

IL-4-Induced Priming of Human Intestinal Mast Cells for Enhanced Survival and Th2 Cytokine Generation Is Reversible and Associated with Increased Activity of ERK1/2 and c-Fos¹

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In synergy with stem cell factor (SCF), IL-4 strongly enhances mast cell proliferation and shifts IgE-dependent cytokine production in mature human mast cells toward an increased release of Th2 cytokines such as IL-3, IL-5, and IL-13 and a decreased IL-6 expression. In this study we analyzed the kinetics and the mechanisms of these IL-4 effects on mast cells purified from intestinal tissue. If the cells were first cultured with IL-4 for 14 days and then without IL-4 for another 14 days, mast cells lost the capacity of producing higher amounts of Th2 cytokines and regained the capacity of producing IL-6. The IL-4-induced up-regulation of mast cell proliferation and FcεRI expression was also reversible if IL-4 was withdrawn for 14 days. Interestingly, in contrast to IL-4, proliferation and phenotype of human intestinal mast cells were not affected by IL-13 although both cytokines were capable of inducing STAT6 activation. Instead, IL-4 treatment (but not IL-13 treatment) was associated with an increased activity of ERK1/2 and c-Fos, the downstream target of ERK1/2 and component of the transcription factor AP-1. Consistently, mast cell proliferation and cytokine expression in response to IL-4 was blocked by the MEK inhibitor PD98059. In summary, our data show that the IL-4 effects on human intestinal mast cell functions are reversible and accompanied by an increased activity of ERK1/2 and c-Fos. *The Journal of Immunology*, 2005, 174: 6751–6756.

Mast cells are of particular importance in immediate-type allergic and other inflammatory reactions. They are a potent cellular source of multiple cytokines suggesting an important role in immunoregulation and host defense. Moreover, they release a number of preformed and de novo-synthesized inflammatory mediators such as histamine, leukotrienes, proteases, PGs, and TNF-α upon stimulation with IgE-dependent and IgE-independent agonists (1–3). Cytokines were also identified as regulators of human mast cell function, of which stem cell factor (SCF)³ and IL-4 seem to be the most important ones (4–11). We reported recently that IL-4, known to regulate B and T lymphocyte differentiation, strongly enhances growth and IgE-dependent mediator release of human intestinal mast cells (5). Moreover we found that IL-4 shifts human intestinal mast cell cytokine production toward a Th2 profile (6, 7). Long-term challenge for 14 days with IL-4 and SCF compared with SCF alone enhanced the expression of IL-3, IL-5, and IL-13, which became detectable even without stimulation by FcεRI cross-linking, whereas suppressing the production of IL-6.

The transcription factor STAT6 is well known to be activated following cell stimulation with IL-4 and this activation of STAT6 was found to be critical for the enhanced expression of many IL-4

responsive genes (12). This finding was confirmed in vivo by mice deficient in STAT6 showing that STAT6 plays a central role in exerting IL-4-mediated effects such as the production of Th2 cytokines from T cells (13). STAT6 also acts as an antagonist of NF-κB binding and transcriptional activation found to be responsible for the IL-4-dependent suppression of TNF-α-induced E-selectin gene expression (14). IL-13 shares many biologic characteristics with IL-4. Both cytokines use the IL-4R α-chain as a common receptor component and activate STAT6 (15). Therefore, we compared IL-4 and IL-13 with regard to signal transduction and function in human mast cells. Moreover, we studied the reversibility of the IL-4-dependent mast cell priming for a Th2 dominated cytokine profile and enhanced proliferation. We found that IL-4 affects human mast cell functions by a signaling pathway involving rather ERK1/2 and c-Fos than STAT6.

Materials and Methods

Isolation and culture of human intestinal mast cells

Human intestinal mast cells were isolated from surgical tissue specimens (macroscopically normal border sections) derived from patients 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 (Hannover, Germany). The methods of mechanical and enzymatic tissue dispersion yielding single cell preparations containing $4 \pm 2\%$ (mean $\pm$ SD) mast cells have been described in detail elsewhere (4). 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 from Invitrogen Life Technologies), mast cells were enriched by positive selection of *c-kit* expressing cells using magnetic cell separation (MACS system; Miltenyi Biotec) and the mAb YB5.B8 (BD Pharmingen) as described (5, 6). The fraction containing the *c-kit*-positive cells (mast cell purity $60 \pm 25\%$) was cultured at a density of 2×10^5 mast cells per milliliter in medium supplemented with 50 ng/ml recombinant human SCF (Amgen) alone or in combination with 10 ng/ml IL-4 (Novartis Pharmaceuticals) and/or with 50 ng/ml IL-13 (PeproTech). After 2 wk at most, mast cell purity increased

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

from 98 to 100% and cells were used for the described experiments. Cultured mast cells were fed once a week by exchanging half of the culture medium supplemented with cytokines.

Stimulation of mast cells and inhibitors

Mast cells were stimulated 1 wk after the last feeding with 10 ng/ml IL-4, 50 ng/ml IL-13, 50 ng/ml SCF, or by FcεRI cross-linking using 100 ng/ml mAb 22E7 (provided by Hoffmann-LaRoche) directed against a non-IgE binding epitope of the FcεRI α-chain. Mast cells were treated with the inhibitor PD98059 (Alexis) at the concentrations indicated. PD98059 was dissolved in DMSO. Controls were conducted with DMSO at a concentration equivalent to the dilution of the inhibitor (0.1%).

RNA preparation and RT-PCR

Total RNA was prepared from 1×10^5 human intestinal cells containing 98–100% mast cells, and RT-PCR was performed as described (5, 6). The following specific sense and antisense primers were used for the cDNAs: GAPDH (product size, 128 bp) 5'-TGGTCTCCTCTGACTTCAAC-3', 5'-CCTGTTGCTGTAGCCAAATT-3'; c-Fos (268 bp) 5'-CAGTGGAACTGTCAAGAGC-3', 5'-AAGGAAGACGTGTAAGCAGTG-3'; IL-3 (193 bp) 5'-CATCTCTCACACATTCCAGG-3', 5'-CAGTTCACACTCCAGGCAAT-3'; IL-5 (157 bp) 5'-GGAATAGGCACACTGGAGAGTCAA-3', 5'-ACTCTTGACAGTAGCTAGG-3'; IL-6 (226 bp) 5'-GATGGATGCTTCCAATCTGG-3', 5'-TGGCATTTGTGGTTGGGTCA-3'; IL-13 (159 bp) 5'-CATTGCTCTCACTTGCCTTGG-3', 5'-CAGCTGTCAGGTATGCTC-3'; IL-13Rα1 (148 bp) 5'-GGAGAATACATCTTGTTCATGG-3', 5'-GCGCTTCTTACCTATCTTCTTGG-3'; IL-13Rα2 (338 bp) 5'-AATGGCTTTCGTTTGTGG-3', 5'-ACGCAATCCATATCCTGAAC-3'. To quantify the mRNA expression, real-time PCR contained 1.5 μl of cDNA template (sample or standard), 10.5 μl of H₂O, 12.5 μl of SYBRGreen PCR Master Mix (Applied Biosystems), and 0.3 μl of 20 μM sense and antisense primer were performed in optical tubes (Applied Biosystems). Reaction mixture without the cDNA was used as negative control in each run. All reactions were performed in the ABI PRISM 7700 Sequence Detector (Applied Biosystems). Specificity of the reaction was controlled by separation of the PCR products on 1% agarose gel. To calculate the relative transcription level of the genes of interest, the copy number for the mRNA of the gene of interest was calculated and divided by that of GAPDH mRNA.

Western blot analysis

To obtain whole cell extracts $0.5\text{--}1 \times 10^6$ mast cells were lysed in extraction buffer containing 25 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 0.5 mM EGTA, 0.05% Triton X-100, 10 mM 2-ME, supplemented with the protease inhibitor mix Complete Mini (Roche Diagnostics). Protein concentration was determined using Bio-Rad protein assay. Cell extracts (10–20 μg of protein each) were separated on a 12% SDS-polyacrylamide gel and blotted onto a nitrocellulose membrane (Schleicher & Schuell) in 0.1% SDS, 20% methanol, 400 mmol/L glycine, 50 mmol/L Tris-HCl, pH 8.3, at 4°C for 4 h at 40 V by electroblotting using Trans-Blot Cell (Bio-Rad). Membranes were blocked with 5% skim milk in PBS containing 0.1% Tween 20 overnight. Membranes were probed with anti-phospho-ERK (MAPK)-1/2 mAbs and anti-ERK (MAPK)-2 (Alexis) or with anti-phospho-STAT6 pAb (Alexis) and anti-STAT6 polyclonal Ab (Santa Cruz Biotechnology). The membranes were stripped following probing with anti-phospho-STAT6 pAb or anti-phospho-ERK1/2 mAb using Restore Western Blot Stripping buffer (Pierce) and a second time probed with anti-STAT6 polyclonal Ab or anti-ERK2 mAb, respectively. The Ag-Ab complexes were visualized using an electrochemiluminescence detection system as described by the manufacturer (NEN Life Science). The signals obtained were measured by a bioimaging analyzer (Fuji BAS-1000; Raytest).

Immunocytochemistry

Cultured cells were harvested, cytocentrifuged on slides, and fixed in acetone for 10 min. Immunocytochemistry was performed using primary mAbs (overnight incubation at 4°C) against human tryptase (230 ng/ml; Chemicon International) and human chymase (100 ng/ml, Chemicon International) and the labeled streptavidin-biotin LAB-SA detection system (Histostain-Plus kit; Zymed Laboratories) as described (5).

Flow cytometry

For each labeling, 1×10^5 mast cells were washed twice (5 min, 400 g) and resuspended in PBS supplemented with 0.1% BSA, 0.1% sodium azide, and 250 μg/ml rabbit IgG. Cells were labeled using the primary mAb directed against FcεRI α-chain (mAb 22E7, 2 μg/ml; Hoffmann-LaRoche).

Appropriate isotype controls were performed. After incubation at 4°C for 45 min, cells were washed and labeled with the secondary Ab goat anti-mouse IgG1 FITC (Southern Biotechnology Associates). Flow cytometric analysis was performed using the FACSCalibur system (BD Biosciences).

Statistics

Data from multiple experiments were expressed as mean + SD, if not indicated otherwise. Differences between groups were analyzed for statistical significance defined as $p < 0.05$ (one-tailed paired *t* test).

Results

The IL-4-dependent priming of human intestinal mast cells concerning survival, FcεRI expression, and cytokine expression is reversible

To analyze whether the IL-4-dependent mast cell priming is reversible, mast cells purified from intestinal tissue were cultured for 14 days in the presence or absence of IL-4, harvested, washed, and then cultured for additional 14 days in the presence or absence of IL-4. The experimental design is shown in Fig. 1A. After 14 or 28 days, mast cell recovery was determined. After 14 days, mast cell numbers were two to three times higher if the cells were cultured with IL-4 in addition to SCF compared with cells cultured with SCF alone. After 28 days, we found a pronounced enhancement of mast cell numbers if IL-4 was present the 14 days before measurement (1-2 or 2-2). Vice versa, the mast cell numbers were similar if IL-4 was absent the 14 days before analysis (1, 1-1, or 2-1) (Fig. 1B). Real-time RT-PCR analysis revealed that the enhancement of Th2-type cytokines (IL-3, IL-5, IL-13) and the suppression of IL-6 in response to IL-4 were also reversible (Fig. 1, C–F). If IL-4 was present the 14 days before measurement, the expression of Th2 cytokines was up-regulated whereas IL-6 expression was down-regulated (2, 1-2, or 2-2). If the cells were cultured without IL-4 before cytokine measurement, IL-6, but not Th2 cytokines, was up-regulated (1, 1-1, or 2-1). If the cells were first cultured with IL-4 for 14 days and then without IL-4 for another 14 days, mast cells lost the capacity of producing higher amounts of Th2 cytokines and regained the capacity of producing IL-6 (2-1). FACS analysis revealed that the up-regulation of FcεRI was also reversible if IL-4 was withdrawn. FcεRI was up-regulated on mast cells if the cells were cultured for 14 days with IL-4 in addition to SCF compared with cells cultured with SCF alone. The up-regulation of the receptor expression was reversible if the cells were cultured for another 14 days without IL-4 but was unchanged if the culture with IL-4 was continued (Fig. 1G). Interestingly, the IL-4-dependent decrease of mast cells containing both tryptase and chymase from 50 to 60% (0 days) to 40–20% (14 days) was discontinued when IL-4 was withdrawn, but the tryptase to chymase mast cell ratio was not reversible within 14 days (Fig. 1H).

Mast cell proliferation and mast cell phenotype is not affected by IL-13

IL-13 is known to share the IL-4R α-chain with IL-4 (15). Thus, it should be clarified whether IL-13 is also able to affect mast cell proliferation and mast cell phenotype in a similar manner as IL-4. Mast cells express mRNA for the IL-13R components IL-13Rα1 and IL-13Rα2 (Fig. 2A). IL-13Rα1 initially binds IL-13 with subsequent recruitment of the IL-4Rα to transduce a signal, whereas the IL-13Rα2 can bind IL-13 in the absence of IL-4Rα, but its role in IL-13 signaling is still unclear (16). IL-13 alone had no effect on mast cell survival and all mast cells died within 3–7 days of culture (data not shown). Unlike IL-4, IL-13 did not enhance mast cell proliferation after 14 days of culture in combination with SCF (Fig. 2B). Further, IL-13 (in combination with SCF) did not cause phenotypical changes, which are present after culture with IL-4. Mast cell subtype distribution (Fig. 2C), FcεRI expression (Fig.

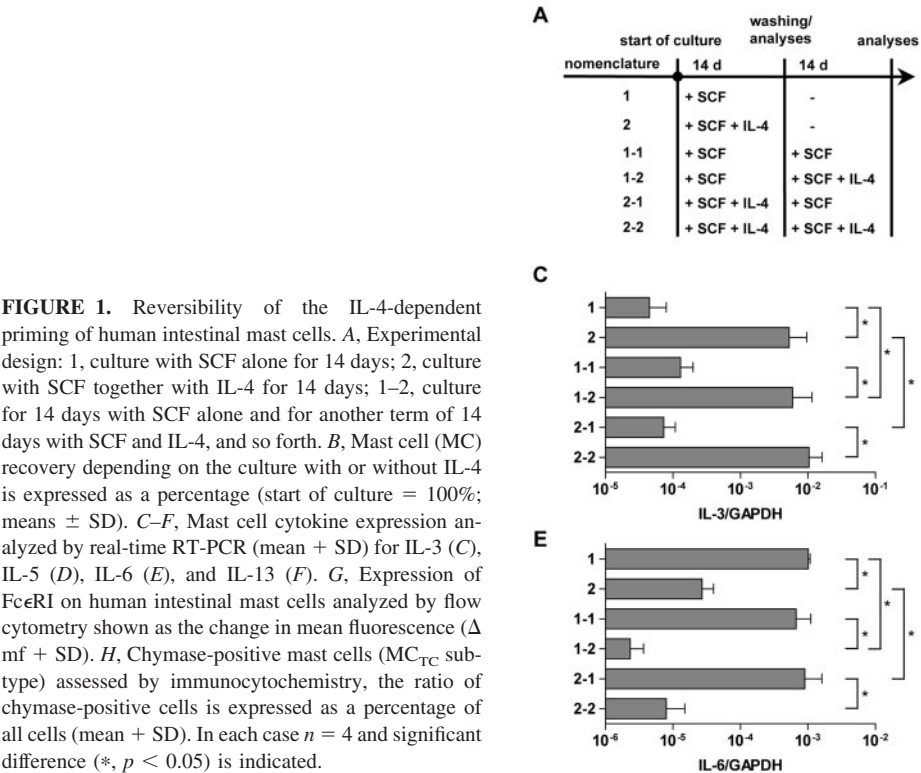


FIGURE 1. Reversibility of the IL-4-dependent priming of human intestinal mast cells. **A**, Experimental design: 1, culture with SCF alone for 14 days; 2, culture with SCF together with IL-4 for 14 days; 1-2, culture for 14 days with SCF alone and for another term of 14 days with SCF and IL-4, and so forth. **B**, Mast cell (MC) recovery depending on the culture with or without IL-4 is expressed as a percentage (start of culture = 100%; means $\pm$ SD). **C–F**, Mast cell cytokine expression analyzed by real-time RT-PCR (mean $\pm$ SD) for IL-3 (**C**), IL-5 (**D**), IL-6 (**E**), and IL-13 (**F**). **G**, Expression of Fc ϵ RI on human intestinal mast cells analyzed by flow cytometry shown as the change in mean fluorescence (Δ mf $\pm$ SD). **H**, Chymase-positive mast cells (MC_{TC} subtype) assessed by immunocytochemistry, the ratio of chymase-positive cells is expressed as a percentage of all cells (mean $\pm$ SD). In each case $n = 4$ and significant difference (*, $p < 0.05$) is indicated.

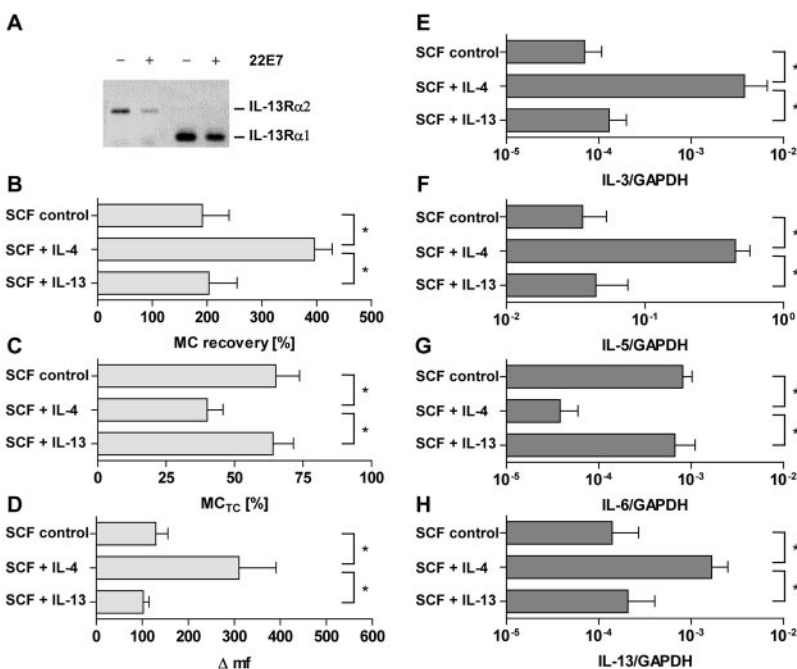


FIGURE 2. Long-term treatment of human intestinal mast cells with IL-13. Mast cells were cultured with SCF alone, with SCF and IL-4, or with SCF and IL-13 for 14 days. **A**, Detection of IL-13R α 1-chain and α 2-chain in human intestinal mast cells by RT-PCR. The IL-13R α 1-chain and α 2-chain were down-regulated in response to Fc ϵ RI cross-linking using 100 ng/ml mAb 22E7 for 90 min. **B**, Mast cell (MC) recovery is expressed as a percentage (start of culture = 100%, mean $\pm$ SD). **C**, Chymase-positive mast cells (MC_{TC} subtype) assessed by immunocytochemistry, in which the ratio of chymase-positive cells is expressed as a percentage of all cells (mean $\pm$ SD). **D**, Expression of Fc ϵ RI on mast cells analyzed by flow cytometry shown as Δ mean fluorescence (Δ mf) $\pm$ SD. **E–H**, Mast cell cytokine expression analyzed by real time RT-PCR (mean $\pm$ SD) for IL-3 (**E**), IL-5 (**F**), IL-6 (**G**), and IL-13 (**H**). In each case, $n = 3$ and significant difference (*, $p < 0.05$) is indicated.

2D), and cytokine expression (Fig. 2, E–H) were unchanged if mast cells were cultured for 14 days with IL-13 and SCF compared with mast cells cultured with SCF alone. Induction of mRNA expression for IL-3, IL-5, and IL-13 in mast cells cultured with IL-13 in addition to SCF was not found. In contrast, if the cells were cultured in the presence of IL-4 in addition to SCF the expression of mRNA for IL-3, IL-5, and IL-13 was induced.

STAT6 becomes activated following short-term stimulation with IL-4 or IL-13 in human intestinal mast cells whereas long-term treatment of mast cells with SCF and IL-4 causes an increased activity of ERK1/2 and c-Fos

The transcription factor STAT6 is well known to become phosphorylated through IL-4 and is critical in the activation or enhanced expression of many IL-4 responsive genes (12). To analyze activation of STAT6 in human intestinal mast cells, mast cells were stimulated with IL-4 or with IL-13. Fig. 3A shows that STAT6 became activated in human intestinal mast cells following stimulation by 10 ng/ml IL-4 for 15 min. The same was found if the cells were stimulated with 50 ng/ml IL-13 for 15 min. After long-term treatment of the cells with IL-4 resulting in the change of cytokine mRNA expression profile, STAT6 was not phosphorylated in mast cells (Fig. 3A). The MAPK ERK1/2 is known to be involved in cell proliferation and cytokine expression (17, 18). To test activation of the ERK1/2 during cell culture, mast cells cultured for 14 days in the presence of SCF were treated 1 wk after the last feeding with 10 ng/ml IL-4, 50 ng/ml IL-13, or 50 ng/ml SCF for 15 min. ERK1/2 phosphorylation was found in response to SCF stimulation but not in response to IL-4 or IL-13 (Fig. 3B).

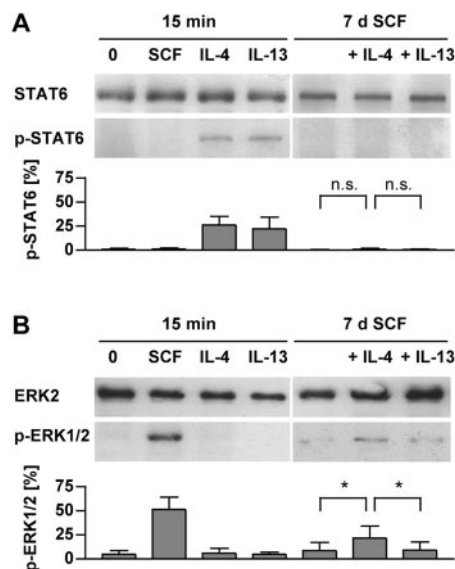


FIGURE 3. Activation of STAT6 by IL-13 and IL-4. *A*, Western blot analysis with Abs against STAT6 or phospho-STAT6 (p-STAT6) using cell extracts of untreated mast cells, mast cells challenged with IL-4 or IL-13, respectively, for 15 min and mast cells after culture with IL-4 or IL-13, respectively, in addition to SCF for 7 days. Additionally, activation of STAT6 is expressed as a percentage of phospho-STAT6 in relation to total STAT6 (mean + SD, $n = 3$). n.s., No significant difference. *B*, Activation of ERK1/2. Western blot analysis with Abs against ERK2 or phospho-ERK1/2 (p-ERK1/2) using mast cell extracts of untreated mast cells, mast cells challenged with IL-4 or IL-13, respectively, for 15 min, and mast cells after culture with IL-4 or IL-13, respectively, in addition to SCF for 7 days. Additionally, activation of ERK1/2 is expressed as a percentage phospho-ERK1/2 in relation to ERK2 ($n = 3$). *, $p < 0.05$ for significant difference.

We found higher levels of activated ERK1/2 in mast cells after culture with IL-4 in addition to SCF compared with cells cultured with SCF alone or with SCF and IL-13 (Fig. 3B). Moreover, we analyzed the mRNA expression for c-Fos, the downstream target of ERK1/2. The activity of c-Fos, a component of the transcription factor AP-1, is regulated by increased transcription (19, 20). Consistent with our finding of an increased activation of ERK1/2 in response to culture with IL-4, we found a clear up-regulation of c-Fos mRNA expression in mast cells cultured with SCF and IL-4 compared with that cultured with SCF alone or with SCF and IL-13 (Fig. 4A).

Mast cell proliferation and cytokine expression in response to IL-4 is corresponding with an increased activity of c-Fos and blocked by the MEK inhibitor PD98059

To examine the time dependency, mast cell cytokine expression was analyzed following culture in the presence of IL-4 for 90 min and 1, 3, and 7 days. The up-regulation of c-Fos mRNA expression started after 1 day of culture (Fig. 4B). The up-regulation of mRNA expression for IL-3 (Fig. 4C), IL-5 (Fig. 4D), and IL-13 (Fig. 4F) as well as the down-regulation of IL-6 (Fig. 4E) started after 3 days of culture. A significant induction of IL-5 mRNA expression was found after 7 days. Thus, the induction of mRNA expression for IL-3, IL-5, and IL-13 was not associated with a phosphorylation of STAT6 after short-term stimulation with IL-4. In contrast, the change of mRNA expression for IL-3, IL-5, IL-6, and IL-13 after 3–7 days corresponds with the increased activity of c-Fos.

To further clarify the involvement of ERK1/2 and c-Fos in the IL-4-activated cell proliferation and cytokine expression, mast cells were cultured with or without IL-4 in addition to SCF in the presence or absence of PD98059, a selective inhibitor of MEK (ERK kinase). PD98059 blocks the activity of MEK and thereby inhibits the phosphorylation and activation of ERK (21). The cells were counted and fed after 6 days, harvested, and analyzed after 10 days of culture. The mast cell proliferation caused by IL-4 was inhibited in response to 1 or 10 μ M PD98059. Fig. 5A shows the result after treatment with 10 μ M PD98059. Following 6 days the mast cell numbers were the same if the cells were cultured with

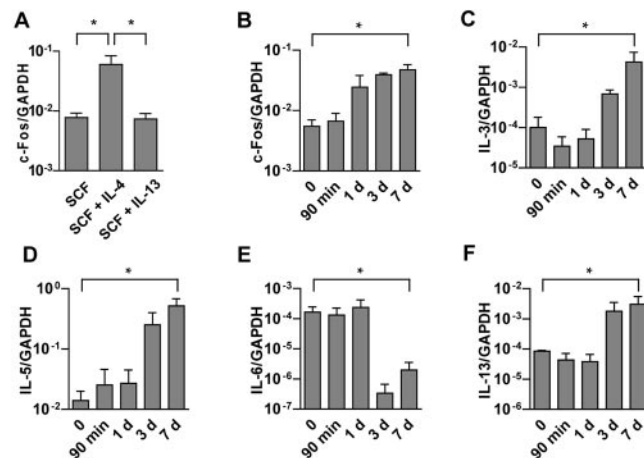


FIGURE 4. Expression of c-Fos and cytokines in response to IL-4. *A*, The mRNA expression for c-Fos determined by real-time RT-PCR (mean + SD; $n = 4$); *, $p < 0.05$, in mast cells cultured with SCF and IL-4, with SCF and IL-13, or with SCF alone for 14 days. *B* and *C*, Time-dependent expression of c-Fos and cytokines in response to culture with IL-4 analyzed by real-time RT-PCR (mean + SD), *, $p < 0.05$, for c-Fos (*B*), IL-3 (*C*), IL-5 (*D*), IL-6 (*E*), and IL-13 (*F*).

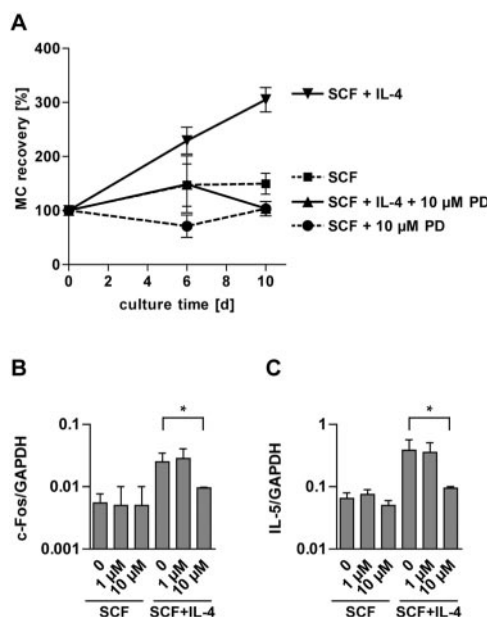


FIGURE 5. Effect of PD98059 on mast cell recovery and expression of c-Fos and IL-5. *A*, Mast cell (MC) recovery of cells cultured with or without IL-4 in the presence or absence of 10 μ M PD98059 (PD) is expressed as a percentage (start of culture = 100%, mean $\pm$ SD). *B* and *C*, The mRNA expression for c-Fos (*B*), and IL-5 (*C*) was analyzed by real-time RT-PCR (mean $\pm$ SD; $n = 3$), *, $p < 0.05$, in mast cells cultured with SCF and IL-4, or with SCF alone for 10 days in the presence or absence of PD98059 (1 or 10 μ M).

SCF alone or with 1 or 10 μ M PD98059 in addition to SCF and IL-4. The mast cell numbers were similar if the cells were cultured with SCF alone, with PD98059 in addition to SCF, or with PD98059 in addition to SCF and IL-4 for 10 days. This observation further supports a role of ERK in the IL-4-dependent increase of mast cells during cell culture. Moreover, Fig. 5*B* shows that the up-regulated c-Fos expression in response to culture with IL-4 was inhibited after treatment with 10 μ M PD98059. The same inhibition pattern was found for IL-5 (Fig. 5*C*).

Discussion

During the last years IL-4 has been recognized as an important regulator of human mast cells derived from intestine, lung, skin, or cord blood (5–11). In synergy with SCF, IL-4 enhances proliferation, mediator release, and Th2 cytokine generation. In this study we show that this IL-4-induced mast cell priming is reversible if IL-4 is withdrawn. Moreover, our data suggest that IL-4 affects human mast cell functions using a signaling pathway involving ERK1/2 and c-Fos.

So far, Th populations were analyzed regarding reversibility of their cytokine profiles (22). It is well known that cytokines are important in the development of different cytokine producing Th1 and Th2 cells. IL-12 promotes Th1 development with IFN- γ secretion, whereas IL-4 promotes Th2 development (23, 24). Th1 cells resulting after 1 wk in the presence of IL-12 plus anti-IL-4 Abs could convert to Th2 cells when restimulated with IL-4, and Th2 cells resulting after 1 wk with IL-4 could give rise to Th1 cells upon restimulation with IL-12 plus anti-IL-4. Interestingly, the cytokine profiles of long-term Th1 and Th2 populations arising originally from repeated stimulation in IL-12 or IL-4 for 3 wk were not reversible (22). In contrast, we could show that the IL-4-induced phenotype of human intestinal mast cells including the cytokine expression profile was almost reversible following 2 wk.

The most prominent IL-4-dependent transcription factor is STAT6. STAT6-deficient mice have a phenotype similar to IL-4-deficient mice or mice after treatment with Abs to IL-4 or to IL-4R. Thus, it has been suggested that STAT6 plays an essential role in exerting IL-4-mediated biologic responses such as the production of Th2 cytokines from T cells (13). IL-13, which is known to use the IL-4R α -chain as a receptor component, shares many biologic characteristics with IL-4 and is known to activate STAT6 as well (15, 16). Interestingly, we found that IL-13 did not influence the cytokine expression profile, the proliferation rate, the expression of Fc ϵ RI, and the chymase expression in human intestinal mast cells although we found that STAT6 becomes equally activated following short-term stimulation of human intestinal mast cells with IL-4 or IL-13. Maybe, STAT6 is not crucial for the IL-4-mediated regulation of human mast cells. STAT6-independent Th2 development has been observed in vivo and in vitro (25). The transcription factor GATA-3, found to be induced by IL-4 through STAT6 early in the Th2 development, was also shown to induce Th2 development in STAT6-deficient T cells (26). The described GATA-3 autoactivation causing the Th2 development and commitment was however different from our findings concerning mast cell regulation, independent from IL-4. Moreover, we did not detect differences of GATA-3 expression in human mast cells dependent on IL-4 stimulation (our unpublished observations). Interestingly in regard to our data, in mice STAT6 was found to be essential for IL-4-induced proliferation of T cells but not for IL-4-induced proliferation of bone marrow-derived mast cells (13, 27–29). The IL-4-induced enhancement of mast cell proliferation and survival occurred in a γ -chain- and a Jak-3-dependent manner, but was independent of STAT6. Noteworthy, IL-4, but not IL-13, was able to induce proliferation and survival of murine bone marrow-derived mast cells (29).

However, IL-4 causes both the shift of the cytokine pattern toward a Th2 phenotype and the increase of proliferation and mediator release in mature human mast cells (5, 6). ERK1/2 is known to be involved in cell proliferation and cytokine expression (17, 18). We found phosphorylation of ERK1/2 in mast cell extracts even 1 wk after the last feeding provided that IL-4 was present during culture. Wery et al. (30) observed increased tyrosine phosphorylation of ERK1/2, which was accompanied by an enhanced catalytic activity of the enzyme following IL-4 stimulation in human keratinocytic cell lines. They also found that in keratinocytes, but not in T lymphocytes, Shc is phosphorylated, suggesting its possible implication in ERK activation in keratinocytes caused by IL-4. The phosphorylation of the adapter protein Shc may be the link between IL-4R and MAPK.

In contrast to the findings observed in keratinocytes we did not detect activation of ERK1/2 in response to short-term stimulation with IL-4. Noteworthy in this respect, Levings et al. (31) reported that the inability of IL-4 to activate Ras/Raf/ERK pathway and subsequently to promote cell cycle progression is complemented by expression of activated N-Ras or conditionally active mutants of Raf1. Moreover, they found that IL-4 and Raf1 not only synergize to promote proliferation, but also to increase JNK activity. Maybe, SCF is responsible for the activation of Ras/Raf in human mast cells that synergize with IL-4 to enhance proliferation and cytokine expression. In accordance with the activation of ERK1/2 we found increased mRNA expression for c-Fos, the downstream target of ERK1/2 and component of the transcription factor AP-1, in response to culture with IL-4. To examine a causal link of IL-4 effects and ERK activation we treated mast cells with the ERK kinase inhibitor PD98059 (21). We found that mast cell proliferation as well as cytokine expression in response to IL-4 was inhibited by PD98059. These findings further suggest a role of

ERK1/2 and c-Fos in IL-4-dependent effects although we cannot exclude an inhibitory effect of PD98059 on other kinases and thus a lack of specificity.

Noteworthy, the increased activity of ERK1/2 and c-Fos corresponds not only with the induced Th2 cytokine expression in response to IL-4 but also with the cytokine expression in response to FcεRI cross-linking suggesting a general role of the ERK1/2 branch of the MAPK pathway for cytokine expression in human mast cells (32).

In conclusion, our data show that IL-4-induced priming of human intestinal mast cells for enhanced survival and Th2 cytokine generation is reversible and that ERK1/2 and c-Fos might be involved in these IL-4-dependent effects. These observations further emphasize that mast cell functions are subject to a considerable diversity and plasticity depending on the cytokine milieu.

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Disclosures

The authors have no financial conflict of interest.

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