

Macrophage subpopulations and macrophage-derived cytokines in sputum of atopic and nonatopic asthmatic subjects and atopic and normal control subjects

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Background: Previous studies have shown a prominent macrophage signal in the bronchial mucosa from nonatopic (intrinsic) compared with atopic (extrinsic) asthmatic subjects. This observation might have represented an expansion of a proinflammatory macrophage population or a homeostatic mechanism to decrease T_H2-type inflammation.

Objective: The aim of the study was to investigate the numbers of macrophages and macrophage subpopulations and the expression of IL-10 and IL-12 in sputum from asthmatic and control subjects.

Methods: Eight atopic asthmatic (AA) subjects, 10 nonatopic asthmatic (NAA) subjects, 6 atopic control (AC) subjects, and 7 normal control (NC) subjects underwent sputum induction.

Macrophages were enumerated by using Romanowsky stain and immunocytochemistry (CD68). RFD1 (interdigitating cell marker) and RFD7 (mature phagocyte marker) mAbs were used for immunocytochemical phenotyping, whereas IL-10 and IL-12 messenger (m)RNA was examined with in situ hybridization by using ³⁵S-labeled riboprobes. The phenotype of cells expressing IL-10 or IL-12 mRNA was examined by simultaneous in situ hybridization and immunostaining.

Results: No differences in the numbers of CD68⁺ macrophages and RFD1⁺, RFD7⁺, and RFD1⁺/RFD7⁺ subpopulations were found between AA, NAA, AC, and NC subjects. However, the numbers of IL-10 and IL-12 mRNA⁺ cells were increased in AA subjects compared with NAA, AC, and NC subjects (*P* < .05). No other differences were found among the groups. Most of the IL-10 and IL-12 mRNA⁺ cells in sputum from asthmatic subjects were macrophages (>80%), with less than 10% of mRNA colocalizing to epithelial cells.

Conclusions: Sputum macrophage numbers, unlike tissue macrophages, as previously reported, were not elevated in NAA subjects. Increased IL-10 and IL-12 expression in atopic asthma may indicate the existence of a homeostatic mechanism to decrease lung inflammation. The lack of such cytokines in intrinsic asthma may predispose to bronchial inflammation in these subjects. (*J Allergy Clin Immunol* 2000;106:697-704.)

Key words: Asthma, atopy, macrophage, IL-10, IL-12

Although many studies have suggested that both allergic and nonallergic asthma share a common immunopathology, increased numbers of CD68⁺ macrophages and cells expressing the GM-CSF receptor α subunit have been found in bronchial biopsy specimens of intrinsic asthmatic subjects compared with their extrinsic counterparts.^{1,2}

The alveolar macrophage pool is heterogeneous and contains subsets of cells expressing different phenotypes and functions.³ These populations may be phenotypically discriminated by using the mAbs RFD1 and RFD7.⁴ RFD1 recognizes a 30-kd epitope within the class II major histocompatibility complex, which appears to be expressed by antigen-presenting cells.⁵ RFD7 sees a 77-kd antigen expressed by mature mononuclear phagocytes,⁴ whereas suppressive macrophages appear to express both antigens.⁶

Alveolar macrophages secrete a number of growth factors, cytokines, and chemokines, including IL-1 β , TNF- α , IFN- γ , IL-6, and GM-CSF,⁷ as well as RANTES, monocyte chemotactic proteins, and eotaxin.⁸ They can also regulate cytokines that decrease T_H2-type inflammation (eg, IL-10⁹ and IL-12¹⁰).

IL-12 is a heterodimer consisting of a p35 and a p40 subunit, which stimulates T_H1 cell differentiation, promotes T-cell production of IFN- γ , induces IL-10 production, and inhibits IgE synthesis.¹¹ IL-10 is mainly an anti-inflammatory cytokine; it inhibits production of proinflammatory cytokines,¹² induces T-cell tolerance to allergens,¹³ and inhibits eosinophil survival and IgE synthesis.¹⁴ IL-10 suppresses the effector and inductive capacities of macrophages and thus may influence the balance of macrophage subpopulations.¹⁵

The aim of this study was to examine macrophages and the macrophage-associated cytokines IL-10 and IL-12 in sputum from intrinsic asthmatic subjects and to compare findings with those of extrinsic, atopic, asthmatic subjects and atopic and normal control subjects.

METHODS

Study subjects

Induced sputum was obtained from eight atopic asthmatic (AA) subjects (*n* = 8), 10 nonatopic asthmatic (NAA) subjects (*n* = 10), 6

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Abbreviations used

AA: Atopic asthmatic
 AC: Atopic control
 NAA: Nonatopic asthmatic
 NC: Normal atopic

nonasthmatic atopic control (AC) subjects ($n = 6$), and 7 nonasthmatic nonatopic control (NC) subjects ($n = 7$). The patients studied were recruited from the Royal Brompton Hospital, London, UK, and 5 of the NAA subjects were recruited from the Sotiria Hospital, Athens, Greece. Asthmatic subjects had a clear history of asthma requiring only intermittent inhaled β_2 -agonists, documented reversible airway obstruction, and a histamine PC_{20} provocation test result of 5 mg/mL or less in the previous 2 weeks. Atopy was defined as a positive skin prick test response to more than one of the extracts from the common local aeroallergens. Nonatopic subjects had negative skin test responses to a wide range of local common aeroallergens, serum IgE concentration within the normal range (0–150 IU), and negative RAST test responses (Pharmacia Diagnostics, Uppsala, Sweden) to 25 common aeroallergens (intrinsic asthmatic subjects from Greece were also tested to olive tree pollen). For all subjects, total serum IgE and RAST measurements were performed in the same laboratory. All subjects were nonsmokers and had not taken oral (2 months) or inhaled (2 weeks) glucocorticoids before the study. The study was approved by the Ethics Committees of the Royal Brompton Hospital, London, UK, and Sotiria Athens Hospital, Greece, and all participants gave informed consent before entering the study.

Sputum induction and processing

Sputum induction was performed by using the method of Pin et al.¹⁶ After pretreatment with 200 μ g of salbutamol, volunteers inhaled gradually increasing concentrations of hypertonic saline solution generated through a low-output ultrasonic nebulizer (Sonix 2000, Medix Ltd, Harlow, UK; output, 0.9 mL/min; median mass diameter, 5.6 μ m). Plugs of sputum were selected and dispersed with 3 volumes of cooled 0.1% dithiotreitol (Sigma Pharmaceuticals, Dorset, UK) freshly diluted in Dulbecco's PBS (Sigma). After 15 minutes on a bench-rocker, an equal volume of Dulbecco's PBS was added, and the sample was filtered through a 48- μ m nylon gauze. Cell viability and total cell count (excluding squamous cell contamination) were estimated by using trypan blue staining (Sigma). The filtered sample was centrifuged at 2000 revolutions per minute for 10 minutes, and then cells were resuspended at 1×10^6 cells/mL in Dulbecco's PBS. Differential cell count was performed on cytocentrifuge preparations stained with Romanowsky stain.¹⁷ With this staining, eosinophils are stained red. All the other cell types (macrophages, epithelial cells, neutrophils, and lymphocytes) were counted by using morphologic criteria. In all cases 400 nucleated nonsquamous cells were counted, and results are expressed as the percentage of total cells or as absolute cell counts by dividing the percentage of each cell type into the total cell counts obtained with trypan blue staining. In addition, in 5 AA subjects and 4 NAA subjects, cell counts for epithelial cells and macrophages were confirmed by immunocytochemistry to cytokeratin or CD68, as described below.

In situ hybridization

On the basis of sequencing, an 883-bp complementary (c)DNA fragment of human IL-10 (3' untranslated region, 36–919; K.M. Moore, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, Calif) and an 860-bp cDNA fragment of human IL-12 p40 subunit (encoding region 542–1302; S.F. Wolf, Genetics Institute, Cambridge, Mass) were inserted into pGEM 7 vectors.

Riboprobes were prepared from cDNA for IL-10 and IL-12, as previously described.^{18,19}

Permeabilization, prehybridization, and hybridization protocols were as described previously.²⁰ Incubation in N-ethyl maleimide, iodoacetamide, and triethanolamine reduced nonspecific binding of the ³⁵S-uridine triphosphate-labeled probes. Negative controls used hybridization with the sense probe and pretreatment of slides with RNase A (Promega, Southampton, UK) before hybridization with the antisense probe. For autoradiography, slides were dipped into K-5 emulsion liquid (Ilford, Basildon, UK) and exposed at 4°C for 2 weeks in absolute darkness in a desiccated environment. The slides were developed (D-19 developing solution; Eastman Kodak Co, Rochester, NY), rinsed, and counterstained with Harris hematoxylin stain. Dense deposits of silver grains on autoradiographs were present over cells expressing eotaxin messenger (m)RNA. Slides were counted in duplicate, blinded to the patients' clinical status, by using an eyepiece graticule. The results were expressed as the number of positive cells per 400 cells. The coefficient of variability of the duplicate counts obtained from all slides was less than 5%.

Immunocytochemistry

The CD68 macrophage marker was examined by using the alkaline phosphatase-antialkaline phosphatase method, as previously described.²¹

RFD1⁺ and RFD7⁺ surface antigens were identified with the immunoperoxidase method (PAP)²² by using mAbs RFD1 (mouse IgM) and RFD7 (mouse IgG₁; Royal Free Hospital, School of Medicine, London, UK). These reagents have been extensively used by many workers to discriminate phenotypically distinct macrophage subsets^{3,4,6} and are commercially available from Serotec Ltd (Oxford, UK). All the other reagents were purchased from Dako (High Wycombe, Great Britain).

First, the slides were incubated with sodium azide in hydrogen peroxide solution to block endogenous peroxidase. Then they were incubated with each antibody (1:5) in PBS for 2 hours at room temperature. After washing in PBS, they were further incubated with a peroxidase-conjugated, rabbit anti-mouse Ig for 45 minutes and then incubated with PAP for 20 minutes. The color reaction was developed by incubation for 15 minutes with diaminobenzidine tetrahydrochloride. After a final wash, the slides were counterstained in hematoxylin stain for 5 minutes, dehydrated in graded alcohol, and mounted with coverslips by using xylene-based mounting media (BDH Chemicals, Poole, UK). Immunoperoxidase staining was read by using an Olympus light microscope. A positive reaction was recorded only in the presence of a dark brown granular staining of the cell membrane for RFD1 and brownish cytoplasmic staining for RFD7. Background staining was identified by comparison with negative control cytospin preparations on which the mAb was omitted. Positive controls were prepared by using sections of human tonsils.

Coexpression of RFD1 and RFD7 antigens on a single cell was identified by immunofluorescence incorporating Ig class-specific second-layer reagents conjugated with fluorescein isothiocyanate and tetraethylrhodamine isothiocyanate, respectively.⁴ A Zeiss fluorescence microscope, equipped with epi-illumination and barrier filters appropriate for fluorescein isothiocyanate and tetraethylrhodamine isothiocyanate, was used for recording RFD1⁺/RFD7⁺ cells. Background fluorescence was identified by using controls, as described above.

Combined immunocytochemistry-in situ hybridization

To identify the cell source of IL-10 and IL-12 mRNA, colocalization studies were performed on cytospin preparations obtained from 5 AA subjects and 4 NAA subjects, as previously described.¹⁹ Briefly,

cell phenotypes were first identified by immunocytochemistry with the alkaline phosphatase-antialkaline phosphatase technique and a phenotype-specific murine mAb. The mAbs used were directed against macrophages (CD68, clone No. EBM 11; Dako, Ely, UK) and human epithelial cells (cytokeratin, clone No. MNF 116, Dako). For negative controls, the primary antibody was replaced with either non-specific mouse Ig or TRIS-buffered saline solution. After developing with Fast Red for cell phenotype, in situ hybridization was performed by using ^{35}S -uridine triphosphate-labeled riboprobes specific for IL-10 and IL-12 mRNA. Cell counts were expressed as the mean percentage $\pm$ SEM of IL-10 and IL-12 mRNA $^{+}$ cells that coexpressed immunoreactivity for CD68 or cytokeratin-positive cells.

Statistical analysis

Data were analyzed with the aid of a commercially available statistical package (Minitab for Windows, Minitab Release 9.2; Minitab Inc, State College, Pa). The Mann-Whitney *U* test was then used for intergroup comparison (with the Bonferroni correction). A *P* value of less than .05 was accepted as statistically significant.

RESULTS

Study population

Although 50 subjects underwent sputum induction, only 31 of them gave an adequate specimen of sputum (viability, $>50\%$; squamous contamination, $<10\%$). Eighteen patients with stable asthma (8 with atopic and 10 with nonatopic asthma) and 13 control subjects (6 atopic and 7 nonatopic subjects) were eventually studied. There were no significant clinical differences between subjects who produced adequate sputum samples and those who did not or between the subjects with intrinsic asthma from the two centers.

Sputum induction was well tolerated by all subjects, none of whom experienced any significant untoward effects. The mean fall in FEV $_1$ (percent predicted) after sputum induction was 5% (range, 4%-10%) in NC subjects, 9% (range, 9%-15%) in AC subjects, 14% (range, 12%-20%) in AA subjects, and 18% (range, 17%-28%) in NAA subjects. In two asthmatic patients FEV $_1$ decreased by more than 20% but returned to normal values after inhalation of salbutamol at the end of the procedure (maximum after 20 minutes).

The asthmatic patients studied had relatively mild disease, and the severity of airflow obstruction and nonspecific bronchial hyperresponsiveness was similar in both the atopic and nonatopic asthma groups. Asthmatic subjects, regardless of atopic status, had elevated blood eosinophils compared with nonasthmatic control subjects ($P < .05$). The atopic subjects, irrespective of whether they had asthma, had clearly elevated median total serum IgE concentrations compared with the NAC subjects ($P < .05$, Table I).

Differential cell count in sputum of AA subjects, NAA subjects, AC subjects, and NC subjects

There were no significant differences in the percentages or the absolute numbers of macrophages counted by using Romanowsky staining between the asthmatic or control groups.

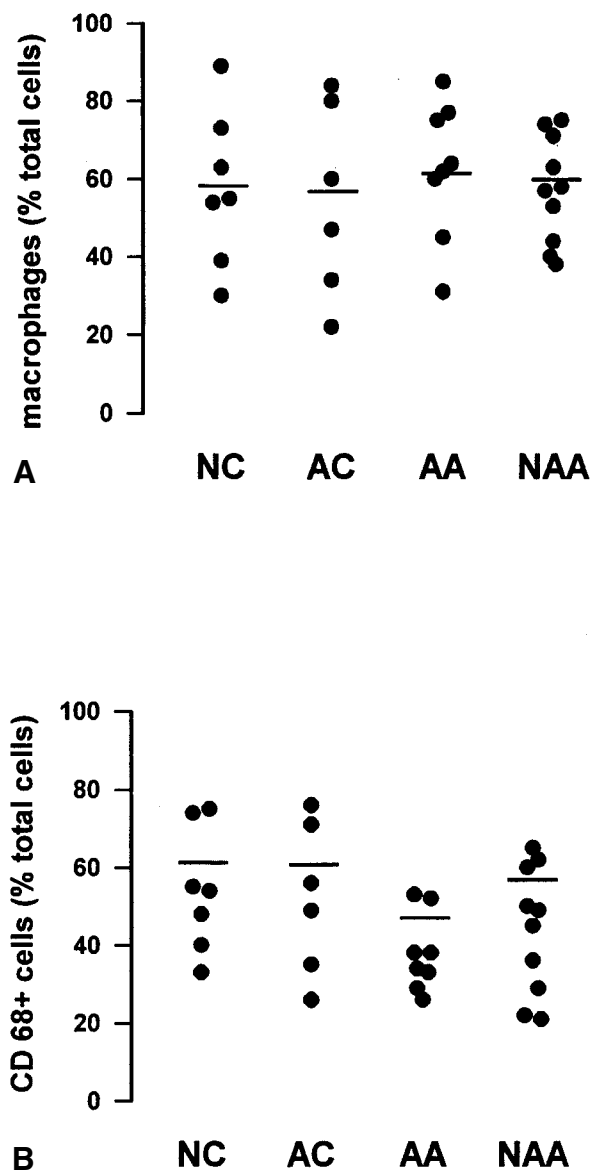


FIG 1. Percentage of macrophages in the sputum from AA subjects, NAA subjects, AC subjects, and NC subjects. **A**, Romanowsky staining; **B**, staining for the CD68 $^{+}$ marker. Comparison between groups was done by using the Mann-Whitney *U* test with the Bonferroni correction.

The median absolute numbers of macrophages were as follows: NAA subjects, 14×10^5 (range, $3.4\text{--}67 \times 10^5$); AA subjects, 8.1×10^5 (range, $0.7\text{--}63 \times 10^5$); AC subjects, 19×10^5 (range, $3.4\text{--}427 \times 10^5$); and NC subjects, 10.7×10^5 (range, $1.7\text{--}20.7 \times 10^5$). This was confirmed by counting percentages of CD68 $^{+}$ cells, which did not differ among the 4 groups (Fig 1).

In contrast, a significant increase in the proportion of eosinophils was evident in the asthmatic groups independently of atopic status compared with nonasthmatic control subjects ($P < .05$). The median percentages of sputum eosinophils were as follows: 6% (range, 2%-44%) in NAA subjects; 5% (range, 0%-17%) in AA subjects; 3%

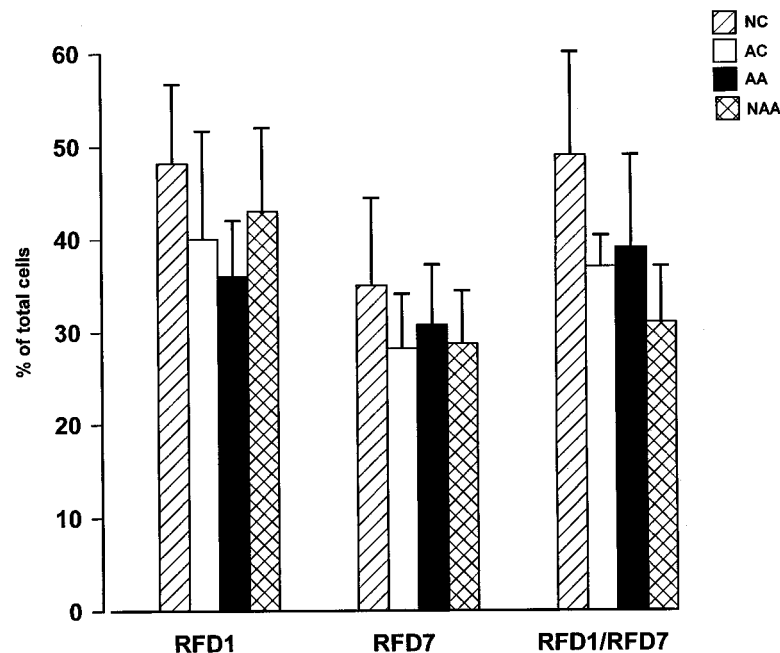


FIG 2. The distribution of macrophage subsets in sputum from AA subjects, NAA subjects, AC subjects, and NC subjects. The figures represent the median values for the proportion of stained cells with macrophage expressing the RFD1⁺ (n = 6), RFD7⁺ (n = 6), and RFD1/RFD7⁺ (n = 4) phenotypes.

TABLE I. Clinical characteristics of study subjects

Subjects	Age (y)	Sex (M:F)	FEV ₁ (% predicted)	Histamine PC ₂₀ (mg/mL)	IgE (IU/mL)	Peripheral blood eosinophils ($\times 10^9/L$)
NC (n = 7)	32 (22-51)	4:3	114 (104-140)	>16	17 (10-99)	0.1 (0.1-0.3)
AC (n = 6)	26 (24-33)	4:2	93 (80-122)	>16	83* (15-136)	0.15 (0.1-0.7)
AA (n = 8)	26 (20-33)	4:4	100 (86-120)	0.75 (0.096-4)	207† (114-632)	0.25‡ (0.2-0.9)
NAA (n = 10)	32 (32-56)	5:5	91.5§ (76-110)	2 (0.02-5)	17.5 (1-83)	0.3‡ (0.1-0.2)

Values are expressed as median (range).

* $P < .01$, AC subjects versus NC and NAA subjects.

† $P < .005$, AA subjects versus AC, NC, and NAA subjects.

‡ $P < .005$, AA and NAA subjects versus NC subjects.

§ $P < .05$, NAA subjects versus NC subjects.

(range, 0%-6%) in AC subjects; and 1% (range, 0%-3%) in NC subjects.

A significant increase in the percentage of epithelial cells was detected in asthmatic subjects (AA and NAA subjects) compared with control subjects ($P < .05$). The median percentages of the epithelial cells were as follows: 5% (range, 2%-13%) in NAA subjects; 4% (range, 1%-9%) in AA subjects; 1% (range, 0%-5%) in AC subjects; and 2% (range, 0%-6%) in NC subjects.

The median percentage of neutrophils was 60% (range, 8%-67%) in NAA subjects, 27% (range, 30%-74%) in AA subjects, 40% (range, 3%-75%) in AC subjects, and 37% (range, 31%-68%) in NC subjects, whereas the median percentage of lymphocytes was 0.5% (range, 0%-2%) in NAA subjects, 1% (range, 0%-5%) in AA subjects, 0.5% (range, 0%-2%) in AC subjects, and 0.7% (range, 0%-3%) in NC subjects. No significant differences were found in the proportions of neutrophils and lymphocytes among the 4 groups.

In all cases similar results were also obtained if numbers of each cell type were expressed as an absolute number.

Macrophage subpopulations in sputum of AA subjects, NAA subjects, AC subjects, and NC subjects

Immunocytochemistry did not reveal any significant differences in the numbers of RFD1⁺ cells between the asthmatic or control groups. Similarly, no significant differences were detected in the numbers of RFD7⁺ and RFD1⁺/RFD7⁺ cells among the 4 groups. The median percentage of RFD1⁺ cells was 55% (range, 10%-67%) in NAA subjects, 30.5% (range, 14%-37%) in AA subjects, 35% (range, 10%-75%) in AC subjects, and 45% (range, 20%-67%) in NC subjects, whereas the median percentage of RFD7⁺ cells was 28.5% (range, 16%-57%) in NAA subjects, 16% (range, 10%-40%) in AA subjects, 33% (range, 12%-44%) in AC subjects, and 32% (range,

13%-60%) in NC subjects. Finally, the median percentage of RFD1⁺/RFD7⁺ cells was 31% (range, 25%-37%) in NAA subjects, 39% (range, 37%-40%) in AA subjects, 37% (range, 30%-44%) in AC subjects, and 49% (range, 38%-60%) in NC subjects (Fig 2). Results were still the same if numbers of each macrophage phenotype were expressed as absolute numbers.

IL-10 and IL-12 expression in sputum of AA subjects, NAA subjects, AC subjects, and NC subjects

Analysis of the expression of IL-10 in the 4 groups of subjects demonstrated an increase in the numbers of cells encoding mRNA for IL-10 in AA subjects, which was significant compared with that found in NAA subjects and nonasthmatic control subjects ($P < .05$). No other differences were detected among the groups.

Similarly, the numbers of cells expressing mRNA for IL-12 were significantly elevated in the sputum of AA subjects compared with their NAA counterparts and control groups ($P < .05$). No significant differences were found between the control groups (Fig 3).

Colocalization of IL-10 and IL-12 mRNA to macrophages and epithelial cells

Combined in situ hybridization-immunocytochemistry was used on sputum cytospin preparations from random AA and NAA subjects to identify the phenotype of IL-10 and IL-12 mRNA⁺ cells. As shown in Table II, CD68⁺ cells comprised the larger proportion of IL-10 and IL-12 mRNA⁺ cells in the sputum of AA and NAA subjects. In each group cytokera-tin-positive epithelial cells constituted a smaller percentage of IL-10 and IL-12 mRNA⁺ cells.

Examples of double in situ hybridization and immunocytochemistry for IL-10 and IL-12 are shown in Fig 4.

IL-10 and IL-12 expression and clinical indices of pulmonary function

There were no significant correlations between cells expressing mRNA for either IL-10 or IL-12 and clinical markers of pulmonary function in both atopic and nonatopic asthma (FEV₁ or PC₂₀ to histamine, $P > .05$).

DISCUSSION

In this study we examined the numbers of macrophages and their subtypes, as well as the expression of two main macrophage-derived cytokines, IL-10 and IL-12, in the sputum of AA and NAA subjects and compared these findings with those of AC and NC subjects. We found no significant differences in the total numbers of macrophages counted by using either Romanowsky staining or the CD68 marker. Similarly, we did not detect any differences in either the inductive, phagocytic, or suppressor phenotype between the two types of asthma or between asthmatic and control subjects. However, we found increased expression of mRNA for IL-10 and IL-12

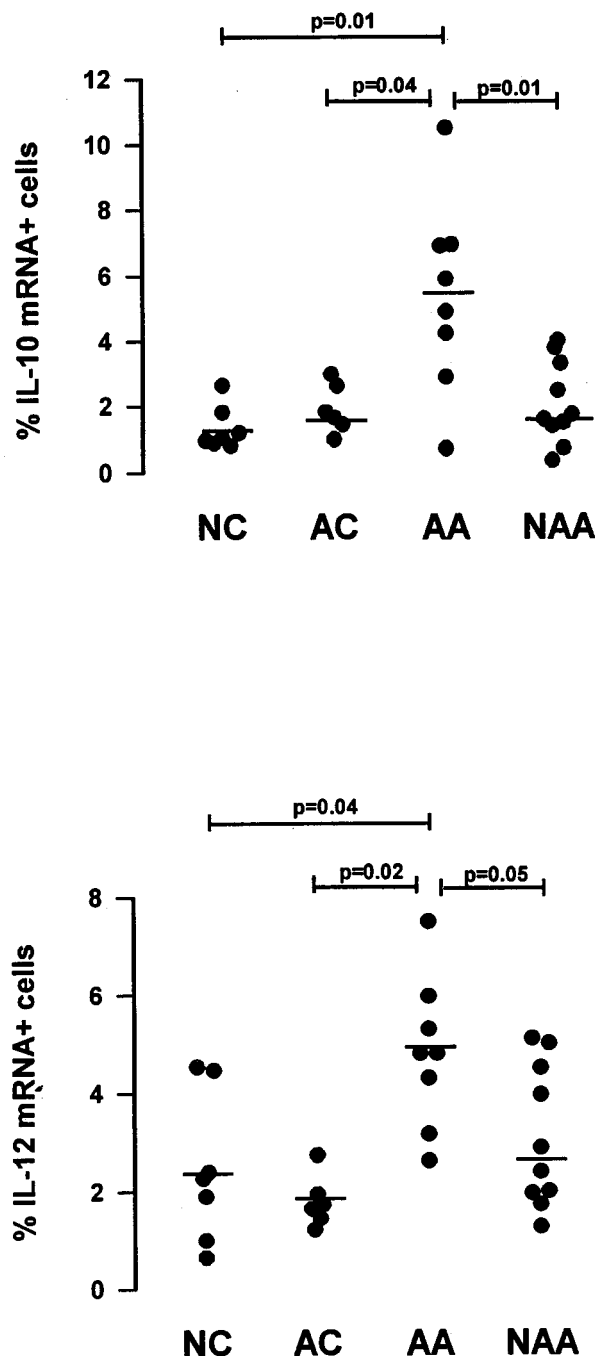


FIG 3. Percentage of cells expressing mRNA for IL-10 and IL-12 in the sputum from AA subjects, NAA subjects, AC subjects, and NC subjects. Comparison between groups was made by using the Mann-Whitney *U* test with the Bonferroni correction.

in the sputum of AA subjects compared with NAA subjects and nonasthmatic control subjects.

In the present study no significant differences in the numbers of macrophages in sputum were detected between atopic and NAA and control subjects. We were also unable to show any reduction in the proportion of

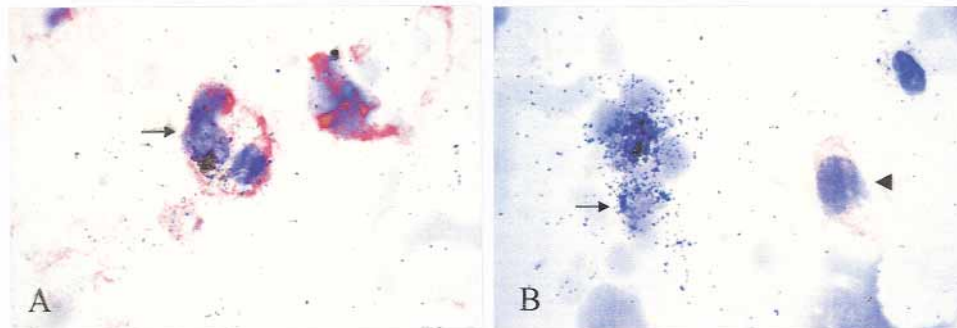


FIG 4. Simultaneous immunocytochemistry and in situ hybridization of sputum cytospin preparations using the alkaline phosphatase-antialkaline phosphatase method and ^{35}S -uridine triphosphate-labeled IL-10 and IL-12 riboprobes. **A**, Double immunocytochemistry-in situ hybridization with anti-CD68 (red staining) and the IL-10 riboprobe (black silver grains) shows a CD68⁺ macrophage expressing IL-10 mRNA (arrow). **B**, Double immunocytochemistry-in situ hybridization with anticytokeratin (red) and the IL-12 riboprobe shows that a cytokeratin-positive epithelial cell (arrowhead) does not express mRNA IL-12 (arrow; magnification 1000 $\times$).

TABLE II. IL-10 and IL-12 mRNA colocalized to CD68⁺ macrophages or to cytokeratin-positive epithelial cells in sputum from AA subjects (n = 5) and NAA subjects (n = 4)

	AA subjects		NAA subjects	
	IL-10 mRNA	IL-12 mRNA	IL-10 mRNA	IL-12 mRNA
CD68 ⁺ macrophages (%)	83.3 (80-84.6)	88.0 (75-90)	86.2 (62.5-88.9)	83.8 (77-90)
Cytokeratin-positive epithelial cells (%)	8.3 (0-20)	10.0 (0-16.7)	9.3 (6.7-12.5)	9.6 (10-14.2)

Results are expressed as median (range).

immunosuppressive macrophages, as has been previously described in bronchial biopsy specimens from AA subjects compared with normal lung tissue.²³⁻²⁵ It is clear that sputum and bronchial mucosa represent two different samples and consist of different cell types and proportions of cells. Therefore macrophages in sputum may reflect a different population than that of the bronchial mucosa or bronchoalveolar lavage fluid.²⁶ In a recent study no significant differences in the numbers of macrophages were found in the sputum of AA subjects compared with those found in the sputum of NC subjects.²⁶

We found diminished expression of IL-10 in the sputum of subjects with intrinsic compared with extrinsic asthma. The relative absence of IL-10 in this type of asthma may permit the continued expression of allergenic cytokines, such as IL-4 and IL-5, as well as proinflammatory cytokines, including IL-1, TNF- α , IL-6, and GM-CSF.⁷ Indeed, in all previous studies on intrinsic asthma, there was always a trend of increased expression of T_H2-type cytokines and C-C chemokines.^{8,27,28}

In human subjects the role of IL-10 in allergic inflammation is as yet undefined. Borish et al²⁹ found a diminished concentration of IL-10 in the bronchoalveolar lavage fluid of AA subjects compared with that of normal subjects,²⁹ whereas Robinson et al⁹ reported increased expression of IL-10 in the bronchoalveolar lavage fluid of baseline AA subjects, as well as after antigen challenge. In another study an elevated concentration of IL-10 was found in the serum of subjects with intrinsic asthma,³⁰ whereas Koning et al³¹ found decreased IL-10

mRNA expression by purified T cells of children with allergic and nonallergic asthma.

When we examined the expression of IL-12 in sputum, we found a significant increase in the numbers of cells encoding mRNA for IL-12 in the AA compared with NAA and control groups. The probe used was specific for the p40 subunit of the IL-12 molecule, which appears to be more specific for the presence of biologically active IL-12 than the p35 subunit.³²

IL-12 has a suppressive role in the development of allergic airway responses.³³ Studies in human subjects have shown that the IL-12 mRNA expression was downregulated in the bronchial mucosa of AA subjects but increased after steroid treatment¹⁰ or after successful grass pollen immunotherapy.³⁴

Therefore it seems possible that the increased presence of IL-12 in the airways of AA subjects would have resulted in a reduced T_H2-type response in atopic asthma and would be involved in the resolution of the asthmatic inflammation.³⁵

Because the production of IL-10 was elevated in AA subjects, it is possible that the increased production of IL-12 is due to a positive feedback mechanism.³⁶ Both IL-10 and IL-12 may play a role in the regulation of T_H1/T_H2 responses. It has been proposed that macrophages can enhance the T_H2 response by turning off their secretion of IL-10 and IL-12 or downregulate it by producing these cytokines. Our data may suggest a macrophage dysfunction or failure in producing IL-10 and IL-12 in intrinsic asthma. This may reflect differential cytokine production of macrophages within the local inflammatory environment. In turn, it may influence macrophage development

and therefore determine the airway macrophage phenotype in asthma. Moreover, differences in the numbers of alternative activated macrophages between the two forms of asthma cannot be excluded because this type of cell produces anti-inflammatory cytokines, such as IL-10 and IL-1 receptor antagonist.³⁷

It is clear that the clinical presentation of nonatopic asthma is different from that of atopic asthma, suggesting that there are at least some mechanistic differences between the two conditions, and it is not the first time that differences have been demonstrated between intrinsic and extrinsic asthma.³⁸⁻⁴⁰ In another study Folkard et al⁴¹ showed that the chemokine RANTES may be differentially produced by macrophages in subjects with atopic and nonatopic asthma, as determined by examining the bronchoalveolar lavage fluid.

In asthma CD68⁺ macrophages were the major source (83%) of the IL-10 and IL-12 mRNA⁺ cells, whereas cytokeratin-positive epithelial cells accounted for approximately 9% of these cells. No differences in the distribution of these cytokine-expressing cells were detected between the two types of asthma. The remainder of the IL-10 and IL-12 mRNA⁺ cells might be attributed to eosinophils and lymphocytes. Previous studies have shown that alveolar macrophages, T cells, and epithelial cells are the sources for IL-10 and IL-12 in the lung.^{9,11,12,29} It is worth noting that the great majority of cells present in sputum consist of macrophages, whereas T lymphocytes are detected in tiny numbers (1%).

When comparing clinical indices of pulmonary function with the expression of IL-10 and IL-12 in asthma, we found no significant correlations. This finding is in agreement with previous studies in AA subjects, which failed to find any association with IL-10 expression and alterations in lung function.^{9,34} This is possibly explained by the multiple actions of IL-10 and IL-12 on various cell types. Although not correlated to markers of asthma severity, expression of IL-10 and IL-12 may be important in the pathogenesis of asthma, irrespective of atopic status.

In conclusion, although we did not confirm differences in macrophage numbers or subtypes, we made the observation of increased IL-10 and IL-12 in atopic, but not in nonatopic, asthma. This may have relevance to continued airway inflammation in intrinsic asthma in the absence of an obvious external antigen stimulation.

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