

IL-9 and its receptor in allergic and nonallergic lung disease: Increased expression in asthma

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Background: Bronchial asthma is a chronic inflammatory disease associated with genetic components. Recently IL-9 has been reported as a candidate gene for asthma and to be associated with bronchial hyperresponsiveness and elevated levels of total serum IgE.

Objective: To investigate the contribution of IL-9 to the pathogenesis of asthma, we examined the expression of IL-9 and its receptor (IL-9R) in bronchial tissue from subjects with atopic asthma (n = 10), chronic bronchitis (n = 11), and sarcoidosis (n = 9) and from atopic (n = 7) and nonatopic (n = 10) healthy control subjects.

Methods: Bronchial biopsy specimens were examined for the presence of IL-9 and IL-9R protein and messenger RNA (mRNA) by immunocytochemistry and in situ hybridization, respectively. To phenotype the cells expressing IL-9 in asthmatic tissue, combined in situ hybridization and immunocytochemistry was also performed.

Results: There was a highly significant difference ($P < .001$) in the expression of IL-9 mRNA in asthmatic airways (20.6 ± 4.0 cells/mm of basement membrane) compared with chronic bronchitis (5.6 ± 4.4), sarcoidosis (2.5 ± 1.8), atopic control subjects (7.7 ± 2.2), and healthy control subjects (2.7 ± 2.3). The number of IL-9 immunoreactive cells was also greater in asthmatic patients compared with the other groups ($P < .05$). Although the level of IL-9R mRNA expression did not differ in any of the groups ($P > .05$), IL-9R immunoreactivity was significantly higher in asthmatic compared with control subjects. Furthermore, IL-9 mRNA expression levels were also significantly correlated with FEV₁ ($P < .05$) and the airway responsiveness to methacholine producing a 20% fall in FEV₁ ($P < .01$). The cells expressing IL-9 mRNA in asthmatic tissue were CD3⁺ lymphocytes (68%), major basic protein⁺ eosinophils (16%), and elastase⁺ neutrophils (8%).

Conclusion: The results of this study demonstrate the potential of IL-9 to be a marker for atopic asthma and furthermore suggest an important role for this cytokine in the pathophysiologic

mechanisms of this disease. (*J Allergy Clin Immunol* 1999;104:108-15.)

Key words: Asthma, IL-9, IL-9 receptor, atopy

Asthma is a chronic inflammatory disease characterized by episodic airways obstruction, bronchial hyperresponsiveness, and an infiltration of the airways by activated inflammatory cells, particularly eosinophils, T lymphocytes, and mast cells.^{1,2} At the site of inflammation these cells are thought to act mainly through the release of cytokines, which have been documented to be central mediators in the regulation of inflammatory responses. A number of studies have demonstrated that in bronchial biopsy specimens from asthmatic patients there is a predominance of T helper (T_H2)-type cytokines, particularly IL-4, IL-5, and IL-13.³⁻⁶ Recently it has been suggested that the T_H2 cytokine IL-9 is also involved in allergic responses.⁷⁻⁹ In fact, IL-9 has been linked to the genetic predisposition to asthma because its encoding gene was located in the chromosomal region (5q23-q33) that includes the cytokine gene cluster implicated in bronchial inflammation associated with this disease.¹⁰⁻¹²

A pleiotropic role for IL-9 in allergic responses is suggested by several independent studies describing its functions in vitro. IL-9 has been reported to be a T cell-derived cytokine with various effects on a variety of cell types associated with allergic inflammation. IL-9 has been shown to stimulate the proliferation of activated T cells, enhance the production of IgE from B cells, and promote the proliferation and differentiation of mast cells and hematopoietic progenitors. In addition, IL-9 has been shown to affect IgE-mediated responses by up-regulating the α chain of the high-affinity IgE receptor.^{13,14}

The in vivo importance of IL-9 in the pathophysiologic mechanisms of asthma has been suggested mainly by animal models of airway inflammation. Genetic mapping studies in mice demonstrate linkage between the regulation of airway hyperresponsiveness and the IL-9 locus.¹⁵ Moreover, lung IL-9 protein levels are highly correlated with airway responsiveness in mice.^{9,15} Studies with IL-9 transgenic mice show that overexpression of IL-9 selectively within the lungs results in a characteristic human asthmatic phenotype including elevated IgE levels, mucus hypersecretion, and dramatically increased bronchial hyperresponsiveness.^{7,8} IL-9 has also been

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TABLE I. Clinical characteristics of study population

	Nonatopic controls	Atopic controls	Atopic asthmatics	Chronic bronchitis	Sarcoidosis
Sex (male/female ratio)	6:4	4:3	7:3	11:0	6:5
Age (y)	25-41	21-39	26-46	42-65	20-49
FEV ₁ (% predicted)*	122 ± 10	101 ± 9	82 ± 8	64 ± 14	97 ± 5
PC ₂₀ (mg/mL)	>16	>16	3.5 ± 1.34*	ND	ND
Total IgE (IU/mL)*	36.2 ± 12	588 ± 153†	901 ± 206†	78.2 ± 22	ND
Skin prick tests	Negative	Positive	Positive	Negative	Negative

PC₂₀: Methacholine dose producing 20% fall in FEV₁; ND, not done.

*Data presented as mean ± SEM.

†P < .001 compared with normal control subjects.

Abbreviations used

BM: Basement membrane
cRNA: Complementary RNA
ICC: Immunocytochemistry
IL-9R: IL-9 receptor
ISH: In situ hybridization
MBP: Major basic protein
mRNA: Messenger RNA
PC₂₀: Methacholine dose producing a 20% fall in FEV₁

reported to promote increased numbers of airway mast cells and eosinophils in IL-9 transgenic mice. In fact, intra-tracheal administration of recombinant IL-9 in naive mice results in airway eosinophilia.⁹ On the basis of these findings and on its pleiotropic functions, IL-9 has been presumed to be associated with the pathogenesis of asthma.

To date, the expression of IL-9 has not been investigated in human allergic disease; therefore in the current study we examined IL-9 and IL-9R messenger RNA (mRNA) expression and immunoreactivity in human asthmatic tissue compared with that in control subjects and in patients with nonallergic lung disease such as chronic bronchitis and sarcoidosis. In addition, we set out to phenotype the cells expressing IL-9 mRNA to determine the cellular sources of this cytokine and to determine whether inflammatory cells other than T cells have the ability to produce IL-9.

METHODS

Subjects

The subjects in this study were recruited from the outpatient clinic at the Montreal Chest Hospital and from Calmette Hospital, Department of Pneumoimmunoallergy, in Lille, France. Hospital ethics committee approval was obtained for this study, and all participating subjects gave informed written consent before the study. A total of 49 subjects were included in this study. Clinical and demographic characteristics of the patients are shown in Table I. Patients with atopic asthmatic, chronic bronchitis, and sarcoidosis and atopic nonasthmatic control and nonatopic healthy control subjects were carefully defined as described below. All subjects except patients with chronic bronchitis were nonsmokers.

Atopy was defined by positive skin prick test (>3 mm skin wheal) to one or more of common inhalant aeroallergens or by radioallergen sorbent test for specific IgE (Pharmacia, Uppsala, Sweden).

All atopic asthmatic patients fulfilled the American Thoracic

Society criteria for asthma,¹⁶ had typical clinical symptoms, documented airway reversibility, and increased airway responsiveness to methacholine producing a 20% fall in FEV₁ (PC₂₀) <8 mg/mL. Asthmatic subjects recruited in this study were classified as mild-moderate asthmatics with FEV₁ values ranging from 60% to 95% predicted. The symptoms were controlled by regular use of β_2 -agonists. None of the asthmatic patients had taken corticosteroids 12 weeks before the study.

Chronic bronchitis was also defined according to the criteria of the American Thoracic Society.¹⁶ Briefly, patients were given a diagnosis of chronic bronchitis if they had a history of cough and sputum production on most days of the month for at least 3 months a year during the previous 2 years. In addition, they had a documented lack of β_2 -induced airflow reversibility recorded as a less than 15% change on FEV₁. All the bronchitic subjects had a history of cigarette smoking, no history of asthma, and were nonatopic and not taking oral or inhaled steroids. Subjects with lung disease other than chronic obstructive pulmonary disease or systemic disease affecting the lung were excluded from the study.

The diagnosis of active pulmonary sarcoidosis was based on previously described criteria,¹⁷ including lung or mediastinal lymph node biopsy specimens exhibiting evidence of noncaseating epithelioid cell granulomas.¹⁸ The criteria used to define this patient population were (1) recently developed or increasing cough or dyspnea or (2) systemic symptoms, such as cutaneous lesions, weakness, fever, arthralgia, or (3) increasing opacities on chest radiography. All patients with sarcoidosis were nonatopic, they did not exhibit airflow limitation or restrictive lung disease, and they had not taken corticosteroids within the past year.

Atopic nonasthmatic control subjects had no past or present asthmatic symptoms but had a history of other atopic diseases, including allergic rhinitis, allergic conjunctivitis, and eczema. Nonatopic healthy control subjects had no symptoms and no atopic diseases. All control subjects had normal spirometry, negative methacholine tests, and no signs of infectious respiratory disease during the previous 4 weeks. None of the control subjects was taking any medication for at least 2 weeks preceding the study.

Bronchoscopy and biopsy

Fiberoptic bronchoscopy was performed according to American Thoracic Society guidelines.¹⁹ The procedure was undertaken if the FEV₁ was >60% and platelets and clotting parameters were within normal limits. Premedication consisted of midazolam (1-4 mg) administered intravenously and albuterol (400 μ g) administered by a metered-dose inhaler. Topical anesthesia was achieved with a 4% lidocaine solution. Continuous oximetry was performed, and oxygen was administered throughout the procedure. Biopsy specimens were taken from the segmental divisions of the main bronchi of the right lung with an alligator forceps (Olympus, Tokyo, Japan).

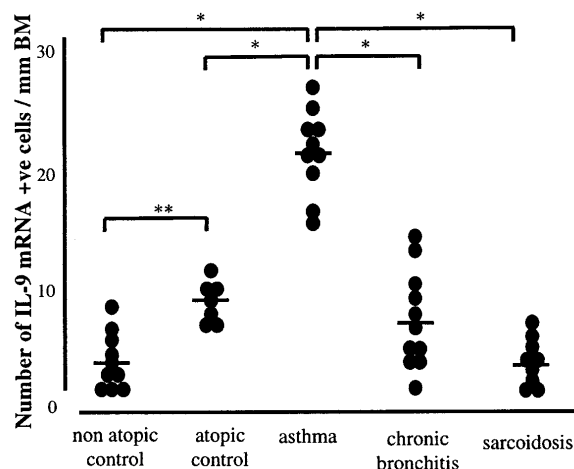


FIG 1 IL-9 mRNA expression in nonatopic and atopic control subjects and subjects with atopic asthma, chronic bronchitis, and sarcoidosis. Results are expressed as numbers of positive cells per millimeter of BM. Asterisk, $P < .001$ for comparison between atopic asthmatics and each of other groups; two asterisks, $P < .05$ for comparison between atopic controls and nonatopic controls (ANOVA).

Tissue preparation

For immunocytochemistry endobronchial biopsy specimens were immediately snap-frozen in isopentane cooled in liquid nitrogen. For in situ hybridization (ISH) biopsy specimens were fixed immediately in a 4% paraformaldehyde/PBS for 2 hours, washed in 15% PBS/sucrose 3 times, embedded in ornithine carbamyltransferase compound (Tissue-Tek, Miles, Elkhart, Ind), and then snap-frozen in isopentane cooled in liquid nitrogen. Cryostat sections 10 μ m thick were cut on 0.1% poly-L-lysine-coated slides, baked in an oven at 37°C, and stored at -80°C until further use.

Probe preparation

Radiolabeled complementary RNA (cRNA) probes coding for IL-9 and its receptor (IL-9R) were prepared from complementary DNA (Magainin Pharmaceuticals, Plymouth Meeting, Pa) as described previously.²⁰ In brief, complementary DNA was inserted into PGEM vectors, linearized, and transcribed in vitro in the presence of sulfur 35-labeled uridine triphosphate and either SP6 or T7 polymerases. Antisense (complementary to mRNA) and sense probes (identical to mRNA) were prepared. The percentage of the incorporation of the radioisotopes was approximately 40% to 50%.

ISH

ISH was performed as previously described.³ Briefly, cryostat sections from biopsy specimens were permeabilized with Triton X-100 and proteinase K solution (1 μ g/mL) in 0.1 mol/L TRIS containing 50 mmol/L EDTA for 20 minutes at 37°C. The samples were subsequently incubated with 0.1 mol/L triethanolamine and 0.5% acetic anhydride for 20 minutes at 37°C to prevent the nonspecific binding of the ³⁵S-labeled cRNA probes. Prehybridization of the samples was carried out in 50% formamide in 2 $\times$ standard saline citrate for 15 minutes at 37°C. Hybridization was carried out with use of a hybridization mixture containing the appropriate sense or antisense probes (0.75×10^6 counts/min per slide). Posthybridization involved high stringency washing of the samples in decreasing concentrations of standard saline citrate at 42°C. To remove any unbound RNA probes, the samples were washed with ribonuclease solution for 20 minutes at 42°C. The samples were then dehydrated with increasing

concentrations of ethanol and air dried. After this, the samples were dipped in Amersham LM-2 emulsion and then exposed for a period of 10 days. The autoradiographs were then developed in Kodak D-19 developer, fixed, and counterstained with hematoxylin. The samples were then mounted with a coverslip and examined under a graduated microscope for positive signals.

Immunocytochemistry

To detect IL-9 and IL-9R immunoreactivity, immunostaining was performed with use of the alkaline phosphatase-antialkaline phosphatase technique as previously described.²¹ Briefly, sections were incubated with the primary antibodies, goat antihuman IL-9 polyclonal antibody (AB-209-NA, R&D Systems, Minneapolis, Minn) and rabbit antihuman IL-9R polyclonal antibody (sc-698, Santa Cruz Biotechnology, Santa Cruz, Calif), at 4°C overnight. Sections were then incubated with the swine antigoat Ig or the swine antirabbit Ig as secondary antibody, followed by the streptavidin complex. The reaction was visualized with fast red alkaline phosphatase substrate. For negative control preparations, the primary antibody was replaced by either nonspecific immunoglobulin or TRIS-buffered saline solution.

Combined immunocytochemistry and ISH

To ascertain the expression of IL-9 mRNA by T lymphocytes, eosinophils, and neutrophils, we simultaneously applied radiolabeled ISH with immunohistochemistry (ICC) for CD3, major basic protein (MBP), and elastase, as previously described.²⁰ Briefly, sections were immunostained with mAbs directed against cell markers specific for T lymphocyte (CD3), eosinophils (MBP), and neutrophils (elastase). After visualization of the positive immunostaining with use of the alkaline phosphatase-antialkaline phosphatase technique, ISH was conducted to detect IL-9 mRNA.

Quantification

Sections were coded and counted in a blinded, random fashion with an Olympus microscope with an eyepiece graticule at $\times 200$ magnification. At least 2 sections were stained, from which 6 to 8 fields were evaluated. Positively staining cells were counted to a depth of 0.45 mm below the basement membrane (BM) and the results were expressed as the mean number of positive cells per millimeter of BM. For the colocalization studies, the results were expressed as a percentage of the IL-9 mRNA positive cells that coexpressed either CD3, MBP, or elastase.

Statistical analysis

The numbers of cells expressing IL-9 and IL-9R mRNA in patients with asthma, chronic bronchitis, and sarcoidosis airways and healthy control subjects were compared with use of a 1-way ANOVA followed by a post hoc *t* test for multiple comparisons. Correlation between the number of IL-9 mRNA positive cells and percent FEV₁ and logPC₂₀FEV₁ were done with use of linear regression analysis. Significance was accepted at the 5% level of confidence. Results were considered statistically significant for *P* values $< .05$. Statistical analyses were performed with use of a standard computer package (Systat version 7.0, Systat, Evanston, Ill).

RESULTS

Hybridization between the labeled cRNA probes and mRNA encoding IL-9 and IL-9R were demonstrated by specific deposits of silver grains in the photographic emulsion overlying the tissue sections. As shown in Fig 1, we demonstrated the expression of IL-9 mRNA in all

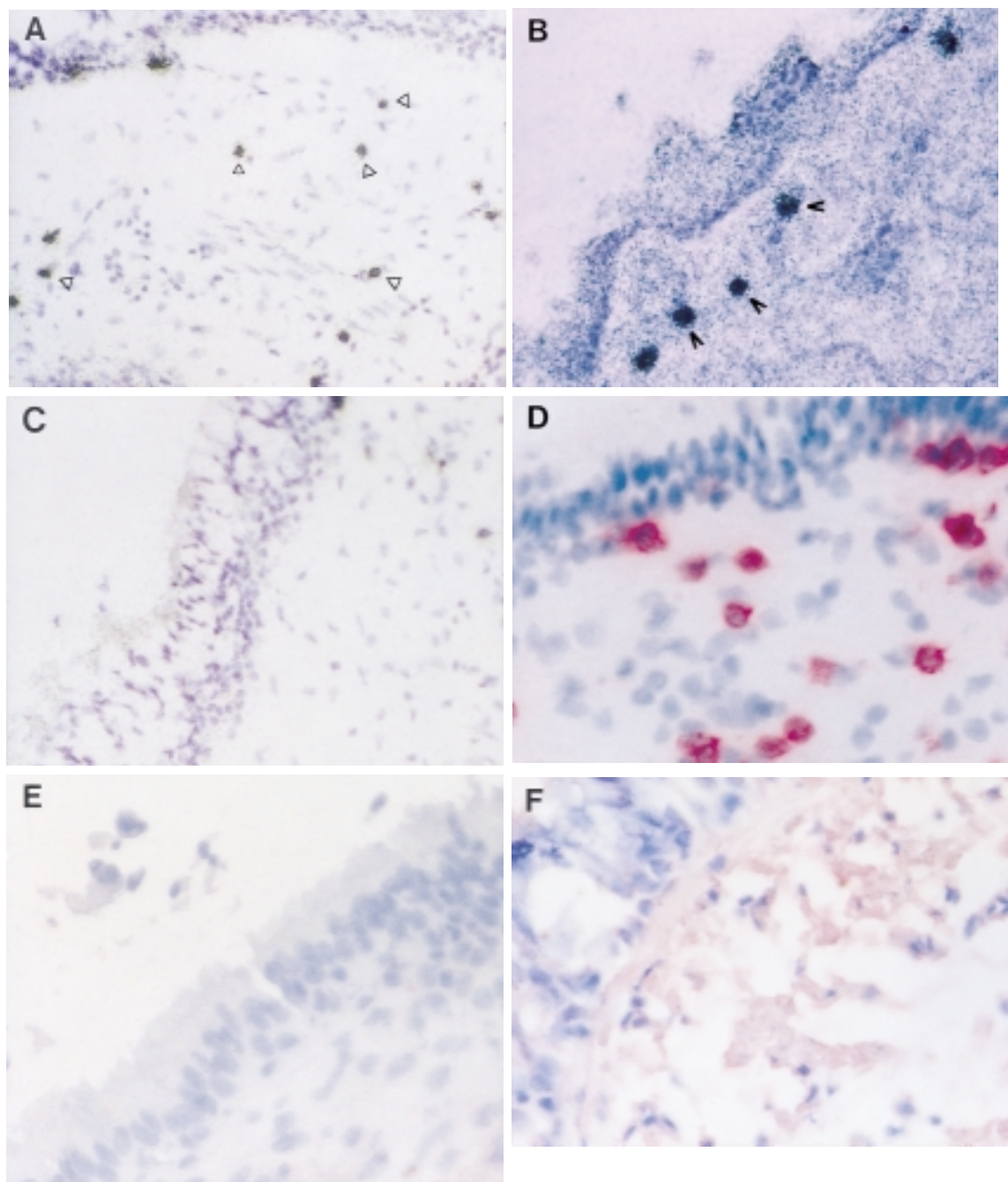


FIG 2. Representative examples of in situ hybridization of IL-9 mRNA by use of ^{35}S -labeled cRNA probes and ICC for IL-9 in bronchial biopsy specimens. In situ hybridization of IL-9 mRNA from asthmatic subject (original magnification $\times 200$) (positive cells indicated by arrows) (**A**), high power of **A** (original magnification $\times 400$) (**B**), and from subject with sarcoidosis (**C**). IL-9 immunoreactivity in bronchial biopsy specimen from atopic asthmatic subject (**D**), from subject with sarcoidosis (**E**), and from nonatopic healthy control subject (**F**).

asthmatic (Fig 2, A and B), atopic, and nonatopic control subjects and subjects with chronic bronchitis (Fig 2, C) and sarcoidosis. However, the level of IL-9 expression in asthmatic patients (mean mRNA positive cells/mm BM $\pm$ SD, 20.6 ± 4.0) was significantly higher compared with the other patient groups such as those with chronic bronchitis (mean $\pm$ SD, 5.6 ± 4.4 , $P < .001$) and sarcoidosis

(mean $\pm$ SD, 2.5 ± 1.8 , $P < .001$) and atopic control (mean $\pm$ SD, 7.7 ± 2.2 , $P < 0.001$) and nonatopic control (mean $\pm$ SD, 2.7 ± 2.3 , $P < .001$) subjects. The expression of IL-9 mRNA was also significantly higher in atopic controls compared with nonatopic control subjects ($P < .05$). Both the degree of airflow obstruction (FEV_1) ($P < .05$, $r = -0.74$) and airway responsiveness to metha-

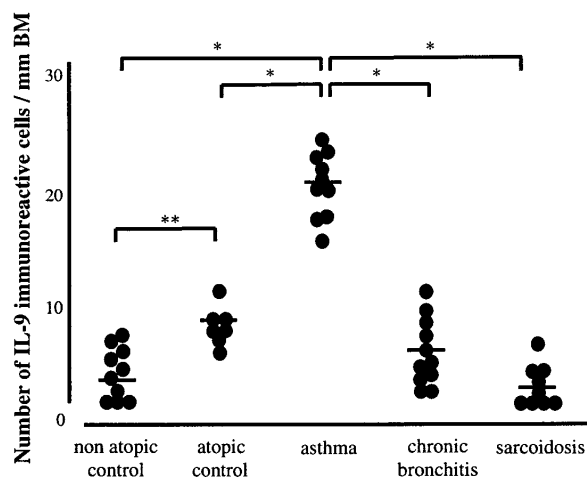


FIG 3. IL-9 immunoreactivity in nonatopic and atopic control subjects and patients with atopic asthma, chronic bronchitis, and sarcoidosis. Results are expressed as numbers of positive cells per millimeter of BM. Asterisk, $P < .001$ for comparison between atopic asthmatics and each of other groups; two asterisks, $P < .01$ for comparison between atopic controls and nonatopic controls (ANOVA).

choline ($\log PC_{20} FEV_1$) ($P < .01$, $r = 0.71$) were significantly correlated with IL-9 mRNA expression in the group of asthmatic patients. No positive hybridization signals were observed when the IL-9 sense probe was used or when the tissues were pretreated with ribonuclease.

As shown in Fig 3, the number of IL-9 immunoreactive cells was also greater in asthmatic airways (mean $\pm$ SD, 26.3 ± 19.1) (Fig 2, D) compared with chronic bronchitis (mean $\pm$ SD, 4.5 ± 3.2) ($P < .001$), sarcoidosis (mean $\pm$ SD, 1.6 ± 1.8) ($P < .001$) (Fig 2, E), atopic controls (mean $\pm$ SD, 7.2 ± 1.5) ($P < .001$) and nonatopic controls (mean $\pm$ SD, 2.5 ± 2.1) ($P < .001$), (Fig 2, F). Furthermore, IL-9 immunoreactive cells were also greater in atopic control subjects compared with nonatopic control subjects ($P < .01$).

As shown in Fig 4, A, IL-9R mRNA-positive cells were detected in all patient groups: asthmatic (mean mRNA positive cells/mm BM $\pm$ SD, 11.8 ± 4.6), chronic bronchitis (mean $\pm$ SD, 12.9 ± 5.0), sarcoidosis (mean $\pm$ SD, 10.0 ± 5.3), atopic control (mean $\pm$ SD, 6.6 ± 4.4), and nonatopic control subjects (mean $\pm$ SD, 9.1 ± 2.1). However, there was no significant difference in the level of expression among these groups. Although IL-9R immunoreactivity was seen again in all groups, asthmatics (mean $\pm$ SD, 11.7 ± 1.6), chronic bronchitis (mean $\pm$ SD, 8.2 ± 1.7), sarcoidosis (mean $\pm$ SD, 7.0 ± 4.8), atopic control (mean $\pm$ SD, 6.8 ± 3.6), and nonatopic control (mean $\pm$ SD, 9.0 ± 2.1), IL-9R protein expression was significantly higher in asthmatic patients compared with nonatopic ($P < .01$) and atopic control subjects ($P < .05$) (Fig 4, B).

When the cells expressing mRNA for IL-9 in asthmatic tissue were phenotyped (Fig 5), combined ICC and

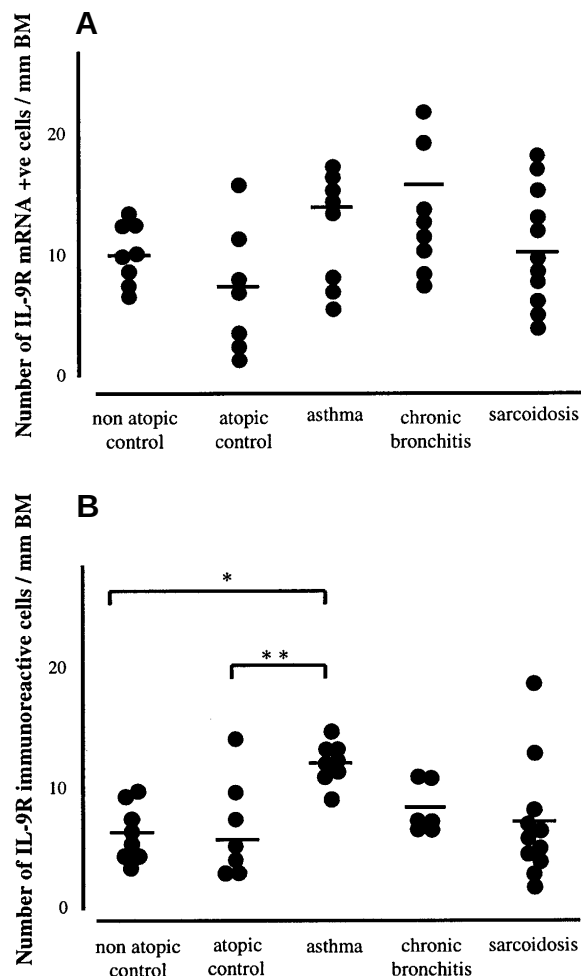


FIG 4. A, IL-9R mRNA expression in nonatopic and atopic control subjects and subjects with atopic asthma, chronic bronchitis, and sarcoidosis. Results are expressed as numbers of positive cells per millimeter of BM. No significant difference among these groups was found. B, IL-9R immunoreactivity. Number of IL-9R immunoreactive cells was significantly higher in atopic asthmatic compared with nonatopic control subjects (asterisk, $P < .01$) and with atopic control subjects (two asterisks, $P < .05$) (ANOVA).

ISH revealed that the majority of IL-9 mRNA-positive cells were CD3⁺ T lymphocytes (mean $\pm$ SD, $68.4\% \pm 10.3\%$), whereas the remainder were MBP-positive eosinophils (mean $\pm$ SD, $15.3\% \pm 3.1\%$) and elastase-positive neutrophils (mean $\pm$ SD, $7.7\% \pm 3.1\%$).

DISCUSSION

In this study we investigated the *in vivo* expression of IL-9 in human bronchial tissue obtained from atopic asthmatic patients compared with atopic and nonatopic control subjects and with subjects with chronic bronchitis and sarcoidosis. With use of *in situ* hybridization and immunocytochemistry staining techniques, our results provide direct evidence that IL-9 is expressed both at the gene and protein level in human airways. Its expression,

however, was found to be up-regulated significantly in bronchial biopsy specimens obtained from subjects with asthma compared with those from controls and from subjects with nonatopic lung diseases. Furthermore, the degree of IL-9 expression correlated with bronchial hyperresponsiveness and to a lesser extent with airway obstruction. Our experiments extend previous preliminary observations in animals and humans of IL-9 associations with asthmatic-like responses and demonstrate the predominant expression of IL-9 at the tissue level in human asthmatic airways. This supports the idea that IL-9 may be a critical factor involved in the pathogenesis of atopic asthma.¹⁵

Bronchial hyperresponsiveness and elevated serum IgE levels are well-known risk factors for asthma thought to be linked and influenced by a genetic background.^{7,9,11,12,15,22,23} Genetic studies have linked asthma phenotypes, including atopy and bronchial hyperresponsiveness, to human chromosome 5q31-q33.^{10,11} The gene for IL-9 is located within that region, and allelic association between IL-9 and total serum IgE levels has previously been demonstrated.^{10,24} Our results demonstrate that the expression of IL-9 mRNA and immunoreactivity was significantly higher in atopic control compared with nonatopic control subjects, suggesting a close association between IL-9 and atopy. Studies have shown in humans and mice that IL-9 regulates serum IgE levels.^{8,9,25} IL-9 has also been reported to up-regulate FcεRIα cell surface expression, enhancing the IgE-mediated release of mast cell mediators.^{13,25}

Genetic studies have also suggested that bronchial hyperresponsiveness is a multigenic process and that its predisposition may be strongly associated with the predisposition to asthma.^{11,12,26-31} Recent genetic studies in murine asthma models identified IL-9 as a candidate gene and have shown an association between lung IL-9 expression with bronchial hyperresponsiveness.¹⁵ Studies with inbred strains of mice demonstrate that bronchial hyperresponsiveness is genetically linked to chromosome 13, which shares homology with human chromosomal region 5q31-q33, on which the murine homolog of the IL-9 gene is located.¹⁵ These studies have shown that reduced expression levels of IL-9 were associated with less responsiveness, whereas overexpression of IL-9 resulted in a marked increase of airway responsiveness.^{7,8,15} Our data demonstrated that the degree of IL-9 expression in human lung correlated with bronchial hyperresponsiveness in asthmatic patients, and these findings are consistent with the above findings suggesting that this cytokine may play a key role in regulating airway hyperresponsiveness.

T_H2 cells and their associated cytokines have been identified in asthmatic airways and are thought to contribute to the development of inflammatory responses in asthma.^{4,32} Experimental data have shown an association between IL-9 production and T_H2 responses *in vitro* and *in vivo*.²⁷ This is consistent with our data showing a low expression of IL-9 mRNA and immunoreactivity in nonatopic diseases such as sarcoidosis and chronic bron-

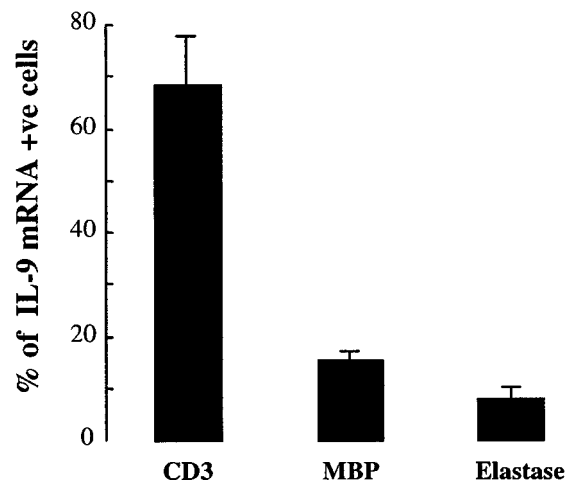


FIG 5. Colocalization of IL-9 mRNA with CD3⁺ T cells, MBP-positive eosinophils, and elastase-positive neutrophils from 5 atopic asthmatic tissues. Results are expressed as percentage of positive cells/total cells.

chitis compared with that seen in asthma, a T_H2-mediated disease. When the cells expressing IL-9 mRNA were phenotyped, we demonstrated that CD3⁺ T cells are the major cellular source of IL-9 *in vivo*. This is consistent with previous data showing that IL-9 is produced *in vitro* and *in vivo* by activated T cells.^{27,33-35} On the basis of these data, we can speculate that the increase in IL-9 expression seen in asthmatic airways is not simply the result of T-cell infiltration, yet it may be a reflection of a T_H2-mediated process. Demonstrating a relatively low expression of IL-9 in chronic bronchitis and sarcoidosis despite the increase in T cells, we can conclude that the change in T-cell numbers alone cannot account for the magnitude of the observed increase in IL-9-positive cells in asthma. Furthermore, we demonstrated that eosinophils and neutrophils also express IL-9 in asthmatic tissue. This novel finding suggests that these cells might contribute to the production of IL-9 in asthma. The activity of IL-9 on eosinophils has recently been shown to promote profound lung eosinophilia in mouse lungs^{7,8}; however, its effects on neutrophils have not yet been reported.

Cytokine mRNA and protein levels do not always correlate because of nontranscriptional regulation and intracellular storage; therefore, to determine whether the increase in IL-9 mRNA also occurred at the protein level, we examined the expression of translated IL-9. ICC analysis thus confirmed a corresponding up-regulation of IL-9 protein in asthmatic airways.

The biologic effects of IL-9 are exerted through the functional IL-9R complex, which consists of a ligand-specific α chain and the IL-2 receptor γ chain. Recently, the human IL-9R gene locus has also been implicated in determining susceptibility to bronchial hyperresponsiveness and asthma.^{22,23} As expected, IL-9R mRNA was detected in asthmatic biopsy specimens; however, the level of expression did not differ significantly from that

in controls and nonallergic diseases. We also examined IL-9R expression at the protein level to verify that the mRNA transcripts being detected are translated into receptor proteins. The only significant difference observed in IL-9R immunoreactivity was between asthmatic patients and healthy control and atopic control subjects. Nevertheless, this difference may be of importance and suggests that biologic variability in receptor function may be determined at the level of receptor protein expression. Recently, Grasso et al²³ have identified a variant IL-9R transcript that encodes for a nonfunctional cell surface receptor that appeared to be more prevalent in healthy individuals. Altered stability of this variant receptor relative to the wild-type receptor protein may explain the observed discordance in asthmatics between IL-9R mRNA and immunoreactivity.

In conclusion, we have demonstrated a significantly higher expression of IL-9 mRNA and immunoreactivity in human airways from atopic asthmatic subjects. The current findings support the idea that IL-9 might be an important mediator for the development of bronchial asthma and possibly other T_H2 -mediated diseases. Further research is needed, however, to elucidate the IL-9 pathway, which may be a key factor in the mechanism for modulating IL-9 signaling. The importance of IL-9 activity needs to be evaluated further by use of IL-9 antagonists in vivo or in systems where T_H2 responses are inhibited. This may provide potentially useful insights into the pathogenesis of asthma, which may further lead to novel therapeutic approaches directed against it.

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