c-kit Ligand: A Unique Potentiator of Mediator Release by Human Lung Mast Cells
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Summary
Mast cells (MC) play a central role in extrinsic allergic reactions such as asthma and may participate in other inflammatory and fibrotic processes. However, with the exception of immunoglobulin E (IgE) receptor-dependent stimulation, no secretagogues of human lung MC have yet been described. It is also unclear whether mediator release can be regulated by certain cytokines as demonstrated previously in basophils and other human inflammatory effector cells. Here, we show that the c-kit ligand (KL), a recently identified stem cell growth factor, at concentrations 10–100 times lower than that required to promote cell proliferation, enhances the release of histamine and leukotriene C4 in response to IgE receptor crosslinking of human lung MC. KL does not induce mediator release per se, but increases the sensitivity of MC to anti-IgE receptor stimulation and also enhances mediator release to maximally effective concentrations of anti-IgE receptor antibody. By contrast, a large number of cytokines examined, including the mast cell growth factors/agonists in rodents, interleukin 3 (IL-3), IL-4, IL-9, and nerve growth factor, were ineffective in this respect. These findings suggest a unique role of KL in regulating effector functions of human mucosal MC.

The role of human mast cells (MC)1 in mediating allergic reactions is well known (1–4). The occurrence of MC mediators such as histamine and arachidonic acid metabolites after in vivo exposure of asthmatic individuals to inhalative allergens implicates MC as major effector cells in allergic lung disease (1). Furthermore, there is increasing evidence that MC are involved in delayed-type hypersensitivity diseases and other pathophysiologic processes such as wound healing, fibrosis, and neuroimmunologic disorders (4–6). MC are found predominantly in the skin, lung, heart, and intestine; they represent the only tissue cell type containing histamine and bearing high-affinity IgE receptors (1–4). In rodents, two subpopulations of MC have been identified: connective tissue-type MC, found in the skin and the peritoneal cavity, and mucosal MC, found predominantly in the respiratory tract and gastrointestinal mucosa (3, 4). In humans, the existence of a similar heterogeneity of MC is suggested by histochemical and functional studies (4). Whereas MC from human skin respond to various stimuli such as anti-IgE, the anaphylatoxins C3a and C5a, and the neuropeptide substance P (7, 8), IgE receptor crosslinking represents the only known way to induce mediator release in mucosal-type human lung MC.

Several studies have shown that the effector function of inflammatory effector leukocytes such as neutrophils, eosinophils, and basophils is regulated by distinct sets of cytokines (9–15). For example, basophil mediator release in response to IgE receptor crosslinking and to diverse other IgE-independent agonists is strongly modulated by the hematopoietic growth factors IL-3, IL-5, granulocyte/macrophage CSF (GM-CSF), and nerve growth factor (NGF) (13–15; Bischoff, S. C., and C. A. Dahinden, manuscript submitted for publication). In the rat, peritoneal MC release histamine in response to NGF (16). By contrast, it is largely unknown whether cytokines or growth factors affect MC mediator release in humans.

Recently, a novel hematopoietic growth factor named stem cell factor, mast cell growth factor, or c-kit ligand (KL) has been identified in rodents. In the rodent system, KL is involved in hematopoiesis, gametogenesis, and development of MC and melanocytes (17). By use of KL cDNA as a probe, the human homologue was cloned and the soluble form of recombinant human KL protein (rhuKL) was expressed and purified (18). However, with the exception of a stimulatory activity on colony formation in bone marrow cultures in synergy with IL-3, G-CSF, GM-CSF, and erythropoietin (18–20), the biological effects of KL in the human system are poorly defined.

In this study we show that low concentrations of rhuKL strongly enhance IgE-dependent mediator release by human lung MC. This observation suggests that KL has an important role in MC-associated lung diseases, such as asthma.
Materials and Methods

Reagents. Commercial reagents were obtained from the following sources: Hepes, Calbiochem-Behring Corp. (La Jolla, CA); EDTA, Fluka AG (Buchs, Switzerland); Percoll and gelatin, Pharmacia Fine Chemicals (Uppsala, Sweden); DNase I, pronase, collagenase D, elastase, and fatty acid–free BSA, Boehringer Mannheim Inc. (Mannheim, FRG); chymopapain, Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest purity available.

Buffers. Tyrode buffer contains 137 mM NaCl, 2.7 mM KCl, 0.36 mM Na2HPO4, and 5.55 mM glucose. TE buffer is Tyrode buffer containing 2 mM EDTA. TGMD buffer is Tyrode buffer supplemented with gelatin (1 mg/ml), 1.23 mM MgCl2, and 15 μg/ml DNase. Hepes buffer contains 20 mM Hepes, 125 mM NaCl, 5 mM KCl, and 0.5 mM glucose. HA buffer is Hepes buffer plus BSA (0.25 mg/ml). HACM buffer is HA buffer supplemented with 1 mM CaCl2 and 1 mM MgCl2.

Cell Preparation. Human lung MC were isolated by the four-step enzymatic tissue dispersion method described by Schulman et al. (21) with some modifications. Macrophagocytically normal human lung tissue (10-40 g) was obtained from pneumoectomy or lobectomy specimens removed from patients with lung cancer. Immediately after resection the tissue was placed in TE buffer at 4°C. Within 1-2 h, lung tissue was dissected free of major bronchi and blood vessels, cut into fragments of 0.2-1 g, washed twice in TE buffer, and incubated in TE buffer containing pronase (3 mg/ml) and chymopapain (0.75 mg/ml) at room temperature. During this first digestion step, the tissue was chopped finely with scissors. After 20 min the lung fragments were separated from freed cells by filtration through a polyamid Nybolt filter (pore size, 100 μm; Swiss Silk Bolting Cloth Manufacturing Co. Ltd., Zürich, Switzerland), before the fist digestion step under identical conditions. The lung fragments were then washed in TGMD buffer and incubated twice for 20 min at 30°C in TGMD buffer containing collagenase D (1.5 mg/ml) and elastase (0.15 mg/ml). These digestion steps were also followed by separating freed cells from the lung fragments by filtration. The freed cells obtained after digestion steps 3 and 4 were pooled, washed twice in HA buffer (400 g, 10 min, 4°C), filtered through a Nybolt filter (pore size, 100 μm), and finally resuspended in HACM buffer at a cell density of 0.5-2 × 10^6 cells/ml.

Differential counts of cytotoxicity smears stained with May-Grünwald-Giemsa revealed a MC percentage of 4-14% after enzymatic digestion. In some experiments, MC were further purified by discontinuous Percoll gradient centrifugation. Percoll gradients were layered in 2-ml aliquots of 1.090, 1.080, 1.070, and 1.062 g/ml Percoll solutions, the latter containing ~10^7 cells. The tubes were centrifuged (500 g, 40 min, 20°C), and cells at each interface were collected, washed twice in HA buffer (400 g, 10 min, 4°C), and resuspended in HACM buffer. MC found at the 1.070/1.080 and 1.080/1.090 interfaces were free (<1%) of other lung tissue cells, whereas the cell fractions at the 1.062/1.070 and <1.062 interfaces contained variable proportions of tissue cells and 20% and 5% MC, respectively.

Mediator Release Assay. The release experiments were performed in a shaking water bath at 37°C as described (13-15). After a warming-up period of 10 min, the cells were preincubated with cytokines or in buffer for 20 min or as indicated, followed by the addition of the triggering agent. Unless indicated otherwise, the release reaction was stopped by placing the tubes in ice-cold water 40 min after addition of the second stimulus. Cells were separated from supernatants by centrifugation (400 g, 10 min, 4°C). Histamine and sulfidoleukotrienes (LTc4/D4/E4) were measured in the supernatants as described (13-15; Bischoff, S. C., and C. A. Daehinden, manuscript submitted for publication). Histamine content per MC was 2.5 ± 0.3 pg (mean ± SEM from all experiments), as determined by measurement of total histamine per milliliter of cell suspension after lysis. Although no basophils were found in dispersed lung cells by microscopy, as a further control of basophil contamination, the MC were sequentially exposed to IL-3 and FMLP in all the experiments. Under these conditions being optimal for basophil mediator release, no mediator released by MC occurred (15).

Cell Stimuli. The soluble forms of purified recombinant human KL (rhuKL; molecular mass of 18.6 kD; 165 amino acids; end sequences: MEGICRNPVT ...... TKPFMLPPVA, single-letter code) and murine KL (rmuKL; molecular mass of 18.4 kD; 165 amino acids; end sequences: MKEICGNPVT ...... TKPFMLPPVA) were kindly provided by Hoffman-La Roche (Basel, Switzerland). In some experiments, unpurified COS-supernatants containing recombinant murine KL provided by the Genetics Institute (Cambridge, MA) was used. Dilutions at 1:200 of the COS-conditioned medium gave half-maximal activity as measured by the MO-7 proliferation assay (22). KL dilutions were made in Hepes buffer containing BSA (1 mg/ml). All other cytokines were used in the recombinant human form: IL-1 (15, 23), IL-3 (13-15), IL-5 (15), IL-8 (24) GM-CSF (13, 15, 25), TNF-α, (23), and NGF (Bischoff, S. C., and C. A. Daehinden, manuscript submitted for publication); IL-4 and TGF-β were provided by Sandoz Ltd. (Basel, Switzerland); INF-γ was from Hoffmann-La Roche; IL-9, macrophage CSF (M-CSF), and leukemia inhibitory factor (LIF) were gifts from the Genetics Institute; IL-2, IL-6, and granulocyte CSF (G-CSF) were purchased from Amgen, Inc. (Thousand Oaks, CA); IL-7 was from Bissendorf Biochemicals (Hanover, FRG); fibroblast growth factor (FGF) and insulin-like growth factor I (IGF I) were from Boehringer Mannheim. MC were triggered for mediator release by the purified mAb 29C6 directed against a non-IgE–binding epitope of the high-affinity IgE receptor α chain (anti-IgE Ab). This antibody, which binds with high affinity (Ka = 3.2 nM) to the IgE receptor, was a generous gift from Hoffmann-La Roche (Nutley, NJ). Anti-IgE mAb LE27 (anti-IgE Ab) was purified as described (25); polyclonal anti-IgE serum was purchased from Serotec, Inc. (Oxford, UK). The complement-derived anaphylatoxins C3a and C5a were purified from human serum (14, 25). Platelet-activating factor C18 (PAF) was purchased from Novabiochem (Läufelfingen, Switzerland), substance P from Boehringer Mannheim, and FMLP from Bachem AG (Bubendorf, Switzerland). All substances were stored in small concentrated aliquots at −70°C and thawed once before use.

Data Presentation. All experiments were performed in duplicate and repeated at least three times. Data from multiple experiments are presented as mean ± SEM. Histamine release is expressed as nanograms of histamine/10^5 MC, leukotriene release as picograms of sulfidoleukotrienes/10^5 MC.

Results

KL Enhances MC Mediator Release in Response to IgE Receptor Stimulation. Fig. 1 shows the release of histamine and leukotrienes induced by a maximally effective concentration of anti-IgE antibody (Ab) in dispersed human lung MC preincubated for 20 min in buffer or with an optimal concentration of human KL. The experiments demonstrate that KL enhanced IgE receptor–dependent mediator release in all 12 experiments performed with MC isolated from different lung specimens, albeit to a somewhat variable degree (24.5 ± 4.2 vs. 62.3 ± 6.2 ng of histamine/10^5 MC; 712 ± 161 vs.
Figure 1. Effect of KL preincubation on mediator release by human lung MC. Dispersed human lung MC were preincubated in buffer or with an optimal concentration of human KL (10 ng/ml) for 20 min and then stimulated with a maximally effective concentration of anti-IgE receptor mAb 29C6 (aR, 100 ng/ml) for another 40 min. Histamine (left) and leukotriene C4 (right) release is shown. Each point represents the mean of two determinations from one experiment; 12 experiments with different MC preparations were performed. Data with and without KL of the same MC preparations are connected by lines.

2,006 ± 358 pg LTC4/10⁶ MC; mean ± SEM without and with KL). KL augmented the release of both preformed and newly synthesized mediators to a similar extent (histamine, 226 ± 56%; LTC4, 242 ± 36% enhancement). KL by itself, even at 10-fold higher concentrations than required for maximal synergistic effects, failed to induce any detectable LTC4 generation and caused minimal histamine release in only 5 of 12 experiments (0.49 ± 0.25 ng of histamine/10⁶ MC). The potentiation of mediator release by KL was not affected by the presence of contaminating lung tissue cells, since identical results were obtained by unfraccionated dispersed lung cells and MC preparations depleted of tissue cells by Percoll gradients (not shown).

Fig. 2 shows the effect of preincubation with KL on the concentration-dependent mediator release in MC triggered with anti-IgEAb. Preincubation with KL not only considerably enhanced histamine as well as leukotriene release in response to optimal concentrations of anti-IgEAb, but also rendered the MC responsive to Ab concentrations that were otherwise unable to induce mediator release. In the presence of KL, ~30-fold lower concentrations of anti-IgEAb were required to induce the release of comparable amounts of mediators. Qualitatively identical results were obtained by stimulating MC with polyclonal or monoclonal anti-IgEAb instead of anti-IgEAb. However, the release induced by anti-IgEAb was clearly more variable among different MC preparations, and in some experiments MC mediator was detectable only in the presence of KL (data not shown).

KL Dose Response. Fig. 3 shows the dose-dependent potentiation of IgE-dependent histamine and LTC4 release after preincubation of human lung MC with variable concentrations of human KL and murine KL. KL affected MC mediator release at concentrations considerably (10–100-fold) lower than previously established for synergistic bone marrow progenitor cell proliferation (18–20). The enhancement of mediator release was dose dependent from 100 pg to 1 ng/ml of rhuKL and became maximal at 3.3 ng/ml (Fig. 3). It is of interest that murine KL was nearly as active as the human protein on human lung MC. Similar results were obtained with unpurified COS supernatants transfected with soluble murine KL, giving half-maximal activity at a 1:200 dilution in the MO-7 proliferation assay (22). For MC mediator release, the ED₅₀ ranged from 1:30,000 to 1:3,000, and the ED₅₀ from 1:3,000 to 1:1,000 (three experiments, not shown), again indicating that even in the heterologous mouse–human MC assay, mediator release is enhanced at considerably lower concentrations of KL than required for cell proliferation.

Kinetics of MC Mediator Release. Human lung MC released histamine within 1–3 min of exposure to anti-IgEAb. The onset of release of the newly synthesized LTC4 occurred...
somewhat later, and 10 min were required for maximal release (Fig. 4). The spontaneous mediator release remained close to zero over 0–40 min of incubation. In the presence of human KL, the release of both preformed and newly synthesized mediators was strongly enhanced, but the time course of the release reaction was not altered.

**Variation of the Time Interval between Addition of KL and Anti-IgER Antibody.** Fig. 5 shows that the amount of mediators released by lung MC varied depending on the time interval of addition of KL and anti-IgER. The optimal intervals between the addition of the stimuli were different for the enhancement of histamine and leukotriene release. Maximal potentiation of MC degranulation occurred when KL and anti-IgER Ab were added simultaneously to the MC. In contrast, a KL preincubation period of ~10 min was required for an optimal increase of LTC₄ release. Curiously, at an optimal priming time for LTC₄ release, we repeatedly observed a concomitant, reversible reduction of the KL-induced en-

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**Table 1. Mast Cell/Basophil Mediator-releasing Substances**

| Agonist | Human lung mast cells | Human basophils |
|---------|-----------------------|-----------------|
| Anti-IgE | ++ + + + + + + + + + + | 12,13,15         |
| Anti-IgER| ++ + + + + + + + + + + | *              |
| FMLP    | - ND + + + + + + + + + + | 13,15          |
| C5a     | - ND + + + + + + + + + + | 14             |
| C3a     | - ND + + + + + + + + + + | 25             |
| PAF     | - - + + + + + + + + + + | 23             |
| SP      | - - (+) + + + + + + + + | *              |
| rhuIL-1 | - - - + + + + + + + + + | 15,35,43       |
| rhu IL-2| - - - - - - - - - - - - | *              |
| rhu IL-3| - - - + + + + + + + + + | 12-15          |
| rhu IL-4| - - - - - - - - - - - - | *              |
| rhu IL-5| - (+) - + + + + + + + + | 15             |
| rhu IL-6| - - - - - - - - - - - - | *              |
| rhu IL-7| - - - - - - - - - - - - | *              |
| rhu IL-8| - - + + + + + + + + + + | 24             |
| rhu IL-9| - - - - - - - - - - - - | *              |
| rhu G-CSF| - - - - - - - - - - - - | *              |
| rhu M-CSF| - - - - - - - - - - - - | *              |
| rhu GM-CSF| - - + + + + + + + + + + | 12,15,15       |
| rhu TNF-α| - - - - - - - - - - - - | 23             |
| rhu TGF-β| - - - - - - - - - - - - | *              |
| rhu IFN-γ| - - - - - - - - - - - - | *              |
| rhu NGF | - - - + + + + + + + + + | 5              |
| rhu FGF | - - - - - - - - - - - - | *              |
| rhu IGF I| - - - - - - - - - - - - | *              |
| rhu LIF | - - - - - - - - - - - - | *              |
| KL      | - ++ + - - - - - - - - | *              |

A + as "trigger" means a direct effect on histamine or LTC₄ release within 40 min of incubation; a + as modulator indicates enhancement of mediator release in response to appropriate stimuli within 20 min of preincubation; a − indicates no effects.

* Our unpublished results.

† Mediator release only after preincubation with a basophil modulator.

Concentrations: anti-IgE and anti-IgER Ab (100 ng/ml), FMLP (2.5 × 10⁻⁶ M), C₃a (10⁻⁶ M), C₅a (10⁻⁸ M), PAF (10⁻⁶ M), SP (2 × 10⁻⁵ M), cytokines (1–100 ng/ml).
hancement of histamine release. A slight enhancement of mediator release over that observed by anti-IgE Ab stimulation alone could even be found when KL was added shortly after IgE receptor stimulation (Fig. 5, negative time values). A constant preincubation time of 20 min was selected for the experiments shown in the previous sections, because of the discrepancy in optimal priming times for the enhancement of histamine and LTC₄ release.

**Comparison of Mediator Substances for Human Lung MC and Basophils.** Several cytokines such as IL-1, IL-3, IL-9, GM-CSF, and NGF have been suggested as MC growth factors and/or secretagogues in the rodent system (4, 16, 26). Some of these (IL-1, IL-3, GM-CSF, NGF) have been found to enhance basophil responsiveness (13–15; Bischoff, S. C., and C. A. Dahinden, manuscript submitted for publication), while little is known about their effects on human lung MC. Therefore, many cytokines and basophil agonists were examined for their ability to modulate or trigger mediator release by human lung MC under the incubation conditions used in this study. Experiments were performed at least three times, and anti-IgE Ab stimulation with and without KL preincubation served as positive controls for the induction of mediator release and modulatory effects, respectively. Of the stimuli shown previously to induce mediator release in basophils, only IgE-dependent activation resulted in mediator release by human lung MC. Since certain basophil agonists (i.e., C₃a, PAF, IL-8) efficiently induce basophil mediator release only in the presence of IL-3, IL-5, GM-CSF, or NGF (23–25), we examined whether KL renders human lung MC responsive to the IgE-independent agonists listed in Table 1. However, the results were negative. The only cytokine found to reproducibly modulate IgE-dependent mediator release in MC was KL, with the possible exception of a minimal enhancement of mediator release by IL-5, and none of the examined cytokines triggered MC mediator release by itself. Finally, NGF, a potent activator of rodent MC and modulator of human basophils (16; Bischoff, S. C., and C. A. Dahinden, manuscript submitted for publication) failed to modulate or induce mediator release of human lung MC, even after KL preincubation, up to concentrations exceeding by 100-fold that maximally effective in human basophils (data not shown).

**Discussion**

The multiple functions of KL, a gene product of the Steel locus in the mouse, are emphasized by the facts that the KL-encoding gene is highly conserved in mammals and that Steel mutations lead to various symptoms such as anemia, deficiencies in MC and leukocyte progenitor cells, alterations of the coat color, and defective gonadal development (17, 18). Whereas potent stimulatory activities of KL on human bone marrow progenitor cell proliferation, particularly in synergism with other growth factors, are well established (18–20), further biological activities in the human system are not yet known. The present study shows that KL may have an important proinflammatory effect by strongly enhancing the release of preformed and de novo synthesized inflammatory mediators in mature human lung MC triggered by IgE receptor stimulation.

MC priming by human KL occurs at surprisingly low concentrations (0.1–3 ng/ml), a fact emphasizing the possible in vivo importance of KL in regulating mature MC function. The low concentrations and the short time required for KL priming indicate that the effect of KL is specific and is mediated through the KL receptor c-kit proto-oncogene (27). Indeed, our findings are consistent with the presence of a cell surface marker shared by human early myeloid progenitors and MC, as defined by a mAb recently shown to react with the human c-kit proto-oncogene (28, 29). Whereas soluble rat KL has activity similar to that of human KL in human bone marrow culture assays, ~1,000-times greater concentrations of human KL are necessary to induce proliferation in the murine MC/9 cell line (18). Our results further demonstrate a high cross-species activity of rodent KL on human cells, since dose–response studies revealed a similar potency of human and murine KL on human lung MC, albeit at concentrations one to two orders of magnitude below that reported to induce cell proliferation (18–20).

The kinetics of the release reaction in human lung MC are similar to those previously observed in human basophils and lung-derived MC in response to IgE receptor–dependent activation (13, 30, 31). Preincubation of human MC with KL did not accelerate the kinetics of mediator release upon IgE receptor stimulation, in contrast to IL-3 preincubation in human basophils (13). For this study we preferred to use anti-IgE Ab instead of the anti-IgE Ab used by other groups for activation of MC through the IgE receptor, since the extent of mediator release in response to anti-IgE Ab was often higher, and the variability of the response was less in comparison to anti-IgE Ab, possibility because of independence of receptor crosslinking from surface-bound IgE. Moreover, cell activation by a mAb directed against the α chain of the IgE receptor is probably the most specific way to activate MC, since these cells are the only cell types in the lung known to express high-affinity IgE receptors.

The difference in optimal KL priming times for enhancement of histamine and LTC₄ release may reflect different intracellular signaling pathways for degranulation and lipid mediator synthesis in human MC. Indeed, in previous studies with human basophils, we found that histamine release and leukotriene production are separately regulated (14, 15). For example, C₃a is a potent degranulating agent in basophils but is unable to induce lipid mediator synthesis, unless the cells have not been preincubated with IL-3 (14). The influence of the time interval between addition of a modulatory cytokine (KL or IL-3) and a triggering agent upon mediator release by MC and basophils, respectively, is remarkably similar. However, when basophils are activated by a G protein–coupled receptor, such as the C₅a receptor, a short preincubation period with the cytokine is required for agonist-induced LTC₄ formation (14, 15). Nevertheless, when basophils are exposed to IL-3 and anti-IgE Ab at different time intervals, an almost identical pattern of mediator release is obtained (our unpublished data), as shown here for MC.

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A modulatory activity of cytokines on functional cell responses has been described in several inflammatory effector cell types, including human neutrophils, eosinophils, and basophils (9-15). The effect of MC priming by KL is strikingly similar to that of IL-3, IL-5, GM-CSF, and NGF in basophils (13-15; Bischoff, S. C., and C. A. Dahinden, manuscript submitted for publication). In both cases, maximal priming requires preincubation with cytokine for <20 min before addition of the second agonist, and the effect consists of an enhancement of both the release of preformed and newly synthesized mediators in response to optimal concentrations of the triggering agent, as well as an increase in sensitivity to lower concentrations of the secretagogues (13, 15). These analogies between human basophils and MC may suggest a similar mechanism of priming in both cell types. In this regard, it is interesting that the intracellular domain of c-kit has tyrosine kinase activity (27) and that very recently a high-affinity receptor component for NGF has been identified as the irk proto-oncogene, a member of the tyrosine kinase receptor family (32, 33). Furthermore, despite the lack of tyrosine kinase domains in receptors for GM-CSF and IL-3 belonging to the cytokine receptor family, tyrosine phosphorylation has been demonstrated in IL-3-dependent cell lines (34). These observations suggest that tyrosine phosphorylation events may be critical in regulating release of mediators by mature human basophils and MC, a hypothesis consistent with our unpublished results.

In contrast to basophils and other inflammatory effector cells, little is known about cytokines capable of enhancing mediator release in human mucosal MC. Only IL-1 has been reported to augment lipid mediator synthesis in lung-derived human MC triggered with grass pollen antigen after preincubation of the cells with serum of sensitized individuals (35). The effect of IL-1 on histamine release was not examined. The reason for this discrepancy with our findings is unclear, and may be attributed to a different method of cell stimulation, or to differences in buffer conditions and incubation times. Another indication for cytokine-dependent enhancement of MC mediator release came from studies of Leve-Schaffer et al. (36), who showed that coculture of human lung MC with mouse 3T3 fibroblasts for 7 d not only prolongs MC survival but also enhances lipid mediator release in response to anti-IgE Ab (36). Although a direct comparison of the function of MC cultured in the presence or absence of 3T3 fibroblasts could not be made because MC do not survive without fibroblasts, it is tempting to speculate that this functional enhancement is indeed due to KL expressed in 3T3 fibroblasts (37, 38).

Several cytokines including IL-3, IL-4, IL-9, GM-CSF, and NGF have been reported to act as growth factors and/or secretagogues for MC in rodents (4, 16, 26), but whether these cytokines affect human MC growth or function is largely unknown. Parallel to KL, a large number of these and other cytokines were examined under identical experimental conditions and were found to be ineffective in either inducing or modulating release of human MC mediators. Of particular interest is the ineffectiveness of NGF, M-CSF, and IL-3 on human lung MC function. NGF promotes release of mediators from peritoneal rat MC and induces a change in the phenotype of murine mucosal MC to connective tissue MC (16, 39), similar to KL in the murine system (40). M-CSF, a growth factor of the monocyte lineage, and its receptor show homologies to KL and c-kit, respectively, suggesting similarities in the mode of action by these two cytokines (27, 41). Therefore, the negative results obtained with M-CSF may indicate a lack of M-CSF receptors on MC from human lung. Finally, IL-3, a major growth factor of mucosal MC in rodents, was also ineffective on human lung MC, a finding consistent with a recent report demonstrating the lack of IL-3 receptors on human lung MC (42).

Some of the cytokines acting on murine MC (IL-3, GM-CSF, and NGF) appear to affect human basophil function (13-15; Bischoff, S. C., and C. A. Dahinden, manuscript submitted for publication) but seem ineffective in human lung MC. These observations indicate that data obtained from rodents are not easily transferable to the human system, and that murine mucosal MC have more functional resemblance to human basophils than to human mucosal MC. Furthermore, human basophils and lung MC differ in their responsiveness towards IgE-independent agonists, since C3a, C5a, FMLP, PAF, and IL-8, all of which activate basophils (13-15, 23-25), are unable to induce release of mediators in human lung MC. It is clear, however, that the list of negative results presented in this study does not exclude the possibility that some of these bioactive molecules may affect human lung MC under different experimental conditions or with regard to other cellular responses.

Murine KL is expressed in a biological active form as a membrane-bound protein and is also active in a soluble form (22, 38). The extent of KL expression in human adult lung tissue is unknown. The fact that KL promotes a phenotypical alteration of mucosal MC to connective tissue MC in the rodent system (40) may indicate that KL is not strongly expressed in lung tissue, since lung MC are predominantly of the mucosal phenotype. Nevertheless, it cannot be excluded that the KL-induced potentiation of mediator release in dispersed lung MC demonstrated in this study is due to their separation from lung tissue cells expressing KL. However, the fact that KL, at very low concentrations, strongly enhances MC mediator release, even in unpurified MC preparations containing other lung tissue cells, argues against this possibility. In any case, our study raises the important question of whether expression of KL can be upregulated in human lung diseases such as asthma and other MC-associated pathologies.
We thank Drs. M. Schreier and E. Liehl (Sandoz Ltd., Basel, Switzerland and Vienna, Austria), Drs. H. W. Lahm and H. Langen (Hoffmann-La Roche, Basel, Switzerland), Dr. S. Clark (Genetics Institute, Cambridge, MA), Drs. M. Cronin and G. Burton (Genentech, Inc., San Francisco, CA), and Amgen, Inc. (Thousand Oaks, CA) for generous gifts of cytokines; Drs. J. Hakimi and R. Chizzonite (Hoffmann-La Roche, Nutley, NJ) for providing anti-IgE receptor mAb; A. Raaffa-Sohn, J. Zingg, and P. Winkler for technical assistance; doctors from the Department of Heart and Chest Surgery (Director, Dr. U. Althans) for support; and nurses from the Operation Unit (E West, Inselspital Bern) for excellent cooperation.

This work was supported by the Swiss National Science Foundation (grant 31 32470.91) and by a grant from the Deutsche Forschungsgemeinschaft to Dr. Bischoff.

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Received for publication 23 September 1991.

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