Activated Mast Cells Produce Interleukin 13
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Summary
When mast cells are activated through their immunoglobulin (IgE) receptors, release of low molecular weight mediators like histamine is followed by secretion of multiple cytokines, including interleukin (IL)-3, IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor. Here we report that stimulated mast cells also synthesize IL-13 mRNA and protein; secretion of this cytokine may be of particular importance because of its ability to stimulate IgE expression. IL-13 transcripts detected by a semiquantitative reverse transcriptase–mediated polymerase chain reaction assay were induced within 30 min after stimulation of mast cells by dinitrophenyl plus monoclonal IgE anti-dinitrophenyl, and peaked at about 1 h. Within 3 h of IgE stimulation, secreted IL-13 bioactivity, estimated by proliferation of an IL-13–dependent cell line, reached levels equivalent to 1–2 ng/ml of IL-13. When added to human B lymphocytes, the mast cell–derived IL-13 activity (like bone fide IL-13) induced Ig Ce transcripts, DNA recombination characteristic of the isotype switch to Ce, and the secretion of IgE protein. These results suggest a model of local positive feedback interactions between mast cells and B cells, which could play a role in the pathogenesis of atopy.

Mast cells are now recognized as an important source of cytokines possessing proinflammatory, differentiative, and growth-promoting capabilities (reviewed in references 1 and 2) and have been postulated to participate in immunoregulatory cytokine cascades, both as initiators (3), as well as amplifiers (1) of cytokine responses. Moreover, through direct cell contacts mediated by specific cell surface structures, mast cells and their secreted products are speculated to participate in a form of the immunological synapse, complexing with or modifying the functions of other cells under a variety of circumstances (1).

The spectrum of cytokines elaborated by mast cells in response to immunological and pharmacological stimulation is quite broad and includes IL-3, IL-4, IL-5, and GM-CSF. The genes for these cytokines are all colocalized on mouse chromosome 11 (4–6) and possess genomic structural features suggesting that they may have arisen through gene duplication. Recently, an IL-4–like cytokine termed IL-13 has been identified (7), and its gene has been shown to lie on chromosome 11, near the IL-4 gene (8). This same region is associated with certain forms of atopic allergic disease in humans (9). IL-13 shares many biologic properties with IL-4, including suppressive effects upon macrophage activation (10, 11), proliferative and differentiative effects upon hematopoietic progenitor cells (12), and promoting B cell growth and immunoglobulin class switching to IgE (references 7 and 13, reviewed in reference 14). Unlike IL-4, however, IL-13 cannot stimulate T cells. Thus, IL-13 may mediate functions associated with IL-4 under conditions in which IL-4–mediated T cell effects would be disadvantageous.

In this report, we show that human mast cell and basophilic cell lines, primary cultures of murine bone marrow–derived cultured mast cells (BMMC),1 and the cloned murine mast cell line C1.MC/CS7.1 transcribe IL-13 in response to immunological stimulation. We also demonstrate that murine mast cells secrete significant amounts of IL-13 under these conditions. The potential importance of these findings in immunological function of mast cells is discussed.

Materials and Methods

Mast Cells and Cell Activations. The human mast cell line, HMC-1 (15), was obtained from Dr. Joseph Butterfield (Mayo Clinic, Rochester, MN) and maintained in Iscove's Modified Essential Medium, supplemented with 10% FCS, 1.2 mM α-thioglycerol (Sigma Chemical Co., St. Louis, MO), 100 U/ml penicillin, and 0.1 mg/ml streptomycin. The human basophilic cell line, KU812 (16), was obtained from Dr. John Rivera, National Insti-

1 Abbreviations used in this paper: BMMC, bone marrow–derived cultured mast cells; CSA, cyclosporin A; gCe, germline Ce; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; HMC, human mast cell line; mRNA, messenger RNA; pCe, productive, rearranged IgE; rhIL-13, recombinant human IL-13; RTPCR, reverse transcriptase–mediated polymerase chain reaction.
tutes of Health, Bethesda, MD). The IL-3-independent cloned murine mast cell line, C1.MC/C57.1 (17), was obtained from Dr. Stephen Galli (Beth Israel Hospital, Boston, MA). Primary cultures of IL-3-dependent BMMC were prepared from 6-12-wk BALB/c mice, as follows: Mice were killed by cervical dislocation, and bone marrow was aseptically flushed from femurs and tibias into RPMI 1640 medium containing 4 mM L-glutamine, 5 x 10^{-5} M 2-mercaptoethanol, 1 mM sodium pyruvate, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.1 mM nonessential amino acids, 10% heat-inactivated FCS, and 10% medium conditioned by WEHI-3 cells (TIB 68; American Type Culture Collection, Rockville, MD) as a source of IL-3. The bone marrow cells were then collected by centrifugation at 400 g for 10 min and maintained at a density of 3-4 x 10^6 cells/ml in the same medium with biweekly replacement of used medium with fresh. Flasks (75 cm²) were kept at 37°C in a 5% CO², humidified atmosphere. BMMC obtained after 3-4 wk culture were >95% mast cells, as demonstrated by morphologic analysis. For high affinity IgE receptor (Fc,RI)-dependent activation, mast cells were sensitized with a monoclonal murine IgE anti-DNP antibody (18) obtained from Dr. David Katz (Biomedical Research Institute, La Jolla, CA) (19) and challenged with 40 ng/ml DNP30-40 human serum albumin (Sigma Chemical Co.) at a cell density of 2 x 10^6 cells/ml.

Isolation of RNA and Reverse Transciptase-mediated Polymerase Chain Reaction (RTPCR). Cellular RNA was isolated from mast cells by the guanidine thiocyanate/CsCl centrifugation method, as described (18). Levels of cytokine messenger RNA (mRNA) were assessed by a semiquantitative reverse transcriptase-mediated polymerase chain reaction. As a strategy for obtaining semiquantitative results from our RTPCR reactions, products were detected by quantitating incorporated radioactivity, and the number of amplification cycles chosen empirically for each primer pair was such that the maximum signal intensity for a set of samples was within the linear portion of a product vs. template amplification curve (20). After reverse transcription of each RNA sample using random primers, each sample was subjected to an initial assay for amplifiable cDNA, using primers specific for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA, which is not known to vary under our cellular incubation conditions. Based on the amount of G3PDH PCR product in this assay, aliquots of reverse transcriptase product representing equivalent amounts of G3PDH cDNA were amplified using primers for IL-13, IL-4, and again for G3PDH, as follows: RNA (4 μg) was reverse transcribed, using an Rnase H⁺-reverse transcriptase (Superscript; Life Technologies, Inc., Gaithersburg, MD) according to the manufacturer’s conditions. The resultant cDNA was immediately diluted with H₂O to a final volume of 200 μl, without heating or Rnase H treatment. Polymerase chain reaction was performed in thin wall reaction tubes (Perkin Elmer Cetus, Norwalk, CT) in a reaction mixture (50 μl) containing cDNA, 200 μM each dNTP, 1 μCi [³²P]dCTP (3,000 Ci/mmol, Dupont-NEN, Boston, MA), 1 μM each primer, 5% DMSO (Sigma Chemical Co.), and 2.5 U AmpliTaq DNA polymerase (Perkin Elmer Cetus) in reaction buffer supplied by the manufacturer. Primers are listed in Table 1. Amplifications were performed in a thermocycler (GeneAmp PCR System 9,600; Perkin Elmer Cetus) as follows: 94°C, 3 min; followed by 21-32 amplification cycles (94°C, 45 s; primer annealing temperature as specified in Table 1, 1 min; 72°C, 1 min); and main-

| RNA/DNA detected* | Genbank accession no. | Primer pair (upper strand; lower strand) | Size | Temperature |
|-------------------|-----------------------|------------------------------------------|------|-------------|
| m-IL-4            | X05253                | ATGGGCTCTCAACCCCCCAGCTAGT;               | 399  | 62.5        |
|                   |                       | GCTCTTCTTTTCCAGGAAGTC                    |      |             |
| m-IL-13           | L13028                | ATGGGCCTCCTGAGTCGATCCGTCGCC;             | 392  | 67          |
|                   |                       | GAAGGGCCGTGGCAGAAACAGTTGCG              |      |             |
| m-TNF-α           | Y00467                | CACAGAAAGGACAGATCCGCG;                  | 383  | 59          |
|                   |                       | GGCACCACTAGGGTTTGTGCT                  |      |             |
| m-G3PDH           | M32599                | GCCACCCAGAGACTGGATGCG;                  | 446  | 64          |
|                   |                       | CATGTAAGGATGATGTCACCAC                 |      |             |
| h-IL-13           | L06081                | CCACGTCATTGGTCCTCCATTGCC                | 263  | 66          |
|                   |                       | CTTGTCGCGAGACATTCCGTC                  |      |             |
| h-G3PDH           | X01677                | GCTCAGACACATGGAGAGGT;                   | 470  | 64          |
|                   |                       | GTGGTGACAGGGAGATTGCTA                  |      |             |
| h-gCe             | X56795                | AGGCTCAGTCCCAGCGCCACAGAAT;              | 484  | 67          |
|                   |                       | ACTGTAAGTCCTGCTACCAGGTGCGGCGG         |      |             |
| h-pCe             | M99403 (VH5)          | TCTGAGGTGACAGTGGAGAAGTTGTC             | 657  | 60          |
| J00222 (Ce-1)     |                       | GACGCTGAAGTGTGTCG                     |      |             |
| h-pCe (DNA)       | X56795 (Sp)           | GGCTCCTAGACAAAGGGAGGATTGCTATG          | 0.7-4| 58          |
| J00222 (Se)       |                       | GCCCGAATTCTCTAGGCGGTGTACAGGCG          |      |             |

* Primers designed for detection of RNA unless noted. m, murine; h, human.
tenance at 4°C until analysis. Aliquots (25 μl) of each amplification were analyzed by electrophoresis on 7% acrylamide (Long Ranger; AT Biochem, Malvern, PA) Tris-borate EDTA gels, followed by autoradiography and quantitation by Phosphorimage analysis (Molecular Dynamics, Sunnyvale, CA). DNA sizes were determined using mobility standards derived by T4 DNA polymerase end-labeling of Gel Marker DNA (Research Genetics, Huntsville, AL).

**Measurement of IL-13 Proliferative Bioactivity.** To assay IL-13 bioactivity in crude supernatants, we had to take into account the fact that most of the activities of this cytokine are shared with IL-4, which is known to be secreted by mast cells. To avoid confounding IL-4 effects, we assayed IL-13 activity in supernatants from murine mast cells using human cells as responders, since murine IL-13 cross-reacts with human IL-13 receptors, whereas murine IL-4 is species-specific. The IL-13-responsive human premyeloid cell line TF-1 (21) was obtained from G. Zurawski (DNAX Research Institute, Palo Alto, CA) and maintained in RPMI-1640, supplemented with 10% heat inactivated fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 2.5 ng/ml recombinant human IL-13 (rh-IL-13) (Peprotech Inc., Rock Hill, NJ).

TF-1 cells used to measure IL-13 bioactivity were starved of IL-13 for 48 h before exposure to mast cell supernatants. To assess the proliferative response of TF-1 cells to these supernatants, quadruplicate 100-μl aliquots of serially diluted supernatants were added to 96-well flat-bottom plates containing 40,000 TF-1 cells in 100 μl of RPMI 1640, 10% fetal bovine serum, and antibiotics. Cells were cultured for 42 h and then pulsed for 6 h with 1.0 μCi [3H]thymidine (6.7 Ci/mM; Dupont-NEN), harvested onto glass fiber filter strips with a multiple sample harvester (Skatron, Lier, Sterling, VA) and processed for scintillation counting. The maximal proliferative response of TF-1 cells to IL-13 was three-fold over IL-13-starved cells and was achieved with 1 ng/ml rhIL-13.

**Stimulation of IgE Expression in Human B Lymphocytes.** Human B lymphocytes were purified by positive selection from PBMC obtained by ficoll centrifugation of leukophoresis samples from normal donors. PBMC were incubated with anti-CD19 mAb-coated ferrous beads (DYNAL Inc., Great Neck, NY), followed by magnetic separation of B lymphocytes from nonreacting cells. B lymphocytes were cultured at a density of one million cells/ml in Iscove's medium, containing 10% FCS, 2.5 × 10^{-5} M β-mercaptoethanol, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 2 mM l-glutamine. Cells were stimulated with 2 mg/ml of anti-CD40 mAb 62.6.1 (kindly provided by Dr. J. Banchereau, Schering-Plough Research Institute, Lyon, France) or anti-CD40 mAb EA-5 (obtained from Biosource International Inc., Camarillo, CA). B lymphocytes were cultured with anti-CD40 mAb alone, or with 10 ng/ml of recombinant human IL-4 (kindly provided by Dr. A. Levine, Monsanto Co., St. Louis, MO), or with 50% (C1.MC/C57.1) or 100% (BMMC) culture volumes from either resting or IgE/antigen-stimulated mast cells, as described above.

After 6 d of culture, RNA was prepared from three to five million cells, as described above, and genomic DNA was prepared from a similar number of cells. Reverse transcription of cDNA and semiquantitative PCR analysis for germine Ce (gCe) and productive Ce (pCe) was as described above with primers shown in Table 1, using 60°C annealing steps in both cases, and 28 cycles of amplification for gCe, or 30 cycles of amplification for pCe. To detect μ-e switch recombination, Sμe-Sμe switch junctions were amplified from genomic DNA as described (22). When applied to a population of cells undergoing independent Sμe-Sμe recombination events, this protocol yields a ladder of bands. These bands vary in size, because the size of the composite switch region retained on the chromosome after switching varies over several kilobases, depending on the exact position of the recombination breakpoints within Sμa and Sμe. In brief, 500 ng B cell DNA, determined by fluorometric analysis and dissolved in a 25-μl volume, was amplified for a total of 32 cycles, using a primer 5’ to Sμa and a primer 3’ to Sμe, after which samples were electrophoresed, blotted, and hybridized with a 5’ Sμ probe (22). Primers used are listed in Table 1.

**Measurement of IgE and IL-4 by ELISA.** To measure IgE protein expression, triplicate cultures of 200,000 human B lymphocytes were maintained for each culture condition. After 12 d, IgE levels in supernatants from these cultures were analyzed by ELISA, as previously described (23). Murine IL-4 was assayed using an ELISA kit (Endogen, Inc., Boston, MA) according to the manufacturer’s instructions. Cytokine concentrations in nanograms per milliliter were obtained by regression analyses using a curve generated from standard cytokine preparations.

**Results**

**BMMC and a Mast Cell Line Transcribe IL-13 RNA in Response to Immunologic Stimulation, PMA, or Calcium Ionophore.** Mast cells produce IL-4 after stimulation through their IgE receptors or in response to calcium ionophore (24). We sought to determine whether IL-13, an IL-4-like molecule possessing many biologic properties in common with IL-4, was also produced by mast cells. As only low levels of mRNA specific for IL-4 are observed in mast cells after stimulation, we used a RTPCR assay for detection of IL-13.

We first determined whether IL-13 mRNA was expressed in BMMC stimulated with IgE and specific antigen, or in response to pharmacologic stimulation through protein kinase C (PKC) or calcium flux (24). We sought to determine whether IL-13, an IL-4-like molecule possessing many biologic properties in common with IL-4, was also produced by mast cells. As only low levels of mRNA specific for IL-4 are observed in mast cells after stimulation, we used an RTPCR assay for detection of IL-13.

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Because IL-13 and IL-4 mediate similar immunological phenomena, we also examined the induction pattern of IL-4 in BMMC under these conditions. The induction kinetics of IL-4 mRNA were similar to those of IL-13, but with peak expression occurring somewhat more broadly from 1 to 3 h. Unlike IL-13, IL-4 mRNA was not induced by PMA treatment, but was inducible by calcium ionophore, as demonstrated previously in mast cell lines (24). Also, the induction characteristics of IL-13 and IL-4 were similar for BMMC maintained for as long as 7 wk in culture. Thus, IL-13 and IL-4 appear to be coordinately expressed in response to IgE or calcium-mediated signals, but not to PMA/protein kinase C-mediated signals, suggesting that IL-13 and IL-4 may be separately regulated under some circumstances.

To strengthen the conclusion that mast cells (as opposed to some other minor cell population in our BMMC cultures) are capable of IL-13 RNA synthesis, we investigated whether
Figure 1. RTPCR analysis of mast cells stimulated with IgE and specific antigen, PMA, and calcium ionophore. (A) BMMC cultured for 4 wk in IL-3-containing medium and stimulated with IgE and specific antigen, PMA, or calcium ionophore, as indicated, were analyzed by RTPCR. Amplification cycles used were as follows: IL-D, 21 cycles; IL-4, 31 cycles; and G3PDH, 21 cycles. (B) CI-MC/C57.1 cells stimulated with IgE and specific antigen, PMA, or calcium ionophore A23187, as indicated, were analyzed by RTPCR. Amplification cycle were as in A. (C) KU812 were treated with PMA (10 ng/ml), calcium ionophore (1 μM), and the combination for 6 h. HMC-1 cells were treated similarly, but for 3 h. Amplification cycles used were 32 (IL-13) and 21 (G3PDH).

or not IL-13 was expressed in cloned mast cell lines. IgE-, PMA-, or A23187-mediated stimulation of the cloned mast cell line CI-MC/C57.1 resulted in high level accumulation of IL-13 RNA (Fig. 1 B), whereas little or none was observed in MC/9 or PT-18 (data not shown), two mast cell lines which fail to express many of the cytokines observed for CI-MC/C57.1 (18) and BMMC (P. Burd, unpublished observation). At 21 cycles of amplification, a small amount of IL-13-specific product was detected in unstimulated CI-MC/C57.1, which increased within 15 min after antigen cross-linking of IgE; levels peaked by 2 h (~155-fold induction over unstimulated) and declined thereafter. Treatment with either cycloheximide or cyclosporin A (CSA) substantially interfered with RNA accumulation at 1 h (81% and 93% inhibition, respectively). IL-4 RNA induction in these cells displayed kinetics similar to those of IL-13, but somewhat lesser sensitivities to the effects of cycloheximide (48% inhibition) and CSA (73% inhibition) than were observed for IL-13. RNA stability experiments indicated a half-life of ~50 min at maximal induction for IL-13 mRNA in CI-MC/C57.1 (data not shown). Together, these findings suggest that IL-13 expression in response to IgE-mediated signals may be of short duration. A similar pattern of short RNA half-life and limited period of expression has been observed for other IgE-inducible genes in mast cells, including TNF-α, IL-3, and IL-5 (26).

Expression of IL-13 RNA was not unique to murine mast cells, as IL-13 RNA was detected in response to the combination of PMA and calcium ionophore (but to neither agent alone) in the immature HMC-1. (Induction through IgE was not possible in this cell line, as these cells do not express FceRI [15].) In the human basophilic cell line KU812 stimulated with PMA, IL-13 RNA was also detected, which was further increased by cotreatment with ionophore (Fig. 1 C). These results indicate that mast cells of both murine and human origins are capable of expressing IL-13 RNA in response to IgE or pharmacologic stimulation. Moreover, additional experiments examining the signal requirements for IL-13 expression (data not shown) indicate that BMMC and CI-MC/C57.1 respond similarly to treatment with PMA, calcium ionophore, and the combination, and to the drugs cycloheximide and CSA. These results suggest that CI-MC/C57.1 is an appropriate model for expression of this cytokine by mast cells.

Mast Cells Secrete IL-13 Proliferative Bioactivity. To determine whether the IL-13 mRNA expression we detected in stimulated mast cells was associated with secretion of IL-13 bioactivity, we examined the ability of mast cell-conditioned supernatants to stimulate proliferation of a human IL-13-dependent cell line, TF-1 (21). Direct demonstration of IL-13 bioactivity using antimurine IL-13 neutralizing antibodies could not be performed, as this reagent was unavailable. However, use of a human cell line allowed us to take advantage of the fact that murine cytokines are generally inactive with human cells, but IL-13 shows full cross-species reactivity (14). Thus, while TF-1 responds to several other human cytokines
(including GM-CSF and IL-3), it does not respond to the murine homologues produced in these experiments. Proliferation assays were performed using TF-1 cells maintained in rhIL-13. After a 48-h period of IL-13 starvation, cells were exposed to mast cell-conditioned supernatants for 42 h and then pulsed with [3H]thymidine for 6 h. Fig. 2 shows proliferation data obtained from the same experiments presented in Fig. 1 and is representative of three experiments. IL-13 bioactivity was not observed in supernatants from unstimulated cells, nor from cells exposed to IgE alone. After cross-linking of IgE with antigen, IL-13 bioactivity was not observed at 30 min, by which time preformed stores of TNF-α were secreted (26, 27), but was detected by 1 h, with peak secretion attained by 3 h in BMMC and, later, in C1.MC/C57.1. Proliferation of TF-1 cells in response to rhIL-13 was observed at 1 ng/ml, a threefold induction over growth factor starved cells, and showed a dose response through 0.1 ng/ml under the conditions used. We also used this assay to estimate the amount of IL-3 secreted by determining the amount of mast cell supernatant required to cause maximal TF-1 proliferation. The data presented in Fig. 2 show that maximal proliferation of TF-1 cells equivalent to 1 ng/ml rhIL-13 was achieved using 50% supernatants collected from BMMC at 3, 6, or 12 h, suggesting that BMMC secreted ~2 ng/ml. In separate experiments, C1.MC/C57.1 supernatants from 6 or 12 h induced maximal proliferation at 5%, suggesting that these cells secreted as much as 20 ng/ml IL-13 bioactivity. In comparison, BMMC secreted 0.5 ng/ml IL-4 by 12 h, as shown by an IL-4-specific ELISA. These results demonstrate that mast cells secrete large amounts of IL-13 in response to stimulation through the IgE receptor and suggest that IL-13, like IL-4, may be a primary component of IgE-mediated mast cell responses.

**Mast Cell Supernatants Containing IL-13 Bioactivity Can Regulate IgE Expression.** Using an independent bioassay to verify the presence of IL-13, we investigated whether murine mast cell supernatants were able to direct IgE production in B cells, a documented activity of IL-13 (7). As in the proliferation assay described above, human lymphocytes were used so that other murine cytokines in the mast cell supernatants, particularly IL-4, would not interfere with assessment of IL-13 bioactivity. The isotype switch from expression of IgM to IgE is preceded by a developmental stage in which the Ce gene is transcribed from a promoter upstream of the switch region of the e locus (Se), producing a "sterile" or "germ-line" transcript (gCe). This promoter is regulated by IL-4 and may account for the role of this cytokine in switching to IgE expression. However, while IL-4 (or, perhaps, IL-13) may be necessary for this isotype switch, an additional signal is also required; in our experiments, this signal was supplied by CD40 engagement with an antibody. When switch recombination occurs joining 5μ to Se, the promoter of the gCe is deleted; at this stage, transcription of the Ce is initiated by the promoter upstream of the VH region, yielding productive e heavy chain transcripts (pCe). Using RTPCR assays, we investigated the regulation of gCe and pCe accumulation in B lymphocytes by mast cell supernatants. As shown in Fig. 3, gCe were increased in B cells exposed to either IL-4 or mast cell supernatant. Activation of the mast cells by IgE plus antigen had little effect on the ability of the supernatants to induce gCe (C57 stim. vs. C57), but such activation significantly increased the level of pCe. IL-4 has been found to stimulate gCe accumulation at concentrations at least 100 times lower than those required to induce switch recombination (Thyphronitis, G., F. C. Mills, and E. E. Max, unpublished results). It is possible that IL-13 is similar in this respect, so that even unstimulated mast cells make enough IL-13 to induce gCe expression, but no switching and, therefore, no pCe. It is also possible that other cytokines with weak cross-species reactivity might be present in these supernatants (such as TNF-α or TGF-β) and contribute to gCe accumulation.
Figure 3. Mast cell supernatants contain IL-13 bioactivity regulating IgE expression in human B lymphocytes. Human B lymphocytes purified from PBMC (B0) were stimulated with anti-CD40 alone, or, in addition, cultured with IL-4, supernatants from resting CI.MC/C57.1 (C57), or stimulated CI.MC/C57 (C57 stim.) and examined for expression of germ-line Ce transcripts (gCe), VH5-containing Ce transcripts (pCe), and Sμ-Se DNA class switching. RTPCR samples were normalized for expression of G3PDH. DNA from the IgE-secreting cell lines 2C4 and U266 (diluted 10^-3 with neutrophil DNA) was included to demonstrate detection of Sμ-Se switch recombination.

Discussion

In this report, we demonstrate that mast cells accumulate IL-13 mRNA and secrete significant amounts of IL-13 after stimulation through the IgE receptor. The induction of IL-13 RNA is rapid, beginning as early as 15 min after IgE receptor cross-linking, peaking between 1 and 2 h, and falling substantially by 6 h. This relatively short period of RNA expression is similar to that observed for IL-4 and TNF-α in mast cells, but differs from the time course of IL-13 RNA expression in T cells, which persists at high levels through 72 h after several activation stimuli (14). However, as is seen in T cells, secretion of IL-13 bioactivity by mast cells closely parallels appearance of RNA, occurring in mast cells as early as 1 h after stimulation and reaching levels of bioactivity approaching ~1-2 ng IL-13/10^6 cells. Mast cell-derived IL-13 demonstrates several biologic activities described for T cell-derived IL-13: It supports growth of the IL-13-dependent TF-1 cell line, and, in B cells, induces gCe accumulation, μ-ε switch recombination, and IgE secretion.

The biologic properties of IL-13 and the amounts secreted after stimulation suggest that mast cell-derived IL-13 may be a physiologically significant mediator of mast cell responses. The in vitro experiments presented here indicate that substantial levels of IL-13 are secreted by 3 h after IgE cross-linking, the same time at which in vivo studies of passive

Table 2. Activated CI.MC/C57.1 Supernatants Stimulate IgE secretion

| Donor | Anti-CD40 | Anti-CD40 + IL-4 | Anti-CD40 + C57 | Anti-CD40 + Stim C57 |
|-------|-----------|-----------------|----------------|---------------------|
| Donor 1 | -0.47 ± 0.06 | 17.9 ± 3.7 | 1.19 ± 0.76 | 15.1 ± 3.2* |
| Donor 2 | 0.59 ± 1.28 | 20.6 ± 4.5 | 0.77 ± 1.55 | 3.6 ± 1.1* |
| Donor 3 | 0.10 ± 0.02 | 12.6 ± 5.5 | 0.15 ± 0.14 | 5.7 ± 1.8* |

Purified B cells were seeded at 2 x 10^6 cells/well in microtiter plates and stimulated with anti-CD40 antibodies, rIL-4 (10 ng/ml), or CI.MC/C57.1 supernatants (50%) as indicated. Results are reported as the mean plus standard deviation of quintuplicate determinations and reported as nanograms per milliliter IgE detected by ELISA.

* Significantly greater than corresponding same donor treated with anti-CD40 alone with P <0.05 by Student's t test corrected for unequal variances.

Similarly, mast cell activation was required to elicit significant IgE secretion, in response to either CI.MC/C57.1 (Table 2) or BMMC (Table 3) supernatants. Treatment of B cells with either anti-CD40 antibody alone, or in combination with supernatant from unstimulated mast cells, did not lead to appreciable IgE secretion, whereas cotreatment with supernatants from mast cells treated with IgE plus antigen, like IL-4, did. (In the experiment presented in Fig. 3 and in Table 2 as Donor 2, IL-4 induced more secretion, but less switching, than the supernatant from stimulated CI.MC/C57.1 cells; this was not observed for other donors, and similar quantitative dissociation between secretion and switching has been reported in a murine system [29]). These results show that activated mast cells not only accumulate IL-13 mRNA, but also secrete a protein with two independent IL-13 bioactivities: supporting the growth of an IL-13-dependent cell line and inducing IgE expression.
Table 3. Activated BMMC Supernatants Stimulate IgE Secretion

|                | Anti-CD40 | Anti-CD40 + IL-4 | Anti-CD40 + BMMC | Anti-CD40 + Stim BMMC |
|----------------|-----------|------------------|------------------|-----------------------|
| Donor 4        | -0.093 ± 0.010 | 1.159 ± 0.186 | -0.050 ± 0.019 | 0.615 ± 0.291*       |
| Donor 5        | -0.086 ± 0.004 | 0.466 ± 0.256 | -0.070 ± 0.017 | 0.754 ± 0.376*       |

Purified B cells were treated as in Table 2 with anti-CD40 antibodies, rhlL-4, or BMMC supernatants (100%) as indicated. Results are reported as the mean plus standard deviation of quintuplicate determinations and are reported in nanograms per milliliter IgE detected by ELISA.

* Significantly greater than corresponding same donor treated with anti-CD40 alone with P <0.01 by Student’s t test corrected for unequal variances.

cutaneous anaphylaxis have shown inflammatory cells (monocytes, neutrophils, and others) beginning to infiltrate sites of mast cell degranulation (30, 31). Like IL-4, IL-13 exhibits immunosuppressive effects upon monocytes, inhibiting production of proinflammatory cytokines, chemokines, and hematopoietic growth factors, including IL-1, IL-6, IL-8, IL-10, IL-12, TNF-α, macrophage inflammatory protein-1α, GM-CSF, and granulocyte-CSF (10, 11). IL-13 also decreases NO production and increases parasite survival in macrophages (11).

These properties suggest that one function of mast cell-derived IL-13 may be to inhibit cytokine production by infiltrating inflammatory cells recruited to sites of mast cell degranulation, thereby dampening their potential for amplification of cytokine responses at these sites.

Although we have demonstrated that IL-13 derived from mast cells is able to modulate the IgE response by promoting class switching to IgE, the contribution of this cytokine to IgE responses in vivo is not clear. As murine B cells do not possess receptors for IL-13 (14), mast cell–derived IL-13 could not contribute directly to murine IgE responses. However, mast cell–derived IL-13 may contribute to IgE responses in humans and to the development of elevated IgE levels associated with certain pathologic conditions, such as parasitic infections. In this scenario, long-term or chronic antigenic stimulation of mast cells could result in amounts of IL-13 (as well as IL-4) sufficient to skew the Ig response towards IgE. A physiologic role for IL-13 regulation of IgE is consistent with a reinterpretation of recent data (9) linking the IL-4 gene with a genetic locus regulating serum IgE levels in atopic humans: Since the IL-13 gene is within 30 kb of the IL-4 gene, it is possible that the relevant allelic differences regulate the function of IL-13, rather than IL-4. Other gene linkage studies indicate that mutations within the antigen receptor activation motif of the cytoplasmic tail of the high affinity IgE receptor β subunit may also be associated with the atopic state (32). Such mutations might modulate cytokine production in response to receptor signaling, leading to increased or prolonged production of these IgE-inducible cytokines. By either mechanism, these studies suggest that mast cell cytokine production may be involved in either the establishment or development of allergic diseases.

In summary, we have demonstrated that mast cells produce significant levels of IL-13 after immunological stimulation and that mast cell–derived IL-13 is biologically active to direct B cell Ig class switching to IgE. The high levels of IL-13, relative to IL-4, secreted by mast cells, the shared activities of these two cytokines, and their similar biologic potencies (14) suggest that effects previously ascribed solely to IL-4 during mast cell function may be mediated, in part, through IL-13.

The authors thank Dr. Gerard Zurawski (DNAX Research Institute, Palo Alto, CA) for TF-1 cells, Dr. Stephen Galli for C1.MC/CS.1 cells, Dr. Joseph Butterfield for HMC-1 cells, Dr. John Rivera for KU812 cells, Sandoz Pharmaceuticals for the gift of cyclosporin A, and Drs. Eda Bloom and Mark Hayes for critical reading of the manuscript.

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Received for publication 15 August 1994 and in revised form 15 December 1994.

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