

IL-9 and c-Kit⁺ mast cells in allergic rhinitis during seasonal allergen exposure: Effect of immunotherapy

Kayhan T. Nouri-Aria, PhD, FRCPath,* Charles Pilette, MD, PhD,* Mikila R. Jacobson, PhD, Hiroshi Watanabe, MD, and Stephen R. Durham, MD, FRCP *London, United Kingdom*

Background: IL-9 is an important stimulus for tissue infiltration by mast cells, a feature requiring concomitant activation of c-Kit.

Objectives: We assessed IL-9 expression and c-Kit⁺ mast cells in the nasal mucosa of patients with allergic rhinitis during seasonal pollen exposure and observed the effects of allergen immunotherapy.

Methods: We studied 44 patients with seasonal rhinitis and asthma before and 2 years after a double-blind trial of grass pollen immunotherapy. Nasal mucosal IL-9⁺ cells and c-Kit⁺ mast cells were assessed by means of immunochemistry. Cell types expressing IL-9 protein were determined by means of dual immunofluorescence. IL-9 mRNA-positive cells were assessed by means of *in situ* hybridization, and their phenotype was determined by using sequential immunohistochemistry and *in situ* hybridization.

Results: Nasal mucosal c-Kit⁺ mast cells were increased during the pollen season ($P = .0001$). IL-9 mRNA-positive cells also tended to increase ($P = .1$) and correlated with nasal EG2⁺ eosinophils ($r = 0.47$, $P = .05$) and IL-5 mRNA-positive cells ($r = 0.54$, $P = .02$). The cell sources of IL-9 included T cells, eosinophils, neutrophils, and mast cells. When compared with placebo, successful pollen immunotherapy markedly inhibited seasonal increases in nasal mucosal c-Kit⁺ mast cells ($P = .001$) and the seasonal expression of IL-9 mRNA-positive cells ($P = .06$). Immunotherapy also inhibited IL-9 protein expression from nonendothelial cell sources ($P = .0007$).

Conclusion: IL-9 is upregulated in the nasal mucosa during the pollen season and correlates with tissue infiltration by eosinophils. Successful pollen immunotherapy is associated

with inhibition of seasonal increases in both nasal c-Kit⁺ mast cells and eosinophils. This effect might be explained, at least in part, by the reduced local expression of IL-9. (*J Allergy Clin Immunol* 2005;116:73-9.)

Key words: Allergy, IL-9, mast cells, eosinophils, cytokine

Mast cells, as well as eosinophils and basophils, are important effector cells in human allergic diseases. They mediate immediate responses to allergen exposure triggered by specific IgE and promote allergic inflammation through the release of proinflammatory mediators, including histamine, leukotriene C₄, IL-4, and IL-13.^{1,2} The number of mast cells is increased in tissue sites of allergic inflammation.³⁻⁵

Allergen immunotherapy is highly effective for seasonal allergic rhinitis⁶; clinical improvement correlates with inhibition of the seasonal recruitment of eosinophils and basophils to the nasal mucosa.^{7,8} However, in a previous study we did not observe significant changes in mast cell numbers (tryptase positive) in the nasal mucosa of immunotherapy-treated patients.⁸ Moreover, although reduction in IL-5 expression could account for the inhibition of tissue eosinophilia after immunotherapy,⁷ the influence of immunotherapy on cytokines related to mast cell development has not been assessed in the nasal mucosa.

IL-9 has been identified as a mast cell growth-enhancing factor.⁹ IL-9 also stimulates differentiation of mast cells,¹⁰ which express specific receptors for IL-9.¹¹ Studies on transgenic mice indicate that IL-9 induces the accumulation of mast cells in mucosal tissues,^{10,12} with this effect requiring the concomitant activation of the stem cell factor (SCF)/c-Kit pathway.¹⁰ Further studies confirmed a role for IL-9 in many aspects of allergic airway disease,¹³ including tissue eosinophilia, bronchial hyper-responsiveness,¹⁴ IgE production,¹⁵ and mucus hypersecretion.^{16,17} The IL-9 gene has been localized to the human chromosome 5q35-q33 cluster gene region and has been proposed to contribute directly to the genetic link between this important locus and the asthma phenotype.¹⁸ IL-9 expression is increased in atopic asthma^{19,20} and is induced in the lung on local allergen challenge.²¹ However, its expression in the airways after natural allergen exposure and its modulation by means of immunotherapy has not been evaluated.

Therefore we assessed IL-9 as a potential key cytokine driving mast cell infiltration of the nasal mucosa from patients with seasonal allergic rhinitis. We examined nasal

From Upper Respiratory Medicine, Imperial College London at the National Heart and Lung Institute, London, United Kingdom.

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*These authors contributed equally to the present study.

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Reprint request: Stephen R. Durham, MD, FRCP, Upper Respiratory Medicine, Imperial College London, National Heart and Lung Institute, Dovehouse St, London SW3 6LY, United Kingdom. E-mail: s.durham@imperial.ac.uk.

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Abbreviations used

MBP: Major basic protein

SCF: Stem cell factor (or c-Kit ligand)

biopsy specimens obtained from patients with severe seasonal allergic rhinitis during the peak pollen season compared with specimens obtained out of the pollen season for the presence of mast cells expressing c-Kit and for expression of IL-9 protein and mRNA. We also assessed the potential relationship between IL-9 expression and eosinophils in the nasal mucosa and analyzed the effects of 2 years of conventional grass pollen immunotherapy on c-Kit⁺ mast cell numbers and IL-9 expression.

METHODS**Patients**

The clinical features of the 44 patients recruited from the allergy clinic of the Royal Brompton Hospital, London, United Kingdom, have been described previously.²² All subjects had a history of severe summer hay fever that was not controlled by antiallergic drugs and a positive skin response to timothy grass pollen. The study was performed with the approval of the ethics committee of the Royal Brompton Hospital and with the informed consent of all participants.

This was a randomized, double-blind, placebo-controlled, parallel-group study, as previously described.²² After one summer of symptom monitoring and before randomization to immunotherapy or placebo injections, baseline nasal biopsies were performed at a time when patients were asymptomatic. After 2 years of treatment, a second biopsy specimen was taken at the peak of the grass pollen season from the 37 subjects remaining in the study.

Additional biopsy specimens were taken from 9 nonatopic control subjects at the same time as the second (peak seasonal) set of study biopsy specimens to control for the effects of seasonal pollen exposure in nonsensitive subjects.

Immunohistochemistry

Biopsy specimens were divided into 2 halves. One half was immediately mounted in OCT compound (VWR; Lutterworth, Leics, United Kingdom), snap-frozen by means of immersion in isopentane precooled in liquid nitrogen, and then stored at -80°C . Immunohistochemistry was performed on 6- μm cryostat sections fixed in acetone/methanol (60:40) by using the anti-mouse Vector kit (Vector laboratories, Inc, Burlingame, Calif), according to the manufacturer's protocol. Sections were incubated with purified mouse mAb to human IL-9 (clone MH9P3; gift from J. Van Snick, Ludwig Institute for Cancer Research, ICP-Brussels, Belgium) diluted at 2.5 $\mu\text{g}/\text{mL}$ in diluent (DakoCytomation, Cambridgeshire, United Kingdom), followed by incubations with biotinylated horse anti-mouse IgG and avidin-biotin complexes-alkaline phosphatase. The reaction was developed with Fast Red substrate and counterstained with hematoxylin.

Positively stained cells were predominantly endothelial cells. Staining was completely abrogated by means of preincubation of the mAb with the specific epitope peptide (gift from Professor Van Snick). Since IL-9 mRNA was not found in endothelial cells,²³ the IL-9 protein was assessed both in the endothelial cells and separately in the nonendothelial cells. Double immunohistochemistry was performed to determine the phenotype of nonendo-

thelial IL-9 protein-positive cells. Unless stated otherwise, the IL-9 data presented do not include IL-9⁺ endothelial cells.

Immunostaining for c-Kit was performed with a polyclonal rabbit antibody to c-Kit (DakoCytomation) diluted 1:50 and goat anti-rabbit IgG (1:200) as a secondary antibody. Primary controls included isotypic mouse IgG1 (IL-9) and rabbit IgG (c-Kit).

In situ hybridization

The second half of each biopsy specimen was fixed in 4% paraformaldehyde for 2 hours and dehydrated in 15% sucrose-PBS for 1 hour and overnight before being mounted in OCT and snap-frozen as described above. Riboprobes, both antisense and sense, were prepared from cDNA for IL-9 (gift from J. C. Renauld, Ludwig Institute for Cancer Research, ICP-Brussels, Belgium). The cDNA was inserted into different pGEM vectors (Promega, Southampton, United Kingdom) and linearized with appropriate enzymes before transcription. Transcription was performed in the presence of sulfur 35 (³⁵S)-labeled uridine triphosphate and the appropriate T7 or SP6 RNA polymerases. *In situ* hybridization was performed on 6- μm cryostat sections on polysine slides (VWR). Sections were permeabilized with Triton X-100 in PBS, followed by proteinase K digestion. Sections were treated with iodoacetamide and N-ethylmaleimide and then acetic anhydride-triethanolamine to inhibit hybridization binding of ³⁵S. As a negative control, sections were either hybridized with the sense probe or treated with ribonuclease A solution before the prehybridization step with antisense probes.

Colocalization of IL-9 protein and mRNA to leukocytes

Double immunohistochemistry. The cytokine was colocalized to T cells (CD3), eosinophils (major basic protein [MBP]), mast cells (tryptase), and neutrophils (neutrophil elastase) by using double immunofluorescence on acetone-fixed sections to assess the respective contribution of infiltrating leukocytes to IL-9 expression. IL-9 protein was detected by using mouse MH9P3 antibody as stated above, and polyclonal antibodies were used to reveal T lymphocytes (rabbit anti-CD3; Abcam Ltd, Cambridge, United Kingdom), eosinophils (goat anti-MBP; Santa Cruz Biotechnology, Santa Cruz, Calif), mast cells (goat anti-tryptase, Santa Cruz), and neutrophils (goat anti-neutrophil elastase, Santa Cruz). IL-9 protein was detected by using FITC-labeled rabbit anti-mouse antibody, whereas biotinylated anti-rabbit (Vector) or anti-goat antibodies (Strattech Scientific, Ltd, Cambridge, United Kingdom) were used as secondary antibodies for leukocyte phenotyping, followed by Alexa Fluor 594-labelled streptavidin (Molecular Probes, Cambridge Biosciences, Cambridge, United Kingdom).

Sequential immunohistochemistry-in situ hybridization. Colocalization of IL-9 mRNA to infiltrating leukocytes was performed on paraformaldehyde-fixed sections by using immunohistochemistry with the phenotype-specific markers CD3 (T cells), MBP (eosinophils), or neutrophil elastase (neutrophils; DakoCytomation) and alkaline phosphatase-antialkaline phosphatase (DakoCytomation) developed with Fast Red (Sigma, St Louis, Mo). This leukocyte staining was followed by *in situ* hybridization with a ³⁵S-labeled IL-9 antisense probe. Only double-positive cells were counted.

Quantification

IL-9 protein, IL-9 mRNA, c-Kit⁺ cells, and colocalization of IL-9 mRNA to leukocyte subsets were counted at 200 $\times$ magnification along the entire length of the basement membrane at one grid depth (0.45 mm) with an Olympus BH2 microscope (Olympus Optica Company Ltd, Tokyo, Japan). On average, this involved counting cells in 4 fields, which is equivalent to 0.8 mm² (0.2-1.8 mm²) of

subepithelial tissue per biopsy specimen. The results were expressed as the number of positive cells per square millimeter.

For the colocalization studies of IL-9 protein, double-positive cells were counted at 400 $\times$ magnification with a Nikon Eclipse (E400) fluorescent microscope (Tokyo, Japan) under appropriate absorption/emission wavelengths (590/617 nm for IL-9, FITC; 495/519 nm for leukocytes, Alexa fluor) with Lucia 4.60 software (system for Image processing and analysis; Prague, Czech Republic).

Statistical analysis

Statistical analysis was performed with Instat 2.01 statistical software (GraphPad, San Diego, Calif) and a commercial software package (Minitab release 7; Minitab, State College, Pa). Comparisons between measurements obtained before and during the pollen season in the same subjects were analyzed by using the Wilcoxon matched-pairs signed-rank test. Comparisons between immunotherapy- and placebo-treated groups were performed with the Mann-Whitney *U* test. Correlations were performed with the Spearman rank correlation method. *P* values of less than .05 were considered statistically significant.

RESULTS

Nasal IL-9 expression during the pollen season

In the placebo-treated group the number of IL-9 mRNA-expressing cells in the nasal mucosa of allergic patients tended to increase during the grass pollen season compared with baseline values ($P = .1$; Fig 1, B, and 2, B, and Table I), whereas IL-9 protein levels were unaffected by seasonal pollen exposure ($P = .93$; Fig 1, A, and 2, A, and Table I). After 2 years of immunotherapy, there was a significant reduction with regard to seasonal allergic symptoms (50%), medication use (70%), and nonspecific bronchial responsiveness to methacholine.²² IL-9 expression in the nasal mucosa was significantly inhibited at the protein level after immunotherapy ($P = .0007$; Fig 1, A, and Table I); a trend for inhibition was also observed for IL-9 mRNA ($P = .06$; Fig 1, B, and Table I). Similar trends were also obtained for IL-9 protein when counting IL-9⁺ endothelial cells ($P = .1$, data not shown).

Mast cells in the nasal mucosa

When tryptase was used as a mast cell marker, there was only a 30% increase in the number of submucosal mast cells during the pollen season, and no significant effect of immunotherapy was observed.⁸ In contrast, there were highly significant seasonal increases in the number of c-Kit-expressing mast cells in the nasal mucosa of the same patients ($P = .0001$; Fig 1, C, and Table I). Moreover, this seasonal increase in nasal c-Kit⁺ mast cells was significantly reduced after allergen immunotherapy ($P = .001$; Fig 1, C, and 2, C, and Table I).

Relationship between IL-9 expression and cellular infiltration

There was a trend toward a correlation between IL-9 mRNA expression and tryptase-positive mast cells ($r = 0.44$, $P = .06$). The number of nasal mucosal cells expressing IL-9 mRNA also correlated with activated EG2⁺ eosinophils ($r = 0.47$, $P = .05$).⁷ Since it has been

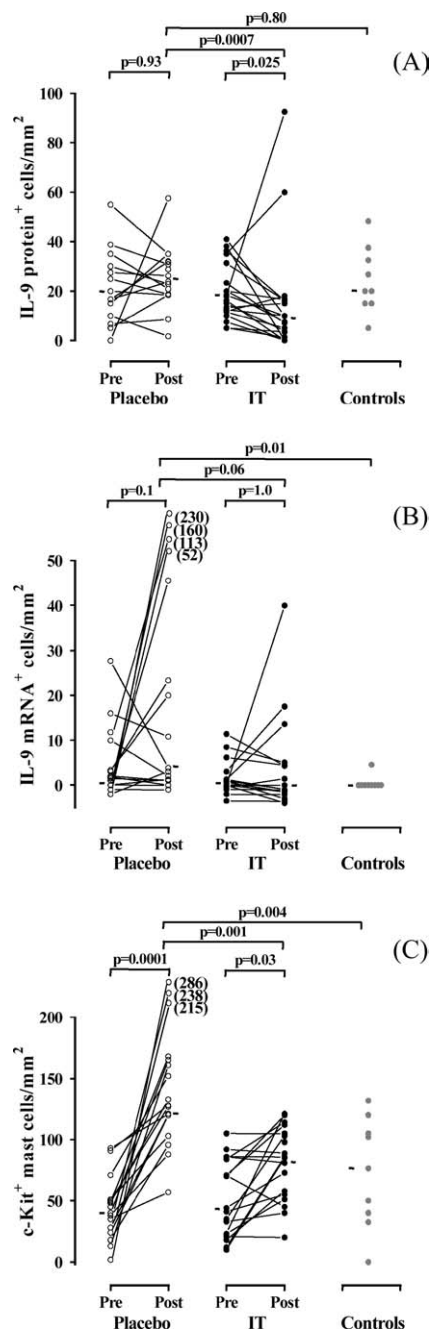


FIG 1. Effects of natural allergen exposure and allergen immunotherapy on IL-9 protein (A), IL-9 mRNA (B), and c-Kit (C) expression in the nasal mucosa. Data shown are for out-of-season (*pre*) and peak-season (*post*) nasal biopsy specimens in immunotherapy- and placebo-treated patients and nonatopic control subjects (peak only). Horizontal bars represent median values.

suggested that the effects of IL-9 on tissue eosinophilia are related to the upregulation of the IL-5 pathway,¹⁷ we addressed this potential relationship by assessing the correlation between IL-9 and IL-5 expression.⁷ IL-9 mRNA-positive cells in the nasal mucosa significantly correlated with cells expressing IL-5 mRNA ($r = 0.54$, $P = .02$).

TABLE I. Local nasal cytokines, c-Kit expression-and EG2⁺ cells before and after immunotherapy

	Immunotherapy		Placebo		Point estimate (95% CI)	P value*
	Before (Winter 1995)	After (Summer 1998)	Before (Winter 1995)	After (Summer 1998)		
IL-9 mRNA/mm ²	0.66 (0, 1.3)	0 (0, 4.4)	1.2 (0, 6.6)	3.6 (0, 48.8)	3.0 (−0.02, 23.3)	.06
IL-9 protein/mm ²	17.1 (11.7, 29.3)	8.75‡ (1.8, 16.3)	20 (10, 30)	25 (18.4, 31.9)	8.33 (−1.67, 18.7)	.08
c-Kit/mm ²	44.3 (23.1, 86)	85.8 (55, 106)	40 (26.2, 50.7)	128§ (111.7, 166.8)	70.8 (36.2, 105.7)	.0001
IL-5 mRNA/mm ²	0 (0, 0)	0.7 (0, 4.6)	0 (0, 1.4)	2.2† (0, 17)	1.25 (0, 7.1)	.16
EG2 cells /mm ²	12 (3.2, 21)	14.2 (9.4, 27)	7.5 (2.8, 28.8)	39.2 (16.4, 76)	17 (1.1, 39.1)	.04

c-Kit-expressing mast cells, EG2⁺ eosinophils, and related cytokines (IL-9 and IL-5, respectively) were assessed in nasal biopsy specimens from patients with allergic rhinitis (n = 37) before (out of the pollen season) and after (during the pollen season) 2 years of grass pollen immunotherapy. Values are medians (interquartile ranges).

*Values refer to between-group differences.

†P = .05, ‡P = .02, and §P = .0001 (within-group comparisons-before-after immunotherapy).

Relationship between IL-9, mast cells, and clinical data

In the placebo-treated group the number of nasal mucosal mast cells expressing c-Kit inversely correlated with nonspecific bronchial responsiveness to methacholine PC₂₀ ($r = -0.76$, $P = .01$) but was not significantly related to symptom scores for rhinoconjunctivitis or medication scores (data not shown). There was also a trend toward a relationship between seasonal symptom scores and peak seasonal IL-9 mRNA-positive nasal cells ($r = 0.42$, $P = .09$), whereas the magnitude of change in IL-9 expression was not significantly correlated with changes in symptoms or rescue medication after immunotherapy (data not shown).

In the immunotherapy-treated patients, a highly significant correlation was observed between the numbers of peak seasonal mucosal eosinophils, and their symptoms were recorded in the 2 days preceding their peak seasonal biopsy ($r = 0.74$, $P < .01$). A significant correlation was also observed between EG2⁺ eosinophils and IL-5 mRNA expression ($r = 0.5$, $P < .05$).

Phenotype of IL-9-expressing cells in the nasal mucosa

Tryptase- or c-Kit-expressing cells infiltrating the nasal mucosa of subjects with allergic rhinitis corresponded, as expected, to mast cells according to morphologic appearance (Fig 2, C). In contrast, different types of cells were found to express IL-9 protein in the nasal mucosa of patients with allergic rhinitis. We carried out colocalization staining to determine the cell source of IL-9 (Fig 3).

We observed that approximately one third of IL-9-expressing cells consisted of CD3⁺ T cells (29.1% ± 6.8% for IL-9 protein and 34.2% ± 10.3% for IL-9 mRNA [mean ± SD], n = 4; Fig 2, D, Fig 3, A and C), with the second main sources being neutrophils and eosinophils, whereas mast cells contributed more marginally to IL-9 expression.

Conversely, only one fifth to one quarter of CD3⁺ T cells produced IL-9, whereas approximately one half of eosinophils and neutrophils expressed this cytokine at the

protein level, as did one quarter of tryptase-positive mast cells (Fig 2, E and F, and Fig 3, B and D).

DISCUSSION

IL-9 is a T_H2 cytokine that contributes during allergic responses, in synergy with the SCF/c-Kit pathway,^{10,24} IL-3 and IL-5 in the tissue accumulation of mast cells and eosinophils. This study shows that expression of IL-9 mRNA is upregulated in the nasal mucosa of patients with allergic rhinitis on natural allergen exposure and that this correlates with tissue infiltration by mast cells and eosinophils. We show for the first time that successful allergen immunotherapy induces inhibition of seasonal increases in c-Kit⁺ mast cells in nasal tissues and significantly reduces the number of IL-9 protein-positive cells. This effect and the reduction in nasal mucosal eosinophils could be related, at least in part, to the modulation of IL-9 expression after immunotherapy.

The differences between the numbers of IL-9 protein- and IL-9 mRNA-positive cells relate potentially to different factors, including active secretion and cytokine binding of IL-9 protein at the cell surface of a variety of cells.

The presence of IL-9⁺ endothelial cells in this study could be explained, at least in part, by direct IL-9 binding to endothelial cells if they expressed the IL-9 receptor or possibly an alternative cross-reacting cytokine receptor. To our knowledge, IL-9 receptor is not found on endothelial cells,²³ whereas IL-7 receptor is expressed and comprises, in addition to a distinct α chain (CD127), a γ chain (CD132), which is shared by the IL-9 receptor.²⁵ The alternative explanation of synthesis of IL-9 by endothelial cells is less likely in the absence of colocalization of demonstrable IL-9 mRNA.

Increases in metachromatic cells on allergen exposure are documented in patients with allergic rhinitis, both after allergen challenge²⁶ and after natural exposure.²⁷ Similarly, accumulation and activation of mast cells can be induced by local allergen provocation to the nose,³ the bronchi,^{4,5} and the skin.⁴ Mast cells differentiate from

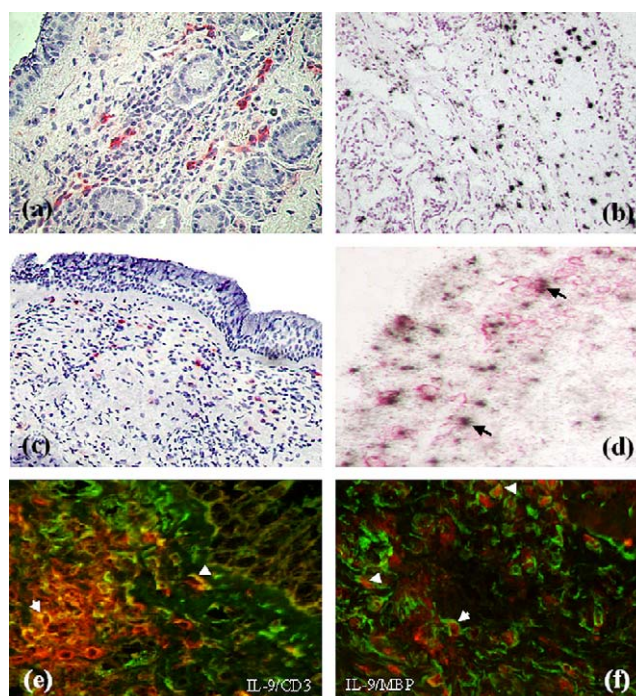


FIG 2. Expression of IL-9 protein (immunohistochemistry [IMH], 200 $\times$ magnification; **a**, IL-9 mRNA (*in situ* hybridization [ISH], 200 $\times$ magnification; **b**, c-Kit⁺ mast cells (IMH, 200 $\times$ magnification; **c**, and colocalization of IL-9 mRNA to CD3⁺ T lymphocytes (IMH/ISH, 400 $\times$ magnification; **d**, and colocalization of IL-9 protein to CD3⁺ T lymphocytes (double immunofluorescence, 400 $\times$ magnification; **e**, and IL-9 protein to MBP⁺ eosinophils (double immunofluorescence, 400 $\times$ magnification; **f**, Arrows show IL-9-expressing cells.

precursor cells, distinct from basophil-eosinophil precursors, under the synergistic influence of specific cytokines, namely IL-3, IL-9, and SCF/c-Kit ligand. We previously showed that IL-3 is increased in the nasal mucosa on allergen challenge.²⁸ Here we show that in patients with grass pollen allergy, IL-9 expression is induced in the nasal mucosa during natural allergen exposure. Moreover, IL-9 expression correlates with mast cell infiltration of nasal tissues, which is consistent with previous *in vitro* and *in vivo* studies^{10,24} showing that c-Kit activation is required for the development of IL-9-induced mucosal mast cell growth. Also, activated mast cells could represent an alternative source of IL-9,²⁹ in addition to T cells, as supported by our colocalization data.

Allergen immunotherapy, which has long-lasting clinical effects in patients with severe hay fever,³⁰ inhibits seasonal increases in nasal epithelial metachromatic cells³¹ and in 2D7⁺ basophils.⁸ In contrast, no significant changes were observed for tryptase-positive mast cells in the nasal mucosa of immunotherapy-treated patients.⁸ In this study we found that seasonal accumulation of c-Kit⁺ mast cells in the nasal mucosa is significantly inhibited after immunotherapy. Moreover, in contrast with the modest increase (approximately 30%) in the number of tryptase-positive cells during the pollen season,⁸ a substantial increase in c-Kit⁺ mast cells (approximately 200%) was observed in the same subjects, further suggesting that tryptase and c-Kit could represent markers of distinct mast cell populations. An alternative explanation

could be that tryptase content of mast cells might be diminished by means of degranulation, and therefore tryptase staining might underestimate the number of mast cells.

Interestingly, in a murine model of ovalbumin allergy, it was recently reported that immunotherapy with immunostimulatory sequences inhibits airway infiltration by mast cells,³² and this reduction was accompanied by reduction in IL-9 and IL-4 expression but not SCF. In our study we did not quantitate SCF levels in the nasal mucosa, which appeared mainly expressed (constitutively) by the epithelium (data not shown). Importantly, c-Kit⁺ mast cell infiltration of the nasal mucosa correlated with nonspecific bronchial hyperresponsiveness to methacholine. Although speculative, this could support a role for mast cells in the documented relationship between the upper and lower airways in terms of symptoms and evolution of seasonal increases in bronchial hyperresponsiveness in patients with rhinitis.

We also observed that IL-9 expression in the nasal mucosa of patients with grass pollen allergy correlates with the number of eosinophils. In addition to effects on mast cells, it was shown in mice that IL-9 promotes tissue eosinophilia, probably through upregulation of the IL-5 pathway.³³ Accordingly, IL-9 expression significantly correlated with that of IL-5, suggesting that IL-9 and IL-5 might have the same main cell source (ie, T_H2 cells), that IL-9 and IL-5 could have reciprocal influences on their synthesis, or both. This latter hypothesis seems,

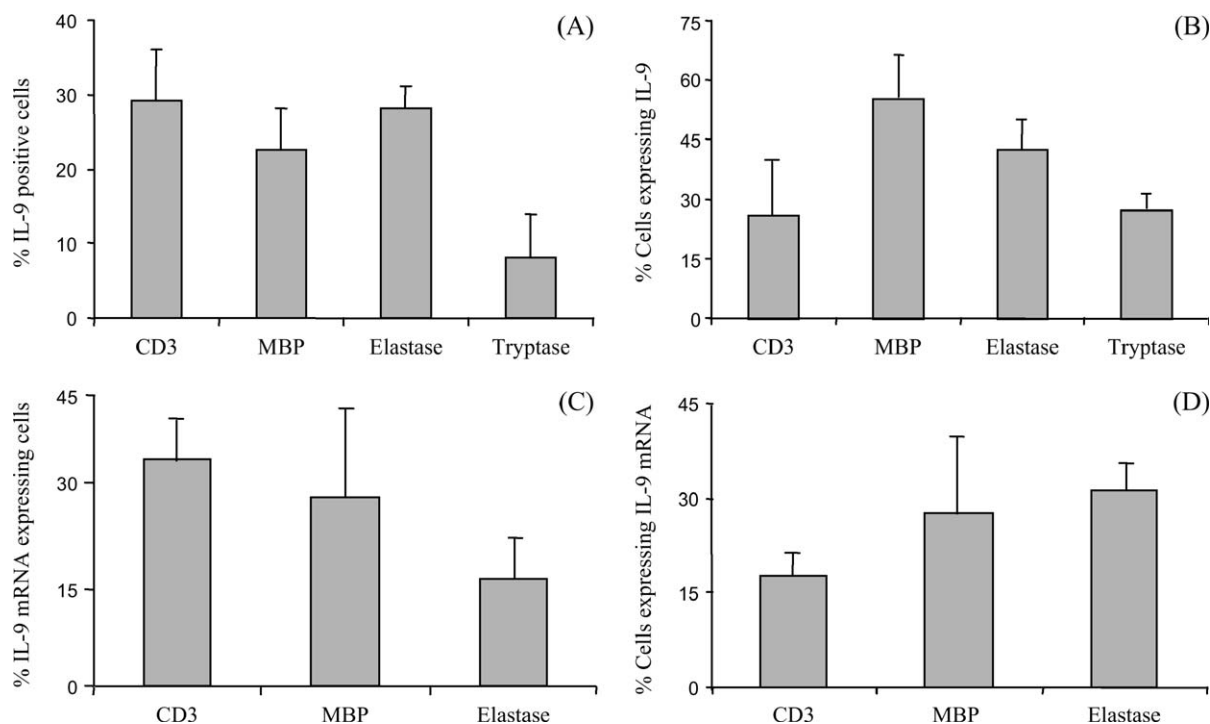


FIG 3. The contributions to IL-9 protein expression by T cells (*CD3*), eosinophils (major basic protein [*MBP*]), neutrophils (*Elastase*), and mast cells (*Tryptase*) are shown (**A**), and the proportions of T cells, eosinophils, neutrophils, and mast cells expressing IL-9 protein are shown (**B**). Contributions to IL-9 mRNA expression and proportions of IL-9 mRNA-expressing leukocytes are also shown (**C** and **D**, respectively).

however, less likely because IL-9 does not appear mandatory to mount T_H2 eosinophilic responses to allergens.³⁴ T cells were shown by means of colocalization studies to represent a main source of IL-9 in the allergic nasal mucosa, suggesting that our findings could reflect immunotherapy-induced modulation of T-cell activation. However, effects on other cell types, such as eosinophils and neutrophils, also contributing significantly to IL-9 expression, cannot be excluded. Thus nearly half of eosinophils and neutrophils infiltrating the nasal mucosa of our allergic patients expressed IL-9 protein.

This study demonstrates seasonal increases in IL-9 expression and c-Kit⁺ mast cells in the nasal mucosa of patients with seasonal allergic rhinitis and suggests that IL-9 contributes *in vivo* to tissue accumulation of mast cells and eosinophils on natural allergen exposure. This is further supported by previous studies^{10,14,33,35} in which similar correlations between the IL-9 expression and the number of eosinophils, IL-5-expressing cells, or both in the tissue were observed. However, other cytokines-factors are also critical to mediate tissue accumulation of eosinophils and mast cells during allergic responses, as suggested by the study of McMillan et al³⁴ using IL-9 knockout mice.

Also, we show for the first time that grass pollen immunotherapy elicits in these patients a prolonged inhibition of IL-9 expression and a reduction in nasal mucosal c-Kit⁺ mast cells. We suggest that c-Kit⁺ mast cells could

represent a particular subpopulation of mast cells that accumulate during the pollen season in the nasal mucosa of allergic patients. These data support the concept that the IL-9 pathway could represent a therapeutic target^{35,36} to dampen chronic allergic inflammation, particularly with regard to mast cells.³⁷

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