Interleukin 4 Is Localized to and Released by Human Mast Cells


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Summary

Recent attention has focused on the T helper type 2 (Th2) lymphocyte as a source of interleukin 4 (IL-4) in allergic disease. However, Th2 cells themselves require a pulse of IL-4 to initiate this synthesis. Here we provide immunohistochemical evidence of IL-4 localization to human mast cells of the skin and respiratory tract, and demonstrate that immunoglobulin E-dependent stimulation of purified human lung mast cells leads to the rapid release of IL-4 into the extracellular environment. We propose that mast cell activation in an allergic response provides a rapid and local pulse of IL-4 into the local environment essential for the triggering of T lymphocytes into sustained IL-4 production and to initiate inflammatory cell accumulation and activation.

Materials and Methods

Human Subjects and Biopsy Specimens. Nasal biopsies were taken from the inferior or inferomedial edge of the inferior turbinate of four patients with allergic perennial rhinitis using 2-mm cupped Hartman’s forceps (Medicom, Tuttlingen, Germany) under tetra-caine local anesthesia. Bronchial biopsies were taken from the right middle lobe carina in four perennial allergic asthmatic patients via a fiber-optic bronchoscope (BFIT20; Olympus Company, Tokyo, Japan) using alligator forceps (Olympus FB15E) (11). All patients gave written informed consent and the biopsy procedures were approved by the Southampton University and Hospitals Ethical Committee. In each instance the biopsy specimens were fixed in ice-cooled acetone containing the protease inhibitors iodoacetamide and PMSF, then stored for 24 h at -20°C before processing into glycol methacrylate (GMA). Biopsies were placed in acetone, then methylbenzoate for 15 min each at room temperature, transferred to GMA JB4 solution (Polysciences, Northampton, UK) at 4°C, followed by embedding in GMA resin.

Immunohistochemistry of Biopsy Specimens. For immunohistochemistry, 2-μm sections were cut by ultramicrotomy, floated on 0.2% ammonia solution in water for 1 min, and dried at room temperature for 1-4 h. Sections were pretreated with 10 mol 0.1% sodium azide solution containing 100 μl 30% hydrogen peroxide for 30 min to inhibit endogenous peroxidase. Mouse IgG1 mAbs 3H4 and 4D9 to human IL-4 (12), AA1 to mast cell tryptase, anti-CD3, and anti-CD4 were applied to sequential sections. A biotinylated rabbit anti-mouse (Dako Ltd., High Wycombe, UK) secondary stage was applied and subsequently demonstrated using the streptavidin-biotin peroxidase complex detection system. Aminoethylcarbazole (AEC) was used as chromogen giving a red reaction product, and sections were counterstained in Mayer’s hematoxylin. Control slides were similarly treated, either with the primary antibody omitted, or in the presence of an unrelated mouse IgG1 mAb at the same concentration as the anti-IL-4 antibodies.
Four double immunostaining sections were initially treated with the first antibody as described using the streptavidin-biotin peroxidase detection system. This process was then repeated for the second antibody but by utilizing the streptavidin-biotin alkaline phosphatase detection system (Dako Ltd.) using fast blue B.B as the chromogen. No counterstain was applied. Controls were performed with no antibody, either the anti-tryptase, anti-CD3, or anti-IL-4 antibody absent, and with each antibody applied first, to show that the detection system for the second antibody did not crossreact with the first antibody.

**Mast Cell Purification.** Foreskin mast cells were dispersed from circumcision specimens using collagenase (1.5 mg/ml) and hyaluronidase (0.75 mg/ml) as previously described (13). The isolated cells were layered onto a discontinuous gradient of 60-80% Percoll (density, 1.076-1.1 g/ml) and centrifuged at 500 g for 20 min at 4°C. Mast cells at the bottom of the gradient and between the 70/80% interface were 90% pure. T lymphocytes were removed by incubation of resuspended cells for 30 min at 4°C with magnetic beads coated with a mAb specific for the CD2 T cell antigen (Dynal, Oslo, Norway). Two 15-min exposures to a magnet removed 99% of CD2-positive T cells. Lung mast cells were dispersed from macroscopically normal human lung, obtained within 1 h of resection as described for the skin above. Due to the different nature of contaminating cells in the dispersate, it was not possible to use density sedimentation alone to purify lung mast cells. Erythrocytes were removed by centrifugation through a 65% continuous Percoll gradient (1.084 g/ml), and the nucleated cells were incubated for 30 min with 5 µg/ml of the mAb YB5.BB (kindly donated by L. Ashman, Melbourne, Australia), which recognizes the mast cell c-kit receptor. Cells were then washed and incubated for 60 min with a 500-µl suspension of magnetic beads coated with goat anti-mouse IgG antibody (Dynal) at 4°C on a roller. Purified mast cells attached to magnetic beads were removed from the suspension with a magnet.

**Immunohistochemistry of Purified Mast Cells.** For immunocytochemical staining, purified mast cells were fixed in buffered 4% paraformaldehyde (20 min), and cytosin preparations were made. The mast cells were then stained for IL-4 with three mAbs, 3H4, 4D9, and 8F12 (12), using the alkaline phosphatase anti–alkaline phosphatase (APAAP) detection system with fast red as the chromogen for skin cells and fast blue as the chromogen for the lung cells to help distinguish cells from dynabeads. Negative controls were performed with primary antibody absent and an unrelated mouse IgG1 mAb. Specificity of immunostaining was demonstrated in Chinese hamster ovary (CHO) cells transfected with cDNA for human IL-4. IL-4 immunoreactivity was shown with the mAb 3H4, which could be effectively inhibited when the 3H4 mAb was preincubated with rhIL-4 (Fig. 1). Positive immunostaining of IL-4-transfected CHO cells was also observed with the anti-IL-4 mAbs 4D9 and 8F12.

**IL-4 Release by Purified Mast Cells.** After purification mast cells were resuspended in cold HBSS* and allowed to warm slowly to room temperature. Aliquots of the cell suspension were made (225 µl containing ∼5-10 x 10⁴ mast cells/tube) and preincubated for 15 min before addition of 25 µl of 10⁻⁴ concentrated anti-IgE or HBSS* (control). Cells were then incubated for 1, 2, 4, or 6 h, and the release reaction was stopped by centrifugation (5 min, 500 g, 4°C). Supernatants were decanted and frozen at −80°C. For histamine analysis, TCA was added to a final concentration of 5% before freezing. Histamine was measured spectrophotofluorimetrically and IL-4 assayed by ELISA. The specificity of our immunostaining for IL-4 is suggested by several factors. First, in the tissue section, the two specific IL-4 antibodies used, 3H4 and 4D9, stain the same cells in spite of reacting with different epitopes on human IL-4, while an isotype control antibody at the same Ig concentration was negative. Second, with the purified cells, the three antibodies used, 3H4, 4D9, and 8F12, all give positive staining, whereas...
Figure 1. Cytocentrifuged IL-4-transfected (CHO) cells (top) and non-transfected CHO cells (middle) showing positive and negative staining, respectively, with mAb 3H4. Incubation of mAb 3H4 with an excess of IL-4 before staining of transfected cells greatly reduced IL-4 immunostaining (bottom).

an isotype control was again negative. Third, CHO cells transfected with the human IL-4 gene and known to secrete large quantities of IL-4 stained positively with the IL-4 antibodies, whereas CHO cells without gene transfection were negative. Incubation of antibodies with IL-4 before staining greatly reduced staining of IL-4-positive CHO cells but was unhelpful in tissue sections due to marked subsequent background staining, probably due to nonspecific binding of immune complexes to resin sections, or binding of immune complexes via the IL-4 receptor, as neither antibodies employed in the tissue sections were neutralizing.

These findings have considerable relevance to the pathogenesis of chronic allergic inflammation and in particular for the role of the tissue mast cell in its initiation. IL-4 has many effects that promote allergic inflammation. These include:

Figure 2. Immunohistological staining with antibodies 3H4 against human IL-4 (top), AAI against mast cell tryptase (middle), and 4D9 also against human IL-4 (bottom) in sequential 2-μm sections of a mucosal biopsy from a patient with atopic asthma showing cellular staining with both anti-IL-4 antibodies and the anti-mast cell tryptase antibody.
upregulation of IgE synthesis (1); stimulation of T cell proliferation and Th2 cell development (6, 7, 16); induction of MHC class II molecules on B cells and monocytes (17, 18); induction of FcERI (CD23) on B cells and macrophages (17, 19); induction of LFA-1 and LFA-3 on B cells (20); activation of macrophages (21); proliferation of fibroblasts (22); induction of VCAM-1 on endothelial cells (23); increased mast cell basal and stimulated histamine release (24); the promotion of eosinophil and mast cell accumulation at the site of allergic reactions (25, 26); and mast cell growth and maturation (27).

The source of IL-4 necessary to maintain these responses is considered to be the T lymphocyte, particularly its Th2 subset (28). However the Th2 lymphocyte itself requires IL-4 to initiate and support its development and cytokine synthesis (6, 7). It is our hypothesis that the release of IL-4 from the mast cell after IgE-dependent activation provides a localized initiating stimulus for the activation of Th2 cells. Furthermore, we suggest that mast cell cytokine release within minutes of allergen provocation plays a major role in the early accumula-

Figure 3. Double immunostaining of 2-μm sections from a nasal biopsy with antibody AA1 against mast cell tryptase and 3H4 against human IL-4 (top), and a bronchial biopsy with AA1 and 4D9 also against human IL-4 (bottom) identifying the localization of IL-4 immunoreactivity to mast cells.

Figure 4. IL-4 immunostaining of human mast cells dispersed and purified from resected foreskin stained with the 3H4 and anti-human IL-4 antibody.

Figure 5. Levels of histamine (expressed as μg/10^6 mast cells; top) and IL-4 (ng/10^6 cells; bottom) in culture supernatants from purified lung mast cells after either saline control (open bar) or anti-IgE challenge (striped bar).
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