Expression of the IL-4 receptor α-subunit is increased in bronchial biopsy specimens from atopic and nonatopic asthmatic subjects

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Background: Recent studies have provided evidence for increased IL-4 expression in the airways of atopic and nonatopic asthmatic subjects. IL-4 is believed to perform important regulatory roles in asthma; however, the expression of the IL-4 receptor has not been investigated. In this study we examined the mRNA and protein expression of the specific α-subunit of the IL-4 receptor (αIL-4R) in bronchial biopsy specimens obtained from atopic and nonatopic asthmatic subjects.

Methods: Asthmatic subjects and nonasthmatic control subjects were recruited, and lung function measurements were performed before bronchoscopy. Endobronchial biopsy specimens were examined for the presence of αIL-4R mRNA and immunoreactivity by using in situ hybridization and immunocytochemistry, respectively.

Results: αIL-4R mRNA-positive and immunoreactive cells were detected in the epithelium and subepithelium in biopsy specimens from all subjects. Expression of αIL-4R mRNA and protein was significantly increased in the epithelium and subepithelium of biopsy specimens from atopic asthmatic subjects compared with atopic control subjects ($P < .05$ and $P < .001$, respectively). Epithelial αIL-4R mRNA expression and immunoreactivity did not differ significantly between nonatopic asthmatic subjects and nonatopic control subjects. Although the numbers of αIL-4R mRNA–positive cells were augmented in the submucosa of intrinsic asthmatic subjects compared with nonatopic control subjects ($P < .05$), αIL-4R immunoreactivity did not differ significantly between these groups. Increased αIL-4R immunoreactive signals were also detected in the endothelial cell layer in both atopic and intrinsic asthmatic subjects compared with atopic and nonatopic control subjects, respectively ($P < .05$). Combined in situ hybridization immunocytochemistry performed on biopsy sections from asthmatic and control subjects demonstrated αIL-4R mRNA expression in CD3-positive T cells and tryptase-positive mast cells, with T cells comprising the larger proportion of αIL-4R mRNA–positive cells. Numbers of αIL-4R mRNA–positive or immunoreactive cells did not correlate with CD3-positive cell numbers, numbers of IL-4 mRNA-positive cells, or indices of pulmonary function.

Conclusion: These results demonstrate constitutive αIL-4R expression in normal airways and enhanced expression in airway tissue from asthmatic individuals. (J Allergy Clin Immunol 1998;102:859-66.)

Key words: *Atopic asthma, intrinsic asthma, IL-4 receptor, IL-4, IgE*

Bronchial asthma is associated with persistent infiltration of the airways with activated $CD4^+$ T lymphocytes exhibiting a T_{H2}-like cytokine profile.[1-3] Recent studies have demonstrated an increased expression of IL-4 and IL-5 in bronchial biopsy specimens from atopic and nonatopic *(intrinsic)* asthmatic subjects.[4] These results suggest that a common pathophysiologic mechanism underlies both variants of the disease. From in vitro studies, it is possible to ascribe several actions of IL-4 to events occurring within asthmatic airways, notably the switching of antigen-specific $CD4^+$ cells to a phenotype of T_{H2}-type cytokine expression.[5,6] In B cells IL-4 induces immunoglobulin heavy chain gene rearrangement to favor IgE production.[7] IL-4 also upregulates vascular cell adhesion molecule (VCAM)-I expression on endothelial cells to facilitate eosinophil and lymphocyte recruitment[8] and induces the expression of eosinophil-associated cytokines and chemokines by epithelial cells.[9,10]

In atopic asthma and allergen-induced rhinitis, a key role for IL-4 is supported by investigations showing that corticosteroid-related improvement of symptoms is concomitant with the attenuation of local increases in IL-4 expression.[11,12] Although these and other pharmacologic

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TABLE I. Clinical characteristics of subjects

	N	Median age and range (y)	Median FEV_1 and range (% predicted)	Median PC_{20} and range (mg/mL)
Nonatopic control subjects	6	22 (19-39)	107.5 (90-119)	>16
Atopic control subjects	7	24.5 (21-42)	107 (81-125)	>16
Atopic asthmatic subjects	8	26 (23-42)	85 (56-104)	1.63 (0.02-3.7)
Intrinsic asthmatic subjects	9	53.5 (39-62)	75.5 (58-96)	0.75 (0.12-6.0)

Abbreviations used
BM: Basement membrane
αIL-4R: α-subunit of the IL-4 receptor
VCAM-1: Vascular cell adhesion molecule-1

studies[13] suggest that IL-4–mediated activities are prominent within asthmatic airways, the evidence implicating IL-4 in the disease pathogenesis remains circumstantial. In particular, studies examining the presence of IL-4 receptor–bearing target cells in the bronchial mucosa are lacking. Our recent investigations of IL-5[14] and GM-CSF[15] receptors have demonstrated that the expression of cytokine receptors can represent potentially important sites of variance between atopic asthmatic subjects, intrinsic asthmatic subjects, and nonasthmatic control subjects.

IL-4 binds with high affinity to the specific α chain of the IL-4 receptor (αIL-4R). Recently it has been suggested that depending on cell type, the αIL-4R subunit can associate with either a γ chain, which is shared among the receptors for IL-2, IL-7, IL-9, and IL-15, or with the α subunit of the IL-13 receptor.[16] In this study we investigated the expression of αIL-4R in bronchial biopsy specimens from atopic and intrinsic asthmatic subjects compared with atopic and nonatopic control subjects. In situ hybridization with radiolabeled antisense riboprobes complementary to αIL-4R and immunocytochemistry with an mAb against αIL-4R were used to determine the presence of αIL-4R mRNA and immunoreactivity, respectively. In addition, we examined the correlation between αIL-4R expression and the severity of asthma as measured by the FEV_1 and airway responsiveness to methacholine.

METHODS

Bronchial biopsies

The study was approved by the Ethics Committees of the Royal Brompton Hospital, London, and the Hochgebirgsklinik, Davos. Thirty subjects were included in this study (Table I), and informed written consent was obtained. Atopic asthmatic, nonatopic asthmatic, atopic nonasthmatic, and normal subjects were carefully defined as described in detail previously.[17] In brief, asthma was defined on the basis of (1) a clear clinical history with current clinical symptoms and (2) a greater than 20% reversibility of FEV_1 spontaneously or after β_2-agonist inhalation and/or a histamine provocation test result with a PC_{20} less than 8 mg/mL in the previous 2 weeks. Atopy was defined by (1) a positive skin prick test response with extracts of 1 or more common aeroallergens and (2) a positive RAST result greater than 0.70 IU/mL to 1 or more of these allergens. Nonatopic subjects had negative skin prick test responses to a wide range of local aeroallergens in the presence of a positive histamine control. The nonatopic control subjects also had negative RAST test results to 25 common aeroallergens and a serum IgE concentration within the normal range for our laboratory (<150 IU/mL). Atopic control subjects were asymptomatic or had mild-to-moderate allergic rhinitis with no history of wheezing or chest tightness, normal baseline lung function with less than 20% reversibility of FEV_1 either spontaneously or after inhaled β_2 agonists, and a histamine provocation result with a PC_{20} greater than 16 mg/mL. All subjects were nonsmokers and had not taken oral corticosteroids for the previous 2 months or inhaled corticosteroids for the previous 2 weeks. Exclusion criteria included the following: age less than 18 years or greater than 65 years, FEV_1 less than 60% of the predicted value on the proposed bronchoscopy day, and evidence of acute or chronic infection, pregnancy, breast feeding, or any chronic medical illness other than asthma.

The technique of fiberoptic bronchoscopy with bronchial biopsy specimens in asthmatic and normal volunteers has been previously described.[2] For each subject, baseline spirometry was recorded, and a methacholine inhalation test was performed. Bronchial biopsy specimens were obtained from the subsegmental airways as previously described.[2] Nebulized salbutamol was given to all subjects (asthmatic and control) before the bronchoscopy procedure. The biopsy specimens were immediately fixed in 4% paraformaldehyde, washed, and frozen in isopentane cooled in liquid nitrogen and stored at –70°C. They were subsequently shipped in dry ice to the Meakins-Christie Laboratories, Montreal, Canada, for processing for in situ hybridization and immunocytochemistry.

In situ hybridization

In situ hybridization was performed as previously described.[18] Briefly, riboprobes (both anti-sense and sense) were prepared from complementary DNA and linearized with appropriate enzymes before transcription. The αIL-4R riboprobe (830 bp; a gift from Immunex Corp, Seattle, Wash) contained the sequence complementary to the exon coding for the transmembrane region of the αIL-4R, thus ensuring specificity for the membrane-anchored isoform of this receptor.[19] Sense and anti-sense riboprobes were also prepared from cDNA coding for IL-4.[4] Transcription was performed in the presence of ^{35}S-labeled uridine triphosphate and the appropriate T7 or SP6 RNA polymerases. Cryostat sections were permeabilized and subsequently treated to prevent nonspecific binding of the ^{35}S-labeled RNA probes. Prehybrization was carried out with 50% formamide and 2× standard saline citrate for 15 minutes at 40°C. Dithiothreitol was included in the hybridization mixture to ensure blockage of nonspecific binding of the probes. Posthybridization washing was performed in decreasing concentrations of standard sodium citrate at 45°C. Unhybridized single stranded RNAs were removed by RNase A. Hybridization signals were visualized by

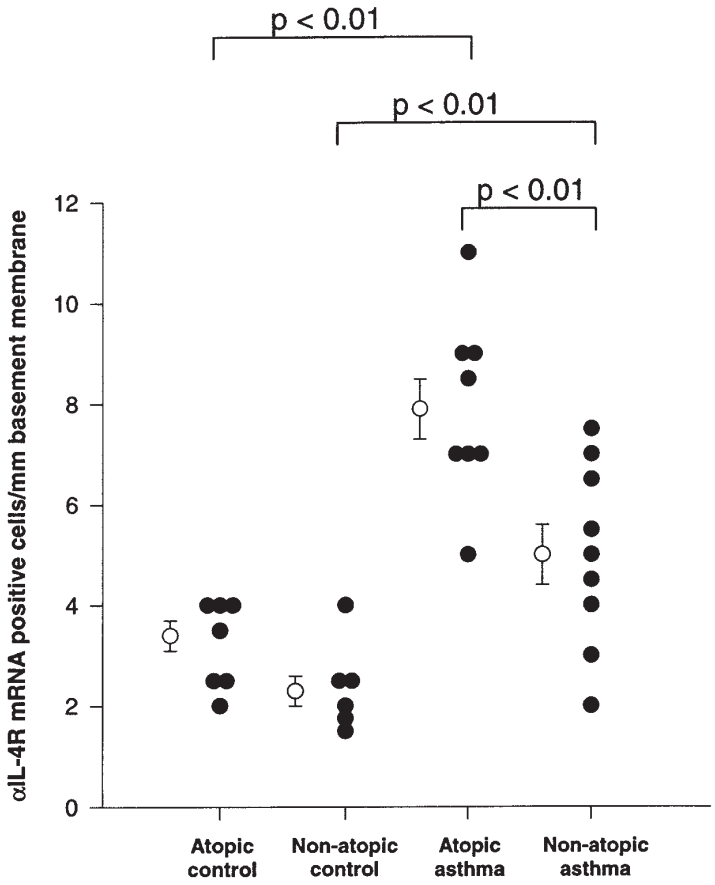

FIG 1. In situ hybridization of bronchial biopsy specimens showing the number of αIL-4R mRNA–positive cells in the subepithelium of atopic (n = 8) and nonatopic (n = 9) asthmatic subjects compared with atopic (n = 7) and nonatopic (n = 6) control subjects, respectively. Individual data points *(filled circles)* are indicated with mean *(open circles)* and SEM *(bars).* There were statistically significant differences in the numbers of αIL-4R mRNA–positive cells between atopic asthmatic and atopic control subjects ($P < .01$) and between intrinsic asthmatic and nonatopic control subjects ($P < .01$). Numbers of αIL-4R mRNA–positive cells were also increased in atopic asthmatic subjects compared with intrinsic asthmatic subjects ($P < .01$).

autoradiography, and the tissue sections were counterstained with hematoxylin. For negative controls, specimens were hybridized with sense probes. In addition, sections were treated with RNase solution before the prehybrization step with antisense probes. Positive cells were only observed when the antisense probes were used; preparations treated with the sense probes and those pretreated with RNase were negative.

Immunocytochemistry

To determine the presence of αIL-4R immunoreactivity or CD3 immunoreactivity in bronchial biopsy specimens from asthmatic and normal subjects, cryostat sections were immunostained with a mouse mAb for the transmembrane region of the α subunit of the human IL-4 receptor (huIL-4R-M57; a gift from Immunex Corp) or mouse anti-human CD3 (Becton Dickinson, Mississauga, Ontario, Canada), respectively. Immunocytochemistry was performed by using the modified alkaline phosphatase anti-alkaline phosphatase technique as previously described,[2] and slides were developed with Fast Red (Sigma Chemical Co, Mississauga, Canada). Negative controls involved the replacement of the primary antibody with tris-buffered saline or an irrelevant mouse isotype-matched antibody, and none exhibited positive staining.

Combined immunocytochemistry–in situ hybridization

To identify the percentage of αIL-4R mRNA–positive cells coexpressing the T-cell marker CD3 or the mast cell marker tryptase, colocalization studies were performed on sections prepared from bronchial biopsy specimens obtained from 3 atopic asthmatic subjects, 3 intrinsic asthmatic subjects, and 3 nonatopic control subjects. This technique has been described elsewhere.[20] The specimens were first hybridized with the radiolabeled αIL-4R antisense probe as described above. To preserve the immunoreactivity of CD3 or tryptase, dextran sulphate was omitted from the hybridization buffer. Immunocytochemistry (APAAP technique) was performed immediately after the standard sodium citrate wash by using an mAb directed against human T-cell receptor (anti-CD3; Becton Dickinson, Mississauga, Ontario, Canada) or an mAb

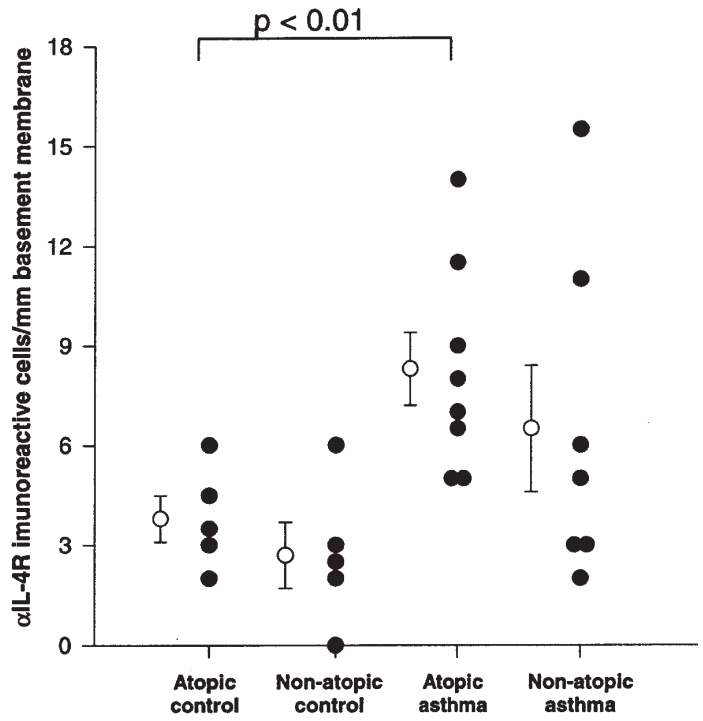

FIG 2. Immunocytochemistry of bronchial biopsy specimens showing the number of αIL-4R-immunoreactive cells in the bronchial mucosa of atopic (n = 8) and nonatopic asthmatic subjects (n = 7) compared with atopic (n = 5) and nonatopic (n = 5) control subjects, respectively. Individual data points *(filled circles)* are given with mean *(open circles)* and SEM *(bars).* There was a statistically significant difference in the numbers of αIL-4R-immunoreactive cells between atopic asthmatic and atopic control subjects ($P < .01$).

TABLE II. Colocalization of αIL-4R mRNA to CD3- and tryptase-positive cells

	Percent of αIL-4R mRNA–positive cells	
	CD3-positive cells	Tryptase-positive cells
Atopic asthma	70.0 ± 2.9	19.7 ± 2.4
Intrinsic asthma	33.0 ± 3.8	7.0 ± 1.5
Nonatopic control subjects	72.3 ± 5.4	5.0 ± 0.6

directed against human mast cell tryptase (anti-tryptase; Chemicon International Inc, Temecula, Calif). Cell counts were expressed as the mean percentage ± SEM of αIL-4R mRNA cells that coexpressed immunoreactivity for CD3 or tryptase and the mean percentage ± SEM of CD3– or tryptase-positive cells coexpressing αIL-4R mRNA.

Quantification

Slides were coded, and positive cells were counted blindly by using 200× magnification with an eyepiece graticule. In the airways subepithelium, cells staining positive were counted to a depth of 115 μm below the basement membrane, and the individual results were expressed as the mean number of positive cells per millimeter of basement membrane (BM) ± SEM. αIL-4 mRNA–positive and/or immunoreactive cells in the epithelium and endothelium were assessed by using a semiquantitative score (0 to 4) based on the percentage of epithelial or endothelial cells exhibiting a positive signal per total epithelium or endothelium.

Statistics

The numbers of cells expressing αIL-4R mRNA, αIL-4R protein, CD3 protein, and mRNA in atopic and intrinsic asthmatic airways and atopic and nonatopic normal control subjects was compared by using a nonparametric Wilcoxon rank-sum test (Systat v5.0; Systat Inc, Evanston, Ill). The coefficients of determination (r^2) and significance values were calculated by using Pearson's product-moment correlation and Bartlett's test. Results were considered statistically significant for P values less than or equal to .05.

RESULTS

Expression of αIL-4R mRNA in bronchial biopsy specimens

Positive signals for αIL-4R mRNA were detected in all biopsy specimens from asthmatic and normal control subjects and were mainly associated with inflammatory cells infiltrating the mucosa. αIL-4R mRNA was also expressed in the epithelium, particularly in biopsy speci-

mens obtained from atopic asthmatic subjects. There was a significantly increased proportion of cells in the airway epithelium expressing αIL-4 mRNA in atopic asthmatic subjects compared with atopic control subjects ($P < .05$). However, increased expression of αIL-4R mRNA was not observed in the epithelium of intrinsic asthmatic subjects compared with nonatopic control subjects. Epithelial αIL-4R mRNA expression did not differ significantly between atopic and nonatopic control subjects. The numbers of αIL-4R mRNA–positive cells in the subepithelium were significantly higher in both atopic (mean ± SEM, 7.76 ± 0.77 cells/mm BM) and intrinsic (mean ± SEM, 4.85 ± 0.61 cells/mm BM) asthmatic subjects compared with atopic (mean ± SEM, 3.45 ± 0.32 cells/mm BM) and nonatopic (mean ± SEM, 2.29 ± 0.32 cells/mm BM) control subjects, respectively ($P < .01$; Fig 1). Subepithelial αIL-4R mRNA–positive cells were also significantly higher in atopic asthmatic subjects compared with nonatopic asthmatic subjects ($P < .01$) but did not differ significantly when atopic and nonatopic control subjects were compared.

Expression of αIL-4R immunoreactivity in bronchial biopsy specimens

αIL-4R immunoreactivity was significantly increased in the airway epithelium of atopic asthmatic subjects compared with atopic control subjects ($P < .01$). This increase was not observed in intrinsic asthmatic subjects when compared with nonatopic control subjects. The numbers of cells expressing αIL-4R immunoreactivity in the subepithelium of bronchial biopsy specimens from atopic asthmatic subjects (mean ± SEM, 8.94 ± 0.97 cells/mm BM) were significantly increased compared with atopic control subjects (mean ± SEM, 3.80 ± 0.68 cells/mm BM; $P < .05$) (Fig 2). Although subepithelial αIL-4R–immunoreactive cells were detected in intrinsic asthma (mean ± SEM, 6.41 ± 1.90 cells/mm BM), the expression was not significantly different in these patients compared with the atopic asthmatic subjects or the nonatopic control subjects (mean ± SEM, 3.30 ± 0.70 cells/mm BM). Immunoreactivity for αIL-4R was also detected in the endothelium in bronchial biopsy specimens and was significantly higher in atopic and intrinsic asthmatic subjects compared with atopic ($P < .05$) and nonatopic control subjects ($P < .05$), respectively. αIL-4R immunoreactivity did not differ significantly between atopic and nonatopic control subjects in the epithelium, subepithelium, or endothelium.

Colocalization of αIL-4R mRNA to T lymphocytes and mast cells

Combined in situ hybridization–immunocytochemistry was used on biopsy sections from randomly selected atopic asthmatic subjects, intrinsic asthmatic subjects, and nonatopic control subjects to identify the phenotype of αIL-4R mRNA–positive cells. As shown in Table II, CD3-positive T cells comprised the larger proportion of αIL-4R mRNA–positive cells in the airway wall of atopic asthmatic subjects, intrinsic asthmatic subjects, and nonatopic control subjects. In each group tryptase-positive mast cells constituted a smaller percentage of αIL-4R mRNA–positive cells. The proportion of CD3-positive cells coexpressing αIL-4R mRNA was 44.0% ± 4.9% in atopic asthmatic subjects, 26.3% ± 2.3% in intrinsic asthmatic subjects, and 8.3% ± 2.7% in nonatopic control subjects. Similarly, differences were found between the groups in the proportion of tryptase-positive cells coexpressing αIL-4R mRNA; 38.0% ± 4.4% in atopic asthmatic subjects, 7.3% ± 1.5% in intrinsic asthmatic subjects, and 9.0% ± 1.5% in nonatopic control subjects. To examine whether differences in T-cell numbers in the bronchial mucosa of atopic asthmatic subjects, intrinsic asthmatic subjects, atopic control subjects, and nonatopic control subjects might contribute to differences in the numbers of αIL-4R mRNA–positive cells, immunocytochemistry was performed with an anti-CD3 mAb. Numbers of CD3-positive cells did not differ significantly in the bronchial mucosa of atopic asthmatic subjects, intrinsic asthmatic subjects, atopic control subjects, and nonatopic control subjects. In addition, numbers of CD3-immunoreactive cells did not correlate with numbers of αIL-4R mRNA–positive or immunoreactive cells ($P > .05$).

Expression of IL-4 mRNA in bronchial biopsy specimens

IL-4 mRNA expression was examined in bronchial biopsy specimens from all patients and control subjects by in situ hybridization. Numbers of IL-4 mRNA–positive cells were significantly higher in the bronchial mucosa of atopic and intrinsic asthmatic subjects compared with atopic ($P < .01$) and nonatopic control subjects ($P < .01$), respectively. Numbers of IL-4 mRNA–positive cells did not differ significantly in atopic asthmatic subjects compared with intrinsic asthmatic subjects or in atopic control subjects compared with nonatopic control subjects. The numbers of cells expressing αIL-4R mRNA or immunoreactivity did not correlate with the numbers of IL-4 mRNA–positive cells in asthmatic or control subjects ($P > .05$).

αIL-4R expression and clinical indices of pulmonary function

There were no significant correlations between cells expressing αIL-4R mRNA or αIL-4R immunoreactivity when examining αIL-4R expression and clinical indices of pulmonary function in both atopic and nonatopic asthma (FEV_1 or PC_{20} to histamine; $P > .05$).

DISCUSSION

Recent studies examining the expression of IL-5 receptor mRNA in asthmatic airways demonstrated a close association between cells expressing isoforms of this receptor and FEV_1.[14] In contrast, the correlation between IL-5 mRNA and FEV_1 was relatively weak,[21] suggesting that receptor expression is more indicative of cytokine action. The importance of αIL-4R has been suggested with the recent identification of a novel αIL-4R allele that is associated with enhanced function and is

strongly related to atopy.[22] By using riboprobes complementary to cellular mRNA encoding the transmembrane region of αIL-4R and through the use of mAbs directed against the same region of the translated protein, we examined expression of the membrane-bound isoform of αIL-4R in bronchial biopsy specimens from atopic and nonatopic asthmatic subjects. Each group was compared with healthy subjects matched for atopic status to control for differences that may have been associated with atopy per se rather than asthma. The results of this study provide evidence for the constitutive expression of αIL-4R in airways of normal individuals and demonstrate that the numbers of subepithelial cells expressing mRNA encoding this receptor are increased in atopic and intrinsic asthmatic subjects. Although subepithelial αIL-4R expression was elevated at the protein level in atopic asthmatic subjects, we were unable to demonstrate this in the bronchial mucosa of patients with intrinsic asthma.

The putative effects elicited by IL-4 on target cells include the switching of $CD4^+$ T cells to a T_{H2} profile of cytokine expressions,[5,6] the upregulation of endothelial VCAM-1 expression,[8] and the induction of B-cell immunoglobulin isotype switching to IgE.[7] These actions are mediated through the IL-4 receptor,[23,24] a complex that includes an IL-4–specific subunit (αIL-4R) and at least 1 other subunit, which may be shared between a number of cytokine receptors.[16] IL-13 has many functional similarities to IL-4, including VCAM-1 upregulation and IgE switching. Unlike IL-4, however, T cells do not respond to IL-13.[25] Functional studies and receptor blocking experiments have suggested that IL-13 also uses αIL-4R in its receptor complex.[16] Using double in situ hybridization, we recently reported that IL-4 and IL-13 mRNA are coexpressed, predominantly by T cells, in the airways of atopic asthmatic subjects.[26] It is therefore possible that both IL-4 and IL-13 act as ligands for αIL-4R investigated in this study.

Prior studies have shown increased expression of IL-4 and IL-5 in bronchial biopsy specimens from both atopic and nonatopic asthmatic subjects,[4] suggesting that the latter is not a distinct immunopathologic entity. The results of our in situ hybridization analyses provide further support for the hypothesis that allergic and nonallergic variants of asthma share some similar pathophysiologic mechanisms because both were found to be associated with increased mRNA expression of αIL-4R. However, αIL-4R mRNA expression was also elevated in the atopic asthmatic subjects compared with the intrinsic asthmatic subjects, possibly suggesting that IL-4–mediated actions are more important in the pathophysiology of allergic asthma. The immunocytochemical data is more difficult to interpret but might be explained by the large variation in αIL-4R immunoreactivity in the intrinsic asthmatic subjects, which may be a reflection of the heterogeneity of the disorder. Other factors that might have influenced the expression of αIL-4R immunoreactivity in intrinsic asthmatic subjects include posttranscriptional regulation and differences in the kinetics of αIL-4R mRNA and protein expression. The precise influences that regulate αIL-4R expression remain to be determined, however.

Various cell types were shown to express αIL-4R in biopsy specimens, including epithelial and endothelial cells, as well as cells within the airway submucosa. Epithelial αIL-4R expression was found to be increased in atopic asthma but not intrinsic asthma. Recent studies have reported that IL-4 upregulates GM-CSF expression by epithelial cells.[9] In vitro epithelial production of GM-CSF is capable of markedly prolonging the survival of eosinophils.[27] It is possible that IL-4/αIL-4R interactions are involved in the induction of GM-CSF–mediated eosinophil survival in atopic asthma. Activated epithelial cells may also comprise a source of chemokines, such as IL-8 and RANTES, which are chemotactic for T lymphocytes and eosinophils.[28,29] Rothenburg et al[30] demonstrated that epithelial expression of eotaxin, a highly specific eosinophil chemoattractant, is induced locally in response to IL-4 in mice. In intrinsic asthma, mechanisms other than IL-4 may be involved in the production of proinflammatory mediators by epithelial cells. IL-4 has also been suggested to promote the accumulation of eosinophils and lymphocytes in asthmatic airways by upregulating endothelial expression of VCAM-1.[8] Indeed, work in a mouse model of asthma has shown an abrogation of airway eosinophilia in animals treated with anti-IL-4 mAbs.[31] Consistent with this, we detected the increased expression of αIL-4R in the endothelium of atopic and intrinsic asthmatic patients compared with control subjects.

αIL-4R has been described in unstimulated T and B lymphocytes, and its expression is strikingly upregulated in vitro in response to IL-4.[32,33] IL-4 mRNA expression was found to be increased in the bronchial mucosa of atopic and intrinsic asthmatic subjects; however, it did not correlate with numbers of αIL-4R mRNA–positive or immunoreactive cells. In this study the majority of cells expressing αIL-4R immunoreactivity and mRNA in the subepithelium exhibited a morphology consistent with that of mononuclear cells, including lymphocytes. Indeed, αIL-4R mRNA colocalized largely to T cells, particularly in allergic asthma where IL-4 is believed to perpetuate the T_{H2}-predominated cytokine response. IL-4 also favors the development of mast cells and upregulates the expression of IgE receptors on these and other cell types.[34] Consistent with these in vitro observations, a significant proportion of αIL-4R mRNA–positive cells were tryptase-positive mast cells in the bronchial mucosa, particularly in atopic asthma.

In atopic asthma CD3-positive T cells and tryptase-positive mast cells accounted for approximately 90% of the subepithelial αIL-4R mRNA–positive cells. The remainder of the subepithelial αIL-4R mRNA–positive cells in allergic asthma might be attributed to eosinophils and B lymphocytes. In contrast to atopic asthmatic subjects, αIL-4R mRNA expression in CD3-positive T cells and tryptase-positive mast cells explained only 40% of the subepithelial αIL-4R mRNA–positive cells in biopsy specimens from intrinsic asthmatic subjects. It is possible that macrophages comprise a distinct population of αIL-4R mRNA–positive cells in intrinsic asthma. Indeed,

macrophage numbers are significantly increased in the bronchial mucosa of intrinsic asthmatic subjects but not atopic asthmatic subjects or normal control subjects.[15] Although the numbers of T cells do not differ significantly between atopic asthmatic subjects, intrinsic asthmatic subjects, and control subjects, the proportion of these cells expressing αIL-4R mRNA varies between the groups. This suggests that differences in αIL-4R expression between the groups cannot be attributed to differences in total T-cell numbers but may be related to other factors such as cellular activation. Similarly, previous studies have shown that numbers of tryptase-positive mast cells do not differ significantly in the bronchial mucosa of atopic and intrinsic asthmatic subjects[35]; however, the percentage of these cells expressing αIL-4R mRNA was approximately 38% in atopic asthmatic subjects compared with 7% in intrinsic asthmatic subjects.

When comparing clinical indices of pulmonary function with the expression of αIL-4R in asthma, we found no significant correlations. This finding is in agreement with previous studies in asthmatic subjects, which failed to find any association with IL-4 expression and alterations in lung function.[4] This is possibly explained by the multiple actions of IL-4 on various cell types and the widespread expression of αIL-4R receptor.[36]

In summary, the results of this study demonstrate increased mRNA expression of αIL-4R in bronchial biopsy specimens from atopic and intrinsic asthmatic individuals and increased αIL-4R immunoreactivity in the former group of patients compared with control subjects. The receptor mRNA and protein were expressed in the airways epithelium, submucosa, and endothelial cells. Although not correlated to indices of pulmonary function, expression of αIL-4R may be important in the pathogenesis of asthma irrespective of etiology.

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REFERENCES

1. Robinson D, Hamid Q, Bentley A, Ying A, Kay AB, Durham SR. Activation of CD4+ T cells and eosinophil recruitment in bronchoalveolar lavage after allergic allergen inhalation challenge in patients with atopic asthma. J Allergy Clin Immunol 1993;92:313-24.
2. Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw A, Assoufi B, et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. Am Rev Respir Dis 1990;142:1407-13.
3. Bentley AM, Menz G, Storz C, Robinson DS, Bradley B, Jeffery PK, et al. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. Am Rev Respir Dis 1992;146:500-6.
4. Humbert M, Durham SR, Ying S, Kimmitt P, Barkans J, Assoufi B, et al. IL-4 and IL-5 mRNA and protein expression in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against "intrinsic" asthma being a distinct immunopathologic entity. Am J Respir Crit Care Med 1996;154:1497-504.
5. Fernandez-Botran R, Sanders VM, Oliver KG, Chen YW, Krammer PH, Uhr JW, et al. Interleukin-4 mediates autocrine growth of helper T cells after antigenic stimulation. Proc Natl Acad Sci USA 1986;83:9689-93.
6. Murphy E, Shibuya K, Hosken N, Openshaw P, Maino V, Davis K, et al. Reversibility of T helper 1 and 2 populations is lost after long-term antigen stimulation. J Exp Med 1996;183:901-13.
7. Del Prete GF, Maggi E, Parronchi P, Chetien I, Tiri A, Machia D, et al. IL-4 is an essential cofactor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. J Immunol 1988;140:4193-8.
8. Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, et al. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. J Immunol 1992;148:1086-92.
9. Nakaniura Y, Azuma M, Okano Y, Sono T, Takahashi T, Ohmoto Y, et al. Upregulatory effect of interleukin-4 and interleukin-13 but not interleukin-10 on granulocyte-macrophage stimulatory factor production by human bronchial epithelial cells. Am J Respir Cell Mol Biol 1996;15:680-7.
10. Garcia-Zepeda EA, Combadiere C, Rothenberg M, Sarafi M, Lavigne F, Hamid Q, et al. Human monocyte chemoattractant protein-4 is a novel CC chemokine with activities on macrophages, eosinophils, and basophils induced in allergic and non-allergic inflammation that signals through the CC chemokine receptors (CCR)-2 and -3. J Immunol 1996;157:5613-26.
11. Masuyama K, Jacobson MR, Rak S, Meng Q, Sudderick RM, Kay AB, et al. Topical glucocorticoid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. Immunology 1994;82:192-9.
12. Robinson D, Hamid Q, Ying S, Bentley A, Assoufi B, Durham S, et al. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5, and interferon-γ cytokine gene expression. Am J Respir Dis 1993;148:401-6.
13. Leung DYM, Martin RJ, Szefler SJ, Shu ER, Ying S, Kay AB, et al. Dysregulation of IL-4, IL-5, and IFN-γ gene expression in steroid-resistant asthma. J Exp Med 1995;181:33-40.
14. Yasruel Z, Humbert M, Kotsimbos T, Ploysongsang Y, Minshall E, Durham S, et al. Membrane-bound and soluble αIL-5 receptor mRNA in the bronchial mucosa of atopic and nonatopic asthmatics. Am J Respir Crit Care Med 1997;155:1413-8.
15. Kotsimbos T, Humbert M, Minshall E, Durham SR, Pfister R, Menz G, et al. Upregulation of αGM-CSF-receptor in nonatopic but not in atopic asthma. J Allergy Clin Immunol 1997;99:666-72.
16. Callard RE, Matthews DJ, Hibbert L. IL-4 and IL-13 receptors: Are they one and the same? Immunol Today 1996;17:108-10.
17. Humbert M, Grant JA, Taborda-Barata L, Durham SR, Pfister R, Menz G, et al. High affinity IgE receptor-bearing cells in bronchial biopsies from atopic and non-atopic asthma. Am J Respir Crit Care Med 1996; 153:1931-7.
18. Hamid Q, Wharton J, Terenghi G, Hassall CJ, Aimi J, Taylor KM, et al. Localization of atrial natriuretic peptide mRNA and immunoreactivity in the rat heart and human atrial appendage. Proc Natl Acad Sci USA 1987;84:6760-4.
19. Renz H, Jujo K, Bradley KL, Domenico J, Gelfand EW, Leung DYM. Enhanced IL-4 production and IL-4 receptor expression in atopic dermatitis and their modulation by interferon-gamma. J Invest Dermatol 1992;99:403-8.
20. Hamid Q, Barkans J, Meng Q, Ying S, Abrams JS, Kay AB, et al. Human eosinophils synthesize and secrete interleukin-6, in vitro. Blood 1992;80:1496-501.
21. Robinson DS, Ying S, Bentley AM, Meng Q, North J, Durham SR, et al. Relationships among numbers of bronchoalveolar lavage cells expressing mRNA for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. J Allergy Clin Immunol 1993;92:397-403.
22. Khurana Hershey GK, Freidrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the α subunit of the interleukin-4 receptor. N Engl J Med 1997;337:1720-5.
23. Gorrone P, Djossou O, Galizzi JP, Banchereau J. A recombinant extracellular domain of the human IL-4 receptor inhibits the biological effects of IL-4 on T and B lymphocytes. Eur J Immunol 1991;21:1365-9.
24. Schnyder B, Lugli S, Feng N, Etter H, Lutz RA, Ryffel B, et al. Interleukin-4 and interleukin-13 bind to a shared heterodimeric complex on endothelial cell mediating the vascular cell adhesion molecule-1 induction in the absence of the common γ chain. Blood 1996;87:4286-95.
25. Zurawski G, de Vries YE. IL-13, an IL-4-like cytokine that acts on monocytes and B cells, but not T cells. Immunol Today 1994;15:19-26.
26. Kotsimbos T, Ernst P, Hamid Q. Interleukin-13 and interleukin-4 are coexpressed in atopic asthma. Proc Assoc Am Physicians 1996;108:368-73.
27. Xaubet A, Mullol J, Lopez E, Roca-Ferrer J, Rozman M, Corrion T, et al. Comparison of the role of nasal polyp and normal nasal mucosal epithe-

lial cells on *in vitro* eosinophil survival by GM-CSF and inhibition by dexamethasone. Clin Exp Allergy 1994;24:307-17.
28. Nakamura H, Yoshimura K, Jaffe A, Crystal RG. Interleukin-8 gene expression in bronchial epithelial cells. J Biol Chem 1991;266:19611-7.
29. Stellato C, Beck LA, Gorgone GA, Proud D, Schall TJ, Ono SJ, et al. Expression of the chemokine RANTES by a human bronchial epithelial cell line. Modulation by cytokines and glucocorticoids. J Immunol 1995;155:410-8.
30. Rothenberg ME, Luster AD, Leder P. Murine eotaxin: an eosinophil chemoattractant induced in endothelial cells and IL-4-induced tumour suppression. Proc Natl Acad Sci USA 1995;92:8960-4.
31. Lukacs NW, Strieter RM, Chensue SW, Kunkel SL. IL-4-dependent pulmonary eosinophil infiltration in a murine model of asthma. Am J Respir Cell Mol Biol 1994;10:526-32.
32. Armitage RJ, Beckmann M, Idzerda RL, Alpert A, Fanslow W. Regulation of interleukin-4 receptors on human T cells. Int Immunol 1990;2:1039-45.
33. Mozo L, Rivas D, Zamorana J, Gutierrez C. Differential expression of IL-4 receptors in human T and B lymphocytes. J Immunol 1993;150:4261-9.
34. Toru H, Ra C, Nonoyama S, Suzuki K, Yata J, Nakahata T. Induction of the high-affinity IgE receptor on human mast cells by IL-4. Int Immunol 1996;8:1367-73.
35. Ying S, Humbert M, Barkans J, Corrigan CJ, Pfister R, Menz G, et al. Expression of IL-4 and IL-5 mRNA and protein product by CD4+ and CD8+ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. J Immunol 1997;158:3539-44.
36. Cabrillat H, Galizzi JP, Djoussou O, Arai N, Yokota T, Arai K, et al. High affinity binding of human IL-4 to cell lines. Biochem Biophys Res Commun 1987;149:995-9.