross-linking using 100 ng/ml mAb 29C6. mRNA expression for IL-5 started 15 min after activation. The peak expression of IL-5 mRNA was at 90 min and could be detected until 6 h after stimulation. We found similar time curves for IL-3 and IL-13 mRNA, whereas IL-9 mRNA expression peaked only after stimulation for 6 h. In contrast, TNF-α mRNA expression peaked already following stimulation for 30 min, and then the expression decreased. To

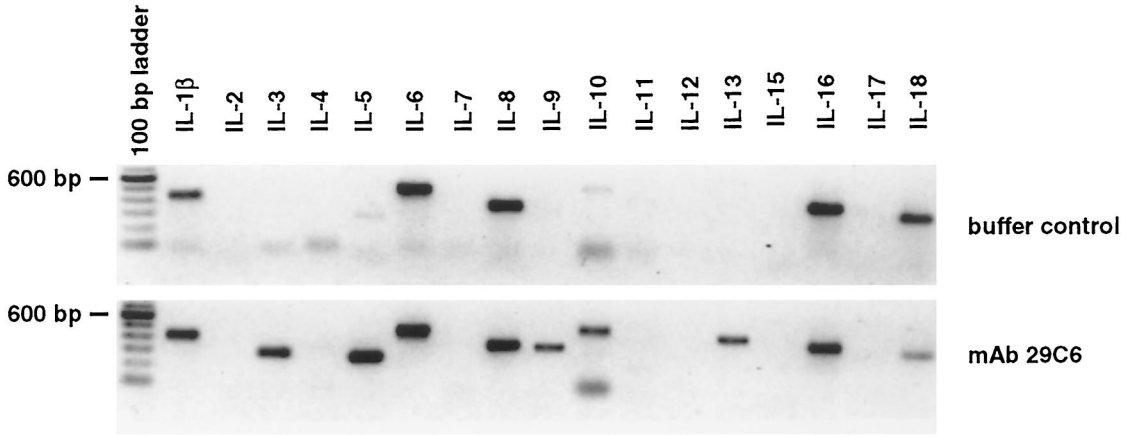

FIGURE 1. Expression of cytokine mRNA in human intestinal mast cells purified to ≥98%. Total RNA was prepared from mast cells challenged with 100 ng/ml mAb 29C6 for 6 h or with buffer control. RT-PCR was conducted using specific primer pairs. DNA fragments obtained after 35 cycles were separated on an 1% agarose gel. One representative of eight experiments is shown.

analyze the dose dependency of cytokine mRNA expression, mast cells were stimulated using 1, 10, or 100 ng/ml mAb 29C6. In this set of experiments, mRNA for IL-3, IL-5, IL-9, and IL-13 was absent in samples of SCF-cultured cells without further stimulation. mRNA expression occurred after activation with a minimal concentration of 1 ng/ml mAb 29C6 for 6 h or 10 ng/ml mAb 29C6 for 1 h, respectively (Fig. 2*B*). The up-regulation of TNF-α mRNA expression occurred on the same conditions.

Detection and in vitro effects of mast cell-derived cytokines

Intracytoplasmic cytokine expression in human intestinal mast cells cultured for 2 wk in the presence of SCF (98–100% pure, $n = 3$) was studied by immunocytochemistry and is summarized in Table I. In accordance with the mRNA data, we found clear immunoreactivity for IL-3, IL-5, IL-9, and IL-13 only if mast cells were treated with 100 ng/ml mAb 29C6 for 6 h. The release of cytokines by human intestinal mast cells (96–100% pure) was measured by ELISA. We tested IL-3, IL-4, IL-5, IL-6, IL-10, and IL-13. Only IL-6 was released spontaneously in 6 h (mean: 36 pg IL-6/10^6 mast cells) with an increase following stimulation by IgE receptor cross-linking (mean: 108 pg IL-6/10^6 mast cells). TNF-α (mean: 181 pg TNF-α/10^6 mast cells), IL-3 (mean: 23 pg IL-3/10^6 mast cells), IL-5 (mean: 367 pg IL-5/10^6 mast cells), and IL-13 (mean: 16 pg IL-13/10^6 mast cells) were released following stimulation. In lysates of mast cells not stimulated by IgE receptor cross-linking, small amounts of TNF-α (8.4 $\pm$ 3.2 pg TNF-α/10^6 mast cells) and IL-6 (4.0 $\pm$ 3.8 pg IL-6/10^6 mast cells) were measured, whereas IL-3, IL-5, and IL-13 were not detected. Following stimulation with mAb 29C6, we measured 50 $\pm$ 32 pg IL-5/10^6 mast cells, 27 $\pm$ 27 pg IL-6/10^6 mast cells, and 48 $\pm$ 43 pg TNF-α/10^6 mast cells. IL-3 or IL-13 were not detected in cell lysates. This finding demonstrates that, in contrast to mast cell mediators

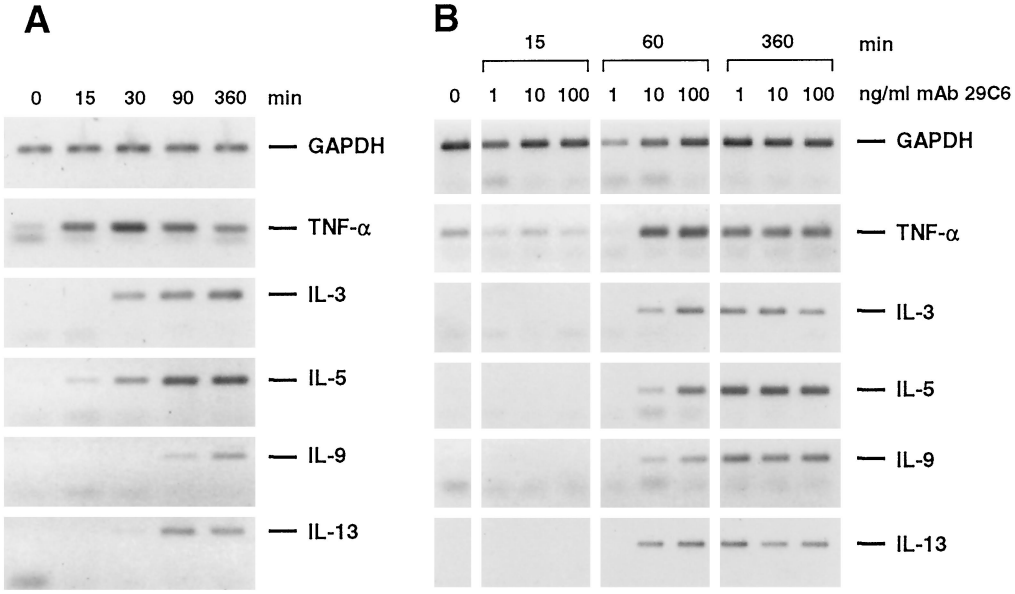

FIGURE 2. Time kinetics and dose dependency of cytokine mRNA expression in human intestinal mast cells. *A*, Total RNA was prepared from mast cells cultured in the presence of SCF without further stimulation (0) or from mast cells stimulated with 100 ng/ml mAb 29C6 for 15, 30, 90, or 360 min. To obtain comparable PCR fragments, RT-PCR was conducted with specific primers and previously determined cycle numbers for GAPDH (27 cycles), IL-5 (30 cycles), TNF-α (33 cycles), and IL-3, IL-9, and IL-13 (35 cycles). *B*, Total RNA was prepared from SCF-cultured mast cells without further stimulation (0) or from mast cells stimulated for 15, 60, and 360 min with 1, 10, or 100 ng/ml mAb 29C6, respectively. RT-PCR was conducted for 27 cycles for GAPDH, 30 cycles for IL-5, 33 cycles for TNF-α, 35 cycles for IL-3 and IL-13, and 37 cycles for IL-9.

Table I. *Intracytoplasmic cytokine expression in human intestinal mast cells*

	Buffer Control[a] (%)	mAb 29C6[a] (%)
TNF-α	56 ± 15	63 ± 17
IL-1β	7 ± 4	12 ± 5
IL-3	0	9 ± 6
IL-4	0	0
IL-5	1 ± 1	15 ± 9
IL-6	5 ± 3	18 ± 8
IL-8	16 ± 9	16 ± 7
IL-9	0	10 ± 8
IL-13	0	10 ± 6
IL-16	6 ± 4	16 ± 8
IL-18	55 ± 25	60 ± 23

[a] Mast cells were challenged with buffer control or with 100 ng/ml mAb 29C6. Cytokines were detected by immunocytochemistry. The percentages of positively stained cells are given in mean ± SD.

such as histamine, a larger part of mast cell-derived cytokines has to be synthesized de novo before release. In all cases, neither IL-4 nor IL-10 were detectable in supernatants derived from mast cells (detection limit: 23.4 pg IL-4/10^6 mast cells, 1 pg IL-10/10^6 mast cells). To analyze in vitro effects of mast cell-derived cytokines, we performed coculture experiments with purified human intestinal mast cells (98–100% pure, $n = 3$) and purified blood eosinophils (89–96% pure, $n = 3$). Mast cells were either challenged with 10 ng/ml mAb 29C6 or buffer control, and eosinophil survival was recorded after 1 wk of culture. In another set of experiments, eosinophils were cultured with or without supernatants derived from mast cells triggered with mAb 29C6. These experiments showed that coculture of eosinophils with mast cells triggered with mAb 29C6 or addition of supernatant derived from stimulated mast cells enhanced eosinophil survival by about 159 ± 19% or 160 ± 27%, respectively, compared with buffer control. Similar effects could be achieved by addition of 2 ng/ml IL-5 (181 ± 34%).

Effect of IL-4 on cytokine expression by human intestinal mast cells

In a recent study, we showed that IL-4 strongly enhances proliferation and mediator release of human intestinal mast cells (22). To analyze the effect of IL-4 on cytokine expression, mast cells were cultured in the presence or absence of IL-4, and the expression of TNF-α, IL-3, IL-5, IL-6, and IL-13 was tested. If the cells were cultured with SCF and IL-4, we found mRNA for IL-3, IL-5, and IL-13 already in cells without further stimulation. Following stimulation with mAb 29C6, mRNA expression for IL-3, IL-5, and IL-13 was further enhanced. Using semiquantitative RT-PCR, we found an IL-4-induced 4-fold increase of mRNA expression for IL-3 and IL-5 in mast cells stimulated by IgE receptor cross-linking (Fig. 3). The data obtained for mRNA expression were confirmed on the protein level by measurement of cytokine content in supernatants of mast cells with or without stimulation by IgE receptor cross-linking. As shown in Fig. 4, the release of histamine in response to IgE receptor cross-linking was about 2-fold increased if mast cells were cultured with IL-4. The release of IL-3, IL-5, and IL-13 in response to IgE receptor cross-linking was also strongly enhanced, the release of IL-3 and IL-13 was about 4-fold higher, and the release of IL-5 was about 2-fold higher than in cells cultured without IL-4. Moreover, IL-5 was released spontaneously by mast cells cultured with IL-4 without further stimulation by IgE receptor cross-linking. In contrast, IL-4 completely inhibited the production of IL-6 in mast cells. Neither mast cells stimulated by IgE receptor cross-linking nor mast cells without further stimulation released detectable amounts of IL-6 if cultured in the presence of IL-4 (Fig. 4). Correspondingly, we found no mRNA for IL-6 in mast cells cultured with IL-4. The expression of mRNA for IL-1β, IL-8, IL-16, IL-18, and TNF-α as well as the release of TNF-α (Fig. 4) were not affected by IL-4.

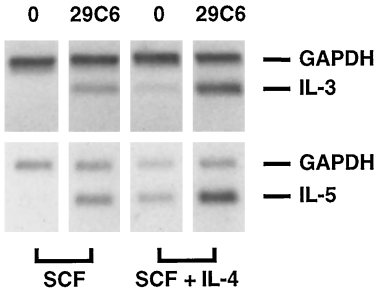

FIGURE 3. Effect of IL-4 on mRNA expression for IL-3 and IL-5 in human intestinal mast cells. Mast cells were cultured for 2 wk with SCF alone or with SCF and IL-4. RNA derived from mast cells, either without further stimulation (0) or stimulated with 100 ng/ml 29C6, was measured semiquantitatively by duplex RT-PCR for GAPDH and IL-3 or IL-5, respectively. Duplex PCRs were started with the primer pairs of IL-3 or IL-5. Following 3 cycles (IL-5) or 8 cycles (IL-3), respectively, the primer set of GAPDH was added. DNA fragments obtained after 29 cycles (IL-5) or after 34 cycles (IL-3) are shown.

Discussion

Recently established methods for obtaining highly purified human intestinal mast cells (19–21) enabled us to analyze the capacity of this cell type to produce cytokines in vitro. Our data demonstrate that mature human mast cells are capable of producing multiple cytokines including those with proinflammatory, chemotactic, and lymphocyte-modulating activities. We found that human intestinal mast cells cultured in the presence of SCF express the proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-18, as well as IL-8 and IL-16, without further stimulation. Moreover, upon activation by IgE receptor cross-linking, mast cells produce the Th2 cytokines IL-3, IL-5, IL-9, and IL-13. As reported for the immature human mast cell line HMC-1 (11, 14), human intestinal mast cells do not produce the Th1-type cytokines IL-2, IL-12, and IFN-γ (data on IFN-γ not shown). In accordance to our data, HMC-1 cells were found to express IL-1β and IL-8 constitutively (11, 14). In contrast, HMC-1 express IL-3 constitutively and IL-6 following stimulation with ionophore (14), whereas human intestinal mast cells express IL-6 without further stimulation, but IL-3 only after activation. Furthermore, IL-5 production was not found in HMC-1 cells (14). The differences in cytokine expression by HMC-1 and mature intestinal mast cells may be due to the fact that HMC-1 had to be stimulated with phorbol myristate acetate or ionophore instead of IgE receptor cross-linking because HMC-1 lack a functional IgE receptor (24). This may also be a reason for the failure to detect IL-5 in HMC-1 cells, because IL-5 expression in isolated human mast cells is induced only following stimulation by IgE receptor cross-linking, as shown here and in previous studies using human bone marrow or cord blood-derived mast cells (8, 25) or human lung mast cells (16).

Nasal mast cells derived from patients with perennial allergic rhinitics and human lung mast cells and were reported to express IL-4 (5, 7, 15, 26). We could not confirm these observations for human intestinal mast. This discrepancy could be due to the heterogeneity of mast cells derived from different tissues (1, 7). Moreover, the mast cells used in our study were isolated from nonallergic donors. Therefore, we cannot exclude the possibility that

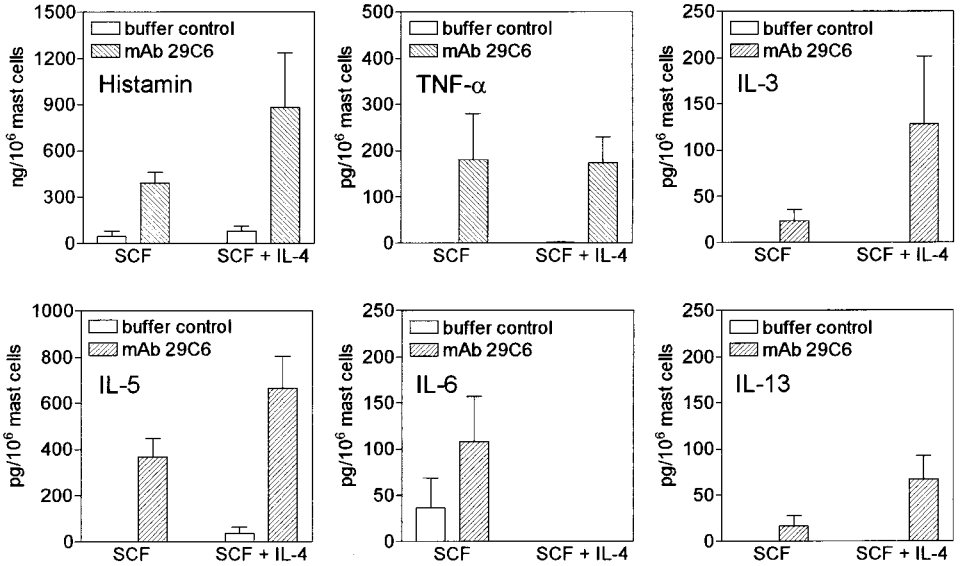

FIGURE 4. Histamine and cytokine release by isolated human intestinal mast cells. Measurement of histamine ($n = 6$), TNF-α ($n = 6$), IL-3 ($n = 3$), IL-5 ($n = 5$), IL-6 ($n = 4$), and IL-13 ($n = 4$) by RIA (histamine) or ELISA in supernatants derived from mast cells (purity 96–100%). Mast cells were cultured for 2 wk in the presence of SCF alone or SCF and IL-4 and then incubated for 6 h with buffer control or 100 ng/ml mAb 29C6. IL-4 and IL-10 were not detected in supernatants ($n = 4$). Detection limits are as indicated in *Materials and Methods*, and mean values (+SD) are shown.

intestinal mast cells in allergic individuals are capable of producing IL-4 as shown previously for nasal mast cells (26). It was reported that HMC-1 (14) but not tissue mast cells produce IL-10. We found IL-10 mRNA expression by intestinal mast cells in four of eight experiments, but did not detect IL-10 protein in mast cell-derived supernatants, which could be due to a late appearance of maximal IL-10 expression following stimulation, as described for HMC-1 (14). Extending previous studies performed with human bone marrow-derived mast cells, we found expression of the $CD4^+$ T cell chemoattractant cytokine IL-16 by intestinal mast cells, which may provide one mechanism for T cell recruitment in mast cell-dependent intestinal inflammation (9). Finally, we report for the first time that mature human mast cells are capable of producing IL-18. IL-18, first described as IFN-γ-inducing factor, ranks with other proinflammatory cytokines as a contributor to systemic and local inflammation because of its ability to induce expression of TNF-α, IL-1β, and both CXC and CC chemokines in human monocytes (27).

The occurrence of IL-3, IL-5, IL-9, and IL-13, which are thought to be produced by lymphocytes of the Th2 phenotype, is a characteristic feature of IgE-mediated allergic inflammation as well as parasitic infection with important pathophysiological implications (28). Here we show that the production of this set of cytokines may occur in a lymphocyte-independent fashion or is at least supported by mast cells, which, by their capacity to store the cytokines to some extent (1), may provide them more rapidly compared with Th2-type cells. IL-3 and IL-5 are known to promote activation and proliferation of eosinophils and basophils (23, 29–30). In a recent study (21), we reported that in vivo IL-5 expression by mast cells was detectable in patients with inflammatory bowel disease and food allergy, but not in healthy controls, suggesting that IL-5 production by mast cells is a typical feature of inflammatory conditions. Possibly, the same is true for other mast cell-derived Th2-type cytokines such as IL-3, IL-9, and IL-13.

IL-4 is known to regulate IgE production in B lymphocytes and development of IL-4- and IL-5-producing Th2-type lymphocytes (31, 32). Recombinant IL-4 was found to down-regulate IL-1β mRNA and to enhance IL-3 and IL-8 mRNA expression in HMC-1 cells (12, 13). Recently, Toru et al. (10) reported an enhanced production of IL-13 following IL-4 priming in immature cord blood-derived cultured mast cells. We could show that IL-4 enhances the release of histamine, leukotriene C_4, and IL-5 in mature human mast cells triggered by IgE receptor cross-linking (22). In this study, we demonstrate that IL-4 modulates the cytokine profile produced by mature human mast cells. IL-4 enhances the release of IL-3, IL-5, and IL-13 induced by IgE receptor cross-linking and, most interestingly, renders mast cells capable of producing and releasing IL-5 even without IgE receptor cross-linking. In contrast, we found that the expression of IL-6 in mast cells was completely inhibited by IL-4. The mechanism of IL-4 effects on cytokine production by human intestinal mast cells is unclear at present. Inhibition studies using actinomycin D indicated that both the up- and the down-regulation of cytokine expression by IL-4 is regulated at the transcriptional level. Because we did not find any IL-4 production by mast cells themselves, IL-4 that may regulate cytokine production in mast cells seems to be produced by other cells such as Th2 lymphocytes or basophils (22, 30, 32).

Furthermore, we could show recently that not only IgE receptor cross-linking and IL-4 but also bacteria effected cytokine production in human intestinal mast cells. We found that Gram-negative bacteria are capable of enhancing TNF-α production in these cells by an IgE-independent means (20). The first evidence for the induction of TNF-α synthesis in mast cells by bacteria came from mice models. In these studies, mast cell-derived TNF-α was found to have a protective role in bacterial infection (33, 34). In contrast, we found no effect of bacteria on the synthesis of Th2 cytokines (data not shown).

Fig. 5 summarizes our findings about the differential cytokine expression by human intestinal mast cells. All experiments were performed with mast cells cultured in the presence of SCF, because SCF is the only cytokine that provides human mast cell survival and is permanently present in tissues containing mast cells, thus presenting some kind of "physiological condition" as suggested earlier (18, 35). Our data suggest that the expression of proinflammatory cytokines occurs constitutively in human intestinal mast cells and may be up-regulated following triggering of the cells

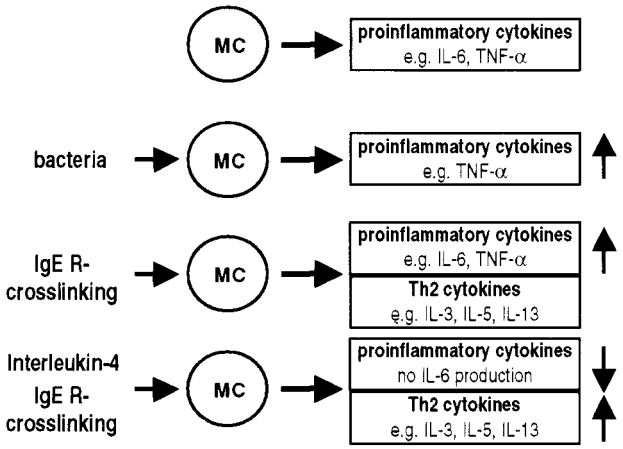

FIGURE 5. Differential cytokine expression by isolated human intestinal mast cells depending on the kind of agonist(s). For explanation, see text.

with IgE-independent agonists such as bacteria (20). Activation of mast cells by IgE receptor cross-linking also results in an up-regulation of proinflammatory cytokines but in addition induces the expression of Th2-type cytokines. IL-4 further enhances the expression of Th2-type cytokines but does not affect or even down-regulates proinflammatory cytokines such as IL-6.

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