

# Allergens, IgE, mediators, inflammatory mechanisms

## Production of IL-5 and granulocyte-macrophage colony-stimulating factor by naive human mast cells activated by high-affinity IgE receptor ligation

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**Background:** The late-phase allergic reaction is an eosinophilic inflammatory response that begins several hours after allergen exposure, may persist for 24 hours, and is an important pathogenic mechanism in allergic disease.

**Objective:** Cultured naive human mast cells were used to investigate whether mast cells are a direct source of the eosinophil-promoting cytokines IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF).

**Methods:** Naive human mast cells were derived from bone marrow mononuclear cells cultured in the presence of stem-cell factor. Cytokine message and protein production in response to high-affinity IgE receptor ligation of cultured mast cells were measured by semiquantitative polymerase chain reaction and ELISA, respectively.

**Results:** IL-5, IL-3, and GM-CSF messenger RNA increased within 2 hours of mast cell activation, with IL-5 and GM-CSF message remaining elevated for 24 hours, whereas IL-3 mRNA rapidly declined. IL-5 and GM-CSF protein were measurable 4 to 6 hours after stimulation and peaked by 24 and 12 hours, respectively. IL-3 protein was not detectable. **Conclusion:** These findings demonstrate that naive mast cells do not constitutively produce IL-5 or GM-CSF protein but are a major source of these eosinophilotropic cytokines on high-affinity IgE receptor ligation. (*J Allergy Clin Immunol* 1997;99:508-14.)

High-affinity IgE receptor (FcεRI) ligation results in mast cell activation, degranulation, and release of preformed and newly synthesized mediators.<sup>1, 2</sup> In vivo, this results in an immediate hypersensitivity reaction, which is often followed several hours later by a late-phase allergic reaction (LPAR) that may persist for more than 24 hours.<sup>1-7</sup> The LPAR is characterized by a leukocytic infiltrate, predominantly comprised of eosinophils and

### Abbreviations used

|                    |                                                  |
|--------------------|--------------------------------------------------|
| FcεRI:             | High-affinity IgE receptor                       |
| GM-CSF:            | Granulocyte-macrophage colony-stimulating factor |
| HPRT:              | Hypoxanthine-guanine phosphoribosyl transferase  |
| LPAR:              | Late-phase allergic reaction                     |
| mAb:               | Monoclonal antibody                              |
| MNCs:              | Mononuclear cells                                |
| MC <sub>T</sub> :  | Tryptase-positive, chymase-negative mast cells   |
| MC <sub>TC</sub> : | Tryptase-positive, chymase-positive mast cells   |
| PCR:               | Polymerase chain reaction                        |
| SCF:               | Stem cell factor                                 |

CD4<sup>+</sup> T cells that secrete a T<sub>H2</sub> profile of cytokines.<sup>1-5, 7-9</sup> Such an eosinophilic inflammatory response is also observed in allergic rhinitis and asthma and correlates with the presence of IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF).<sup>\*</sup> Each of these cytokines has potent bioactivity for eosinophils, with IL-5 being essential for eosinophil accumulation.<sup>1, 2, 12, 14-21</sup> As a major source of IL-5, T<sub>H2</sub> lymphocytes have been implicated as central to the pathogenesis of chronic eosinophilic inflammation in asthma, as well as in antigen-induced pulmonary eosinophilia and airway hyperreactivity in animal models of asthma.<sup>†</sup> However, a potential role for the mast cell has also been demonstrated by attenuation of the eosinophilic inflammatory response during the initial 24 hours after respiratory challenge of antigen-primed mast cell-deficient W/W<sup>v</sup> mice.<sup>28</sup> Furthermore, IL-5 has been localized to mast cells within respiratory tract biopsy specimens, and IL-5 synthesis has been demonstrated with enzymatically dispersed lung mast cells.<sup>11, 13, 29-31</sup>

On the basis of these observations, we propose a paradigm for the pathogenesis of allergen-induced eo-

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<sup>\*</sup>References 1-3, 5, 7, and 9-15.

<sup>†</sup>References 1-3, 11, 12, and 22-27.

sinophilic inflammation wherein activated mast cells would synthesize eosinophilotropic cytokines with kinetics appropriate for the recruitment of eosinophils during the LPAR. Concomitantly,  $T_{H2}$  cells would be activated by antigen-specific stimulation to proliferate and produce eosinophilotropic cytokines, resulting in a more chronic eosinophilic inflammatory response. Herein, we provide the initial investigation of this model by determining the potential for human mast cells to produce IL-5, IL-3, and GM-CSF on ligation of their Fc $\epsilon$ RI. To circumvent difficulties in interpreting data generated from mast cells that have been isolated from tissue and are heterogeneous with regard to prior *in vivo* activation, we used naive mast cells generated *in vitro* during long-term cultures of bone marrow precursor cells under the influence of stem cell factor (SCF). Our results provide the first demonstration that naive human mast cells, although not constitutively producing IL-5, IL-3, or GM-CSF, do upregulate message for each of these cytokines on activation by Fc $\epsilon$ RI ligation but secrete only IL-5 and GM-CSF. Furthermore, activated mast cell production of IL-5 and GM-CSF follows kinetics, which parallel eosinophil accumulation in the LPAR.<sup>1-7,9</sup>

## METHODS

### Mast cell cultures

Mononuclear cells (MNCs) were isolated from bone marrow aspirates of normal volunteers by density centrifugation over Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden). Donors of bone marrow aspirate agreed by informed consent to participate in this study approved by the Baylor College of Medicine Institutional Review Board. MNCs were cultured in RPMI-1640 medium (Gibco) supplemented with 4 mmol/L L-glutamine, 100  $\mu$ g/ml penicillin/streptomycin, 0.1 mmol/L nonessential amino acids, 20 mmol/L N-2-hydroxyethylpiperazine-N-2 ethanesulfonic acid, and 10% fetal bovine serum (complete RPMI) and recombinant human SCF (Immunex, Seattle, Wash.) at a concentration of 50 ng/ml to promote mast cell growth.<sup>32-36</sup> One half of the media was replenished weekly for 12 to 20 weeks. Mast cells were identified on cytopreps as oval to round MNCs filled with cytoplasmic granules, which stained darkly on Wright-Giemsa stain and metachromatically with toluidine blue. Cultures studied for cytokine synthesis were comprised of 88% to 97% mast cells. The few remaining cells were larger and histiocytic-appearing and did not stain metachromatically with toluidine blue. Concurrent control cultures, maintained with complete RPMI in the absence of SCF, were devoid of mast cells but were comprised of histiocytic cells similar to those in SCF-derived mast cell cultures.

### Immunohistochemistry

Tryptase-positive, chymase-negative mast cells (MC<sub>T</sub>) and tryptase-positive, chymase-positive mast cells (MC<sub>TC</sub>) were identified by immunohistochemistry.<sup>35</sup> Briefly, cytopreps were immersed in Mota's fixative (lead subacetate), followed by sequential incubation in a 1:700 dilution of biotinylated anti-chymase monoclonal antibody (mAb) (Chemicon, Temecula, Calif.) and streptavidin-peroxidase (Vectostatin Elite ABC Kit; Vector Laboratories, Burlingame, Calif.) and developed with 3-amino-9-ethylcarbazole substrate (Vector Laboratories). Slides were then incubated overnight with alkaline phosphatase-conjugated anti-tryptase mAb (Chemicon) at a dilution of 1:500 and developed with Fast Blue RR in naphthol buffer (Sigma Chemical Co., St. Louis, Mo.). MC<sub>T</sub> stained blue, and MC<sub>TC</sub> stained brown-red. The histiocytic-appearing, larger cells in SCF-derived cultures were all tryptase- and chymase-negative. Control cultures grown in the absence of SCF were comprised of histiocytic cells and lacked tryptase- or chymase-positive cells.

### Microfluorimetry

Two-color microfluorimetric analysis for CD117 (SCF receptor) and cell-bound IgE was performed on a FACScan (Becton Dickinson, Mountain View, Calif.) by using phycoerythrin-conjugated anti-CD117 mAb (AMAC, Westbrook, Maine) and fluorescein isothiocyanate-goat anti-human IgE antibody (Caltag Laboratories, South San Francisco, Calif.). IgE-sensitized mast cell cultures were stained simultaneously with anti-CD117 mAb and anti-IgE antibody and then fixed in 1% paraformaldehyde. By FACScan analysis, approximately 90% of the cultured cells comprised a uniform, smaller cell population, with the remaining cells segregating as a nonoverlapping population of larger cells. The large population of smaller-sized cells and the small population of larger-sized cells were gated and analyzed separately by microfluorimetry for CD117 and IgE. The large population comprised of smaller-sized cells was CD117<sup>+</sup> and IgE<sup>+</sup>. In contrast, the small population comprised of larger-sized cells was negative for both CD117 and IgE.

Basophils were obtained by density centrifugation of peripheral blood from a patient with chronic myelogenous leukemia and were used as a phenotyping control. The isolated cell population was 80% basophils as determined by histochemistry and, as is characteristic of basophils, were CD117<sup>-</sup> and, after sensitization, IgE<sup>+</sup>.

### Histamine release

Cultured mast cells were sensitized by incubation for 2 hours at 37° C with IgE-containing human serum, washed, and then resuspended in release buffer (AMAC) containing affinity-purified goat IgG anti-human IgG antibody or goat IgG anti-human IgE antibody (0.5  $\mu$ g/ml) (Caltag Laboratories). Exposure of cultured mast cells to only IgE-containing serum or anti-IgE antibody without prior sensitization with IgE did not cause histamine release. Supernatants were assayed for histamine content by enzymatic immunoassay (AMAC).<sup>37</sup>

### Stimulation of cultured mast cells for cytokine synthesis

Mast cell or control cultures were incubated in the presence of IgE for 2 hours at 37° C, washed, resuspended at a cell density of between 1.5 and 3.0  $\times 10^5$  cells/ml, and exposed to goat IgG anti-human IgG or anti-human IgE antibody (Caltag Laboratories) as control or Fc $\epsilon$ RI activation stimuli, respectively. Selected mast cell cultures were stimulated with anti-CD3 mAb 64.1.<sup>38</sup> To maintain mast cell viability during stimulation periods, tested cells were maintained in media with SCF.

### ELISA measurement of cytokine protein levels in culture supernatants

IL-5 ELISAs were performed as previously described.<sup>39</sup> IL-3 and GM-CSF levels were measured by using commercially available ELISAs for IL-3 (R&D Systems, Minneapolis, Minn.) and GM-CSF (Genzyme, Boston, Mass.).

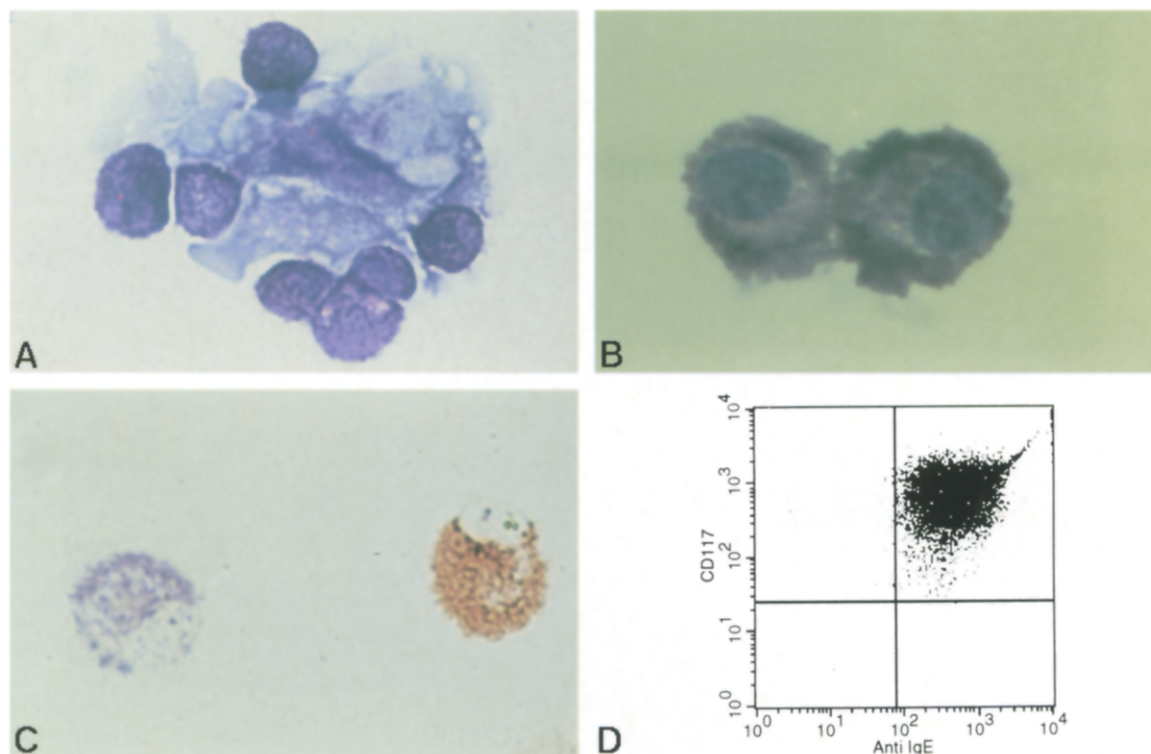


**FIG. 1.** Human mast cells derived from long-term bone marrow MNC cultures maintained with SCF. **A**, Wright-Giemsa stain of a clump of cells from a 16-week-old culture. Mast cells are identified by dark staining of their numerous cytoplasmic granules. The much larger, pale-staining cells are histiocytic-appearing non-mast cells. **B**, Toluidine blue stain of cultured mast cells in which cytoplasmic granules exhibit metachromasia. **C**, Dual immunohistochemical staining for tryptase and chymase. MC<sub>T</sub> stain blue, and MC<sub>TC</sub> stain brown-red. **D**, Microfluorimetric analysis of cultured human mast cells dual-stained for CD117 (y axis) and IgE binding (x axis).

### Semiquantitative polymerase chain reaction (PCR)

RNA was isolated from cells at various time points after stimulation by using the Trisolv method (Biotech Laboratories, Houston, Texas).<sup>40</sup> Complementary DNA was generated by reverse transcription of RNA with a GeneAmp RNA PCR Kit (Perkin-Elmer, Norwalk, Conn.). Primer pairs specific for IL-5, GM-CSF, IL-3, and hypoxanthine-guanine phosphoribosyl transferase (HPRT) were designed according to sequences deposited in GenBank and synthesized by the Nucleic Acid Core Laboratory, Baylor College of Medicine, Houston, Texas. The sequences of the sense and anti-sense primers for IL-5 were 5' CTGCCTACGTGTATGCCATC 3' and 5' AGGAAT-TGGTTTACTCTCCGTC 3', respectively. GM-CSF primer sense and anti-sense sequences were 5' CTGCCTACAGAC-CCGCCTGG 3' and 5' AGGGTCAGTGTGCCAGGGC 3', respectively. IL-3 sense and anti-sense primer sequences were 5' TCCAACCTCCTGGTCCGCCCG 3' and 5' AAGATCGC-GAGGCTCAAAGTCGTCTGTTG 3', respectively. Sense and anti-sense primer sequences for HPRT were 5' CCATTC-CTATGACTGTAGATT 3' and 5' ATTCAAATCCCT-GAAGTATTCA 3', respectively. Expected PCR product sizes for IL-5, GM-CSF, IL-3, and HPRT were 307, 374, 427, and 336 base pairs, respectively, and were confirmed by restriction enzyme analysis. Amplification of cDNA with Taq polymerase (Perkin-Elmer) was performed in a thermocycler (Perkin-Elmer) for 25 to 35 cycles. The number of amplification cycles

used for each primer pair was predetermined such that cytokine product was detected on the linear portion of the amplification curve. Thermocycler settings were 1 minute at 94° C (denaturing), 1 minute and 5 seconds at 55° C (annealing), and 1 minute at 72° C (extension) per cycle. For semiquantitative analysis, PCR products were labeled by the addition of  $\alpha$ -phosphorous 32-labeled deoxycytidine triphosphate (Amersham, Arlington Heights, Ill.) to the PCR mix before amplification. PCR products were electrophoresed through a 9% polyacrylamide gel, which was then stained with ethidium bromide. Bands were analyzed for <sup>32</sup>P incorporation with a Betascope blot analyzer (Betagen Corp., Waltham, Mass.). Semiquantitation of messenger RNA levels was determined by computing the ratio of total radioactive counts detected in any given cytokine band to total counts obtained for HPRT.

### RESULTS

Naive mast cells were derived by long-term in vitro culture of bone marrow MNCs in the presence of SCF. Mast cells were identified as a uniformly sized population of round to oval MNCs containing cytoplasmic granules (Fig. 1, A), which stained metachromatically with toluidine blue (Fig. 1, B) and immunohistochemically for tryptase or chymase (Fig. 1, C). Mast cells comprised 88% to 93% of the cells in long-term SCF-derived cultures, which were used for cytokine analysis.

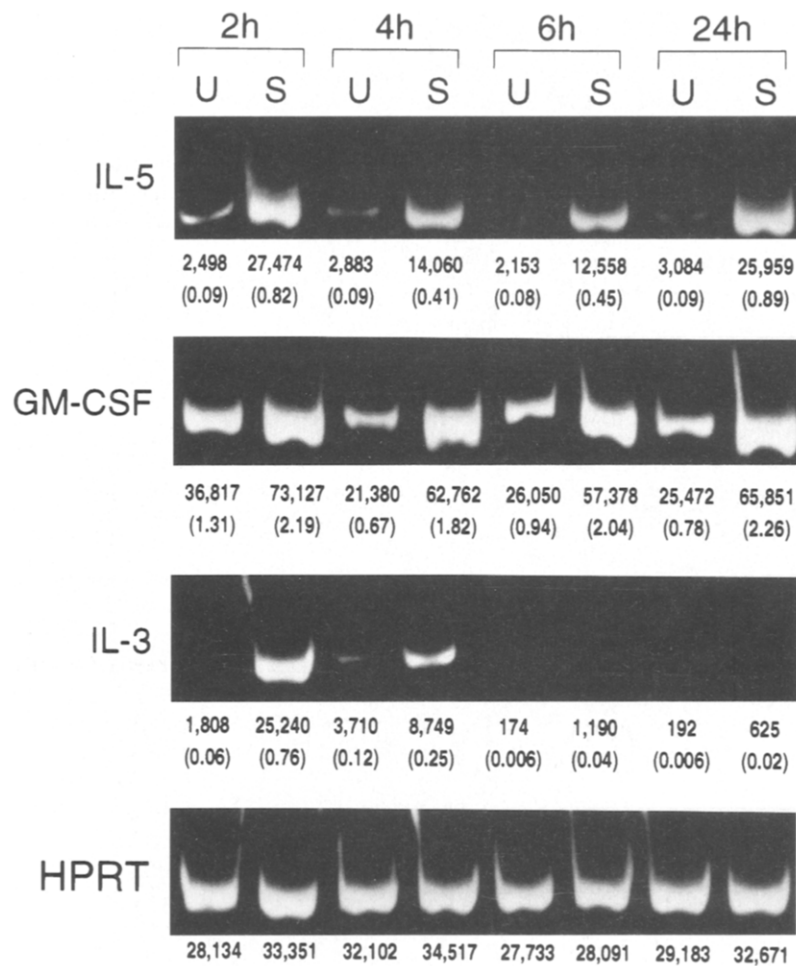


**FIG. 2.** Kinetics for IL-5, GM-CSF, and IL-3 mRNA upregulation after FcεRI ligation of IgE-sensitized mast cells. RNA was extracted from cultured mast cells at indicated time points. PCR was performed with IL-5-, GM-CSF-, IL-3-, and HPRT-specific primers in the presence of  $\alpha^{32}\text{P}$  deoxycytidine triphosphate. After polyacrylamide gel electrophoresis, PCR products were visualized by ethidium bromide staining, and radioactivity of cytokine bands was quantitated by Betascope analysis. U, Unstimulated (IgE-sensitized mast cells + anti-IgG antibody); S, stimulated (IgE-sensitized mast cells + anti-IgE antibody). Radioactivity is expressed as total counts and appears directly beneath the corresponding PCR product. Parenthetical data are ratio of cytokine counts to HPRT (control message) counts.

The few remaining cells in these long-term SCF-derived cultures were large, histiocytic-appearing cells, which were nonmetachromatic with toluidine blue staining and were tryptase- and chymase-negative as determined by immunohistochemistry. By microfluorimetry, approximately 90% of cells comprised a uniform population of smaller-sized cells, which was consistent with the mast cell population delineated by histochemical and immunohistochemical staining. This large population of smaller cells was CD117<sup>+</sup> and bound IgE (Fig. 1, D), confirming their mast cell identity.<sup>34, 41</sup> The small population of larger-sized cells was CD117<sup>-</sup> and did not bind IgE (data not shown). Thus both microfluorimetry and immunohistochemical analyses were concordant in identification of mast cells in SCF-stimulated cultures. In control cultures maintained with complete RPMI and

lacking SCF, no mast cells were present as determined by analysis of Wright-Giemsa- or toluidine blue-stained cytopreps; and no tryptase- or chymase-positive cells were present. The presence of functional FcεRI on cultured mast cells was demonstrated by histamine release after IgE sensitization and exposure to anti-IgE antibody but not to anti-IgG antibody (data not shown). Cultured mast cells not sensitized with IgE did not release histamine on exposure to anti-IgE antibody, confirming their naive status with regard to FcεRI activation. Basophils, eosinophils, and lymphocytes were uniformly absent on Wright-Giemsa-stained cytopreps. Further evidence against the presence of basophils was the absence of CD117<sup>+</sup>, IgE-binding cells, which was the phenotype exhibited by peripheral blood basophils (data not shown<sup>34, 41</sup>). Furthermore, basophils cannot be

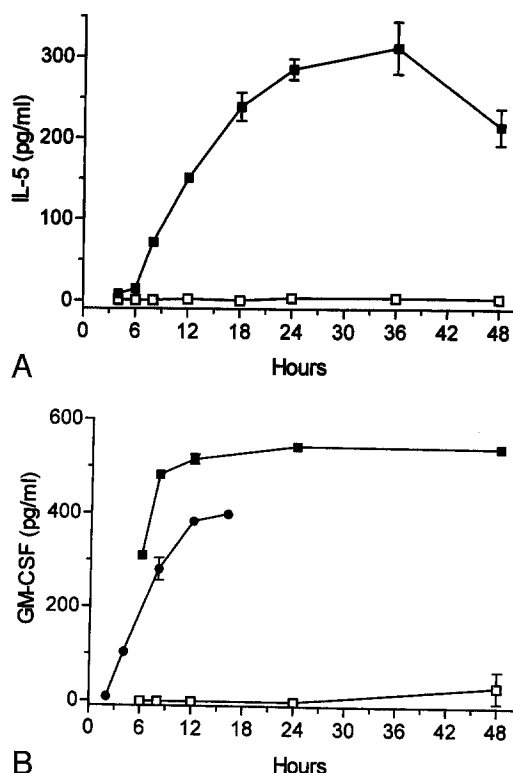


**FIG. 3.** IL-5 and GM-CSF protein secretion by cultured mast cells after Fc $\epsilon$ RI ligation. Cytokine levels were measured in supernatants of IgE-sensitized mast cells at indicated time points after stimulation with anti-IgG antibody (open symbols) or anti-IgE antibody (filled symbols). Data points are expressed as means  $\pm$  SEM. **A**, Kinetics of IL-5 production from a culture comprised of 93% mast cells, of which 60% were MC<sub>T</sub> and 40% were MC<sub>TC</sub>. Sensitized mast cells from this culture demonstrated a net histamine release of 58% after Fc $\epsilon$ RI ligation. Similar kinetics for IL-5 protein production were obtained in three experiments with mast cells derived from independent cultures. **B**, Kinetics of GM-CSF protein production from two separate mast cell cultures. Culture 1 (squares) was the same culture as described above for kinetics of IL-5 protein production. In culture 2 (filled circles), stimulation was limited to anti-IgE antibody because cell numbers were insufficient for generation of control supernatants by stimulation with anti-IgG antibody. Culture 2 was comprised of 88% mast cells, of which 60% were MC<sub>T</sub> and 40% were MC<sub>TC</sub>.

maintained in vitro in the absence of exogenous basophil growth and viability factors for the length of culture time used in these experiments.<sup>34</sup>

These in vitro-derived naive mast cells were studied for their potential to constitutively transcribe message for IL-5, GM-CSF, and IL-3. As shown by ethidium bromide staining of PCR products and semiquantitative PCR analysis, baseline message for these cytokines was detected by PCR; and constitutive message for GM-CSF was greater than that for IL-5, which was greater than that for IL-3 (Fig. 2). A substantial increase in message for all three cytokines occurred 2 hours after stimulation of IgE-sensitized mast cells with anti-IgE antibody (Fig. 2). IL-5 and GM-CSF mRNA levels remained increased for 24 hours after Fc $\epsilon$ RI ligation. However, IL-3 message returned to baseline level by 6 hours after stimula-

tion (Fig. 2). Although the ratio of IL-3 to HPRT mRNA upregulation appeared to be greater than that for either IL-5 or GM-CSF at 2 hours, IL-3 upregulation was not sustained, as was the message for IL-5 and GM-CSF (Fig. 2).

To assess the potential biologic significance of cytokine mRNA upregulation, protein levels for IL-5, GM-CSF, and IL-3 were measured in unstimulated mast cell cultures and at serial time points after Fc $\epsilon$ RI ligation with anti-IgE antibody. Despite the presence of baseline IL-5, GM-CSF, and IL-3 message, none of these cytokines were detected in the supernatants of IgE-sensitized mast cells cultured in media alone or in media containing anti-IgG antibody. However, IL-5 protein was detectable 6 hours after mast cell activation by Fc $\epsilon$ RI ligation and was maximal between 24 and 36 hours after stimulation (Fig. 3, A). GM-CSF protein was first detectable at 4 hours and peaked between 12 and 16 hours after Fc $\epsilon$ RI ligation (Fig. 3, B). In contrast to the several-hour delay in production of detectable quantities of IL-5 and GM-CSF after Fc $\epsilon$ RI ligation, histamine was released by the mast cells within 30 minutes of such an activation signal. These data indicate the lack of constitutive production of IL-5 and GM-CSF and their absence as preformed mediators stored in secretory granules of naive mast cells. This conclusion is also supported by studies demonstrating that tumor necrosis factor- $\alpha$  is stored in mast cell secretory granules and released within 30 minutes after Fc $\epsilon$ RI ligation.<sup>42</sup> In contrast to IL-5 and GM-CSF, IL-3 protein was not detectable despite measurements of supernatants obtained 2, 4, 6, 8, 10, 12, and 24 hours after stimulation (data not shown). The remote possibility that small numbers of phenotypically undetectable T cells could be a source for IL-5 in these cultures was excluded by the inability of anti-CD3 mAb-stimulated mast cell cultures to produce detectable amounts of IL-5 over 24 hours. To enable comparison of IL-5 and GM-CSF production by mast cells versus that reported for other cell types, we normalized our data by calculating the amount of cytokine produced by  $10^6$  mast cells. The amounts of IL-5 and GM-CSF produced by  $10^6$  mast cells were 1334 pg/ml and 3467 pg/ml, respectively, at 48 hours after Fc $\epsilon$ RI ligation.

Control cultures, which were maintained in complete RPMI without SCF, lacked mast cells and were comprised of histiocytic cells. After sequential exposure to IgE and anti-IgE antibody, supernatants were removed at 4, 6, 8, 12, 16, and 24 hours and analyzed by ELISA for IL-5, IL-3, and GM-CSF protein. None of these cytokines was detected at any of the tested time points (data not shown).

## DISCUSSION

These studies demonstrate that mast cells activated by Fc $\epsilon$ RI ligation are a major source of the eosinophilotropic cytokines, IL-5 and GM-CSF. Importantly, the lack of constitutive production of IL-5 and GM-CSF by

naive mast cells indicates that in situ detection of these cytokines within mast cells in tissues or biologic fluids is indicative of prior mast cell activation. The kinetics of IL-5 and GM-CSF production after FcεRI ligation parallel the appearance of eosinophils in the LPAR,<sup>1-5, 7</sup> suggesting that allergen-activated mast cells can directly recruit eosinophils.\* Thus our data support the proposed paradigm that allergen-activated mast cells synthesize eosinophilotropic cytokines with kinetics appropriate for eosinophil recruitment, activation, and survival during the LPAR.

An important aspect of our study was the establishment of a culture system for generation of naive mast cells. Prior investigations, in which mast cells from biopsy specimens of patients with allergic disease were used, have demonstrated immunohistochemically that mast cells are a source of IL-5.<sup>11, 13, 29</sup> In addition, mast cells derived from enzymatically dispersed lung tissue have been shown to produce IL-5 after FcεRI ligation.<sup>30, 31</sup> However, because of their in vivo origin, these mast cells likely reflected heterogeneous stages of cellular activation. The naive state of the mast cells used in this report circumvents potential problems, which may arise from the study of tissue mast cells that have been previously sensitized and activated in vivo. Unlike tissue-derived mast cells, the function and biologic responsiveness of naive mast cells has not been influenced by the plethora of soluble and cellular stimulatory and inhibitory conditions that are present in vivo, particularly in diseased tissue. However, it is possible that SCF, which was present in the media of naive mast cell cultures, may upregulate baseline mast cell expression of mRNA for cytokines.

The eosinophilic response in the LPAR has been increasingly recognized as a critical element of the inflammatory process in allergic diseases such as rhinitis and asthma. Mechanisms whereby IL-5 and GM-CSF may promote eosinophil accumulation in the LPAR include the enhancement of eosinophil adhesion, chemotaxis, transendothelial migration, maturation, and survival.<sup>2, 7, 14-18</sup> Furthermore, IL-5 and GM-CSF both stimulate eosinophil synthesis and release of numerous inflammatory molecules, including major basic protein and leukotriene C<sub>4</sub>.<sup>1, 2, 5, 9</sup> Thus these eosinophilotropic cytokines promote the influx of eosinophils to the site of allergen challenge, prolong their transit time within affected tissue, and upregulate the inflammatory roles of eosinophils in tissue inflammation.

Although mast cells represent only a small percentage of the resident cell population in the usual tissue microenvironment, they are strategically located to promote inflammatory responses. This is perhaps best exemplified by a strong predilection for mast cells to be located along the endothelium and by their characteristically intimate association with endothelial cells. These conditions situate the mast cells to directly interact with the microvasculature and to enhance the bioavailability of

mast cell-derived IL-5 and GM-CSF to eosinophils at the endothelial cell-blood interface.

The amounts of IL-5 and GM-CSF produced by FcεRI-activated naive mast cells are substantial on the basis of comparison with those reported for activated CD4<sup>+</sup> T cells, a recognized major source for both of these cytokines.<sup>12, 15</sup> Polyclonal CD4<sup>+</sup> T-cell lines, derived from patients with atopic asthma and activated with anti-CD3 mAb, have been shown to produce up to approximately 2231 pg/ml of IL-5 per 10<sup>6</sup> cells and 1584 pg/ml of GM-CSF per 10<sup>6</sup> cells at 48 hours.<sup>15</sup> Thus on the basis of in vitro study results, naive mast cells activated by FcεRI ligation produce approximately two thirds as much IL-5 as anti-CD3 mAb-activated CD4<sup>+</sup> T-cell lines but approximately four times more GM-CSF. These relative quantitative comparisons lend support to the importance of the mast cell as a biologically significant cellular source of IL-5 and GM-CSF in allergic inflammation.

In conclusion, our findings support the paradigm in which allergen-activated mast cells synthesize the eosinophilotropic cytokines IL-5 and GM-CSF with kinetics appropriate for promoting eosinophil recruitment, activation, and survival in the LPAR. Thus mast cells can directly promote eosinophil-mediated inflammation in allergic disease. Our studies also establish an in vitro model for determining the biologic effects of diverse agonists and antagonists of mast cell activation.

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