

Images in Allergy and Immunology

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Chemokine expression in allergic diseases

Editor's note: This feature, Images in allergy and immunology, is designed to highlight current concepts of the immunopathology of allergic diseases and other common immunologically mediated diseases. The presentation will appear as sets of images that involve cross-pathology, histopathology, and molecular pathology and will cover a range of topics of interest to allergists and immunologists.

Chemokines are a group of small-molecular-weight cytokines ranging from 8 to 10 kd. They are primarily involved in attracting and regulating leukocyte trafficking into the tissues in a process called chemotaxis. Chemokines can also promote cell growth and differentiation, cytokine production, and membrane receptor expression. There are approximately 50 chemokines described to date, and they are separated out into 4 major families based on the arrangement of the cysteine residues in the molecules: the CC, CXC, CX3C, and XC families. Chemokines can either be released as soluble proteins or fused with mucin-like stalks anchored in the membrane, forming cell-adhesion receptors capable of recruiting cells from the circulation. Chemokines mediate their effects by binding to 7-transmembrane domain receptors coupled to G proteins. Although some chemokines, such as eotaxin (CCL11), bind to only one receptor, many other chemokines can bind to multiple receptors. In addition, many chemokine receptors can bind to multiple different ligands. Therefore there is much functional redundancy present in chemokine biology, making it

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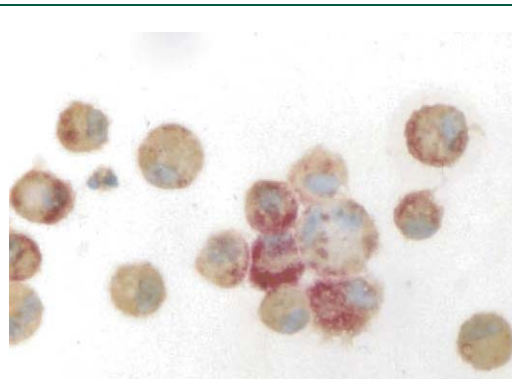


FIG 1. Immunocytochemistry for eotaxin in macrophages was performed on bronchoalveolar lavage cells taken from an asthmatic patient. Eotaxin was stained red by alkaline phosphatase-based immunocytochemistry and colocalized with CD68, which was stained brown.

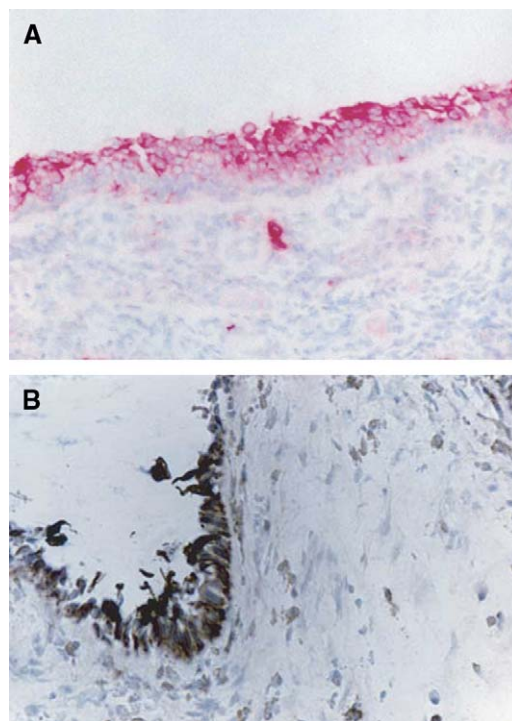


FIG 2. A, Alkaline phosphatase-based immunohistochemistry for eotaxin shows intense red staining of the epithelial layer in the nasal mucosa of an allergic patient during ragweed allergen season. **B,** Similar positivity for eotaxin mRNA, as seen by dark staining, in the epithelial layer is seen after digoxigenin-based *in situ* hybridization.

difficult to ascribe distinct functional attributes to many chemokines.

Many chemokines and their receptors are highly expressed in allergic diseases.¹ Chemokines involved in these diseases have as their major targets T_H cells and eosinophils. These include RANTES (CCL5), eotaxin (CCL11), and monocyte chemoattractant protein 4 (MCP-4; CCL13). Eotaxin was shown to be

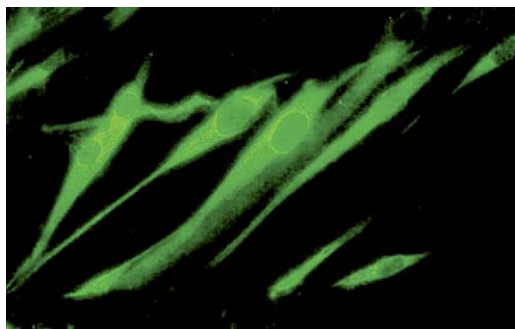


FIG 3. Immunofluorescence staining for eotaxin (green) in human bronchial/tracheal primary smooth muscle cells (Clonetics, Walkersville, Md) grown *in vitro*.

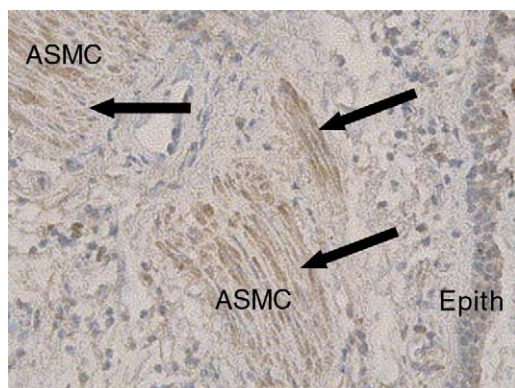


FIG 4. CCR3 immunostaining from a biopsy specimen of an asthmatic subject shows positive signal (brown staining) in smooth muscle bundles, as denoted by the arrows. *Epith*, Epithelium; *ASMC*, airway smooth muscle cells.

produced by macrophages, T cells, and eosinophils in the bronchoalveolar lavage fluid from asthmatic subjects (Fig 1).² Important sources of chemokines, aside from inflammatory cells, are structural cells. Epithelial cells in the airways have been shown to be positive for both eotaxin mRNA and protein (Fig 2). MCP-3, MCP-4, RANTES, and IL-8 are also expressed in the epithelium of asthmatic lungs. A number of chemokines are expressed and produced by smooth muscle cells, including IL-8, RANTES, and eotaxin (Fig 3).³ Not only do these cells produce chemokines, but they also express many chemokine receptors, such as CCR3 (Fig 4).⁴ Structural cell production of chemokines is not limited to large airways; epithelial eotaxin and MCP-4 is also seen in smaller lower airways (Fig 5).⁵ Expression of eotaxin is also detected in epithelial cells and inflammatory cells in the submucosa of the nasal passages in patients with allergic rhinitis and sinusitis, concordantly with an increased number of inflammatory cells (Fig 6). Atopic dermatitis is also associated with increased levels of chemokines, including eotaxin in the epidermis and the subepithelial layer (Fig 7).⁶

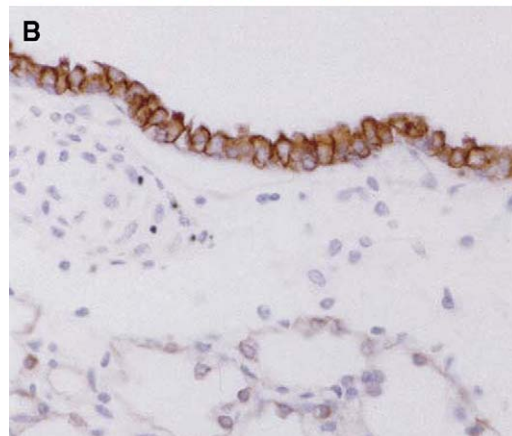
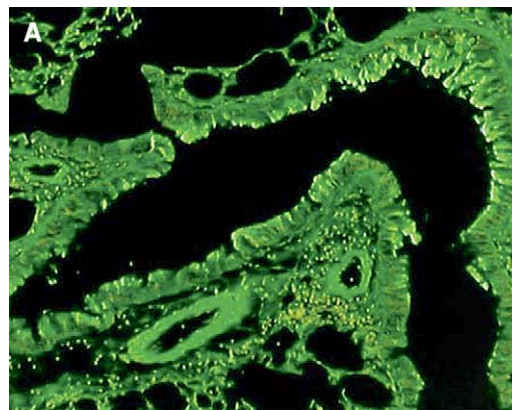


FIG 5. **A**, Immunofluorescence staining for eotaxin (strong green staining) in epithelial cells of the small lower airways of an asthmatic patient. **B**, Alkaline phosphatase-based immunostaining (brown staining) for MCP-4 in epithelial cells of the small lower airways of an asthmatic patient.

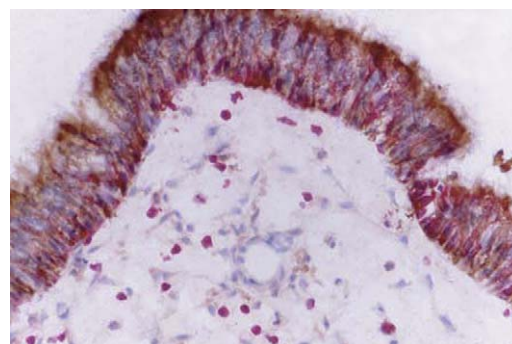


FIG 6. Double immunostaining for eotaxin in red and cytokeratin in brown of nasal biopsy specimens taken from a patient with allergic rhinitis after 24-hour allergen challenge. The epithelial cells are positive for both eotaxin and cytokeratin. Note the presence of eotaxin-positive leukocytes in the mucosa of the biopsy as well.

Corticosteroid treatment of allergic diseases alters the chemokine expression phenotype rather than completely abrogating chemokine expression. A recent study has shown that although corticosteroids decreased expression of eotaxin, MCP-3, and MCP-4 in

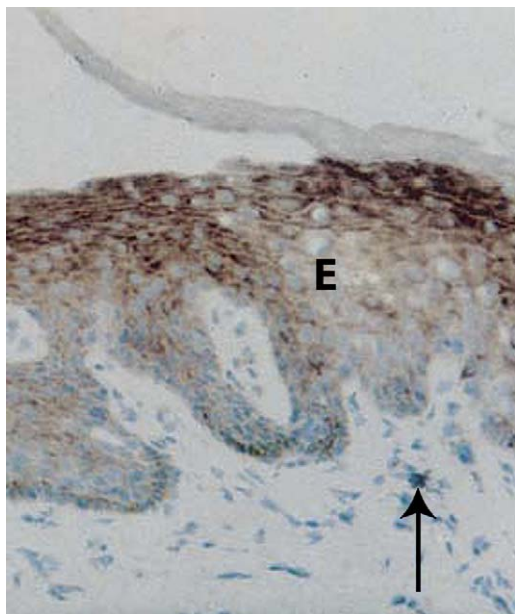


FIG 7. Digoxigenin-based *in situ* hybridization for eotaxin mRNA, as seen by dark brown staining, in a skin biopsy specimen from a patient with chronic atopic dermatitis. The epidermal layer (E) is strongly positive for the probe. Also note an eotaxin mRNA-positive cell in the subepithelial layer (arrow).

asthma, they upregulated epithelial expression of IL-8, IFN-inducible protein 10 (IP-10; CXCL10), and MCP-2 (Fig 8).⁷ These chemokines might contribute to the increased number of neutrophils observed after steroid treatment, particularly in severe asthma, although steroids can directly prolong neutrophil survival. Therapeutic strategies with antibodies to chemokines or their receptors are currently under investigation as alternative treatments for asthma and allergic rhinitis.

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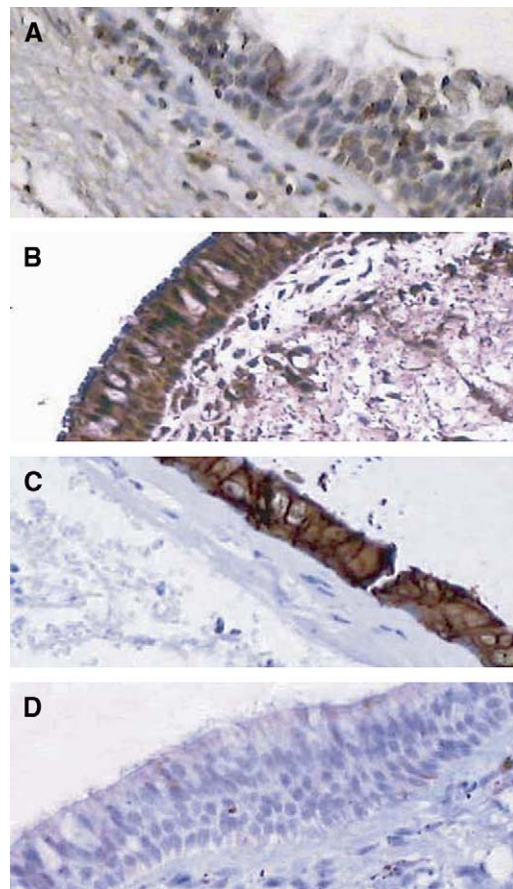


FIG 8. *In situ* hybridization of asthmatic airways by using digoxigenin-labeled chemokine probes. **A**, Baseline *in situ* hybridization for IL-8. **B**, IL-8 expression after corticosteroid (methylprednisolone, 40 mg/d for 2 weeks) treatment. Note increased staining for IL-8 (red-brown staining) in the epithelial layer compared with baseline. **C**, Baseline *in situ* hybridization for eotaxin. **D**, Eotaxin expression after corticosteroid treatment. Note increased eotaxin (red-brown staining) in the epithelial layer.

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