

Bronchoalveolar lavage cytokine profiles in acute asthma and acute bronchiolitis

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Background: The pathogenetic basis for the relationship between acute bronchiolitis and asthma has not yet been completely elucidated.

Objective: The aim of this study was to compare these 2 diseases in terms of their patterns of airway cytokine response (T_H1 or T_H2).

Methods: By using a bronchoalveolar lavage (BAL) technique, this study investigated the cytokine levels of BAL fluid in children with acute asthma who had no identifiable respiratory syncytial virus (RSV) infection ($n = 18$) and in infants with acute bronchiolitis caused by RSV ($n = 22$). Comparisons were made with normal control subjects ($n = 14$). IFN- γ (T_H1) and IL-4 and IL-5 (T_H2) levels were measured in concentrated BAL fluids by means of ELISA.

Results: The IL-5 level ($P < .001$) and IL-5/IFN- γ ratio ($P < .001$) were significantly increased in the asthmatic group with no identifiable RSV infection and in the RSV-induced bronchiolitis group compared with values in the control group. When infants in the bronchiolitis group were divided into eosinophil-positive and eosinophil-negative subgroups, the eosinophil-positive subgroup had significantly increased IL-5 levels ($P < .001$) and IL-5/IFN- γ ratios ($P < .01$) compared with those in the control group, but similar cytokine responses were not induced in the eosinophil-negative subgroup. The percentage of BAL eosinophils correlated significantly with levels of BAL IL-5 in both the asthma group ($r = 0.80$, $P = .000$) and the bronchiolitis group ($r = 0.82$, $P = .000$).

Conclusions: These findings suggest that a subgroup of the RSV-induced bronchiolitis group results in a T_H2 -type response, and this could provide a valuable framework to explain the link between RSV-induced bronchiolitis and asthma. (J Allergy Clin Immunol 2003;112:64-71.)

Key words: Bronchiolitis, asthma, bronchoalveolar lavage, IFN- γ , IL-4, IL-5

Respiratory syncytial virus (RSV) is the most important cause of viral bronchiolitis in young children.¹ Infections with RSV have received much attention because of the clinical analogy, epidemiologic relationship, and similar immunologic phenomena in the airways, such as spe-

Abbreviations used

BAL: Bronchoalveolar lavage

FB: Flexible bronchoscopy

NPA: Nasopharyngeal aspirate

RSV: Respiratory syncytial virus

cific IgE production,² eosinophil activation,³ and generation of chemokines (macrophage inflammatory protein 1 α and RANTES)⁴ and adhesion molecules (soluble intercellular adhesion molecule 1)⁵ between RSV-induced bronchiolitis and childhood asthma. However, the pathogenetic basis for the relationship between acute bronchiolitis and asthma has not yet been completely elucidated.

Over the last decade, interest has been focused on the T_H1/T_H2 paradigm and its relevance to the pathogenesis of asthma. T_H1 responses, characterized by secretion of IFN- γ and IL-2, are involved in infections, whereas T_H2 responses are responsible for the inflammatory response in atopic disease by releasing IL-4 and IL-5.⁶ Indeed, an increase in T_H2 cytokine production has been found in asthma.^{7,8} However, studies to determine whether RSV infections enhance T_H2 responses in human subjects have yielded conflicting results.

Bronchoalveolar lavage (BAL) is a valuable research tool to assess cellular and noncellular components associated with inflammation in the lower respiratory tract.^{9,10} A growing number of studies have used research bronchoscopy with BAL to investigate the mechanisms of airway obstruction in children.¹¹ In asthma studies only adult data have been used in analyzing BAL cytokine data. However, extrapolating from adult data to the pediatric population will likely provide limited and potentially erroneous information. In bronchiolitis studies no BAL cytokine data are available currently, and cytokine results obtained from blood cultures or nasopharyngeal secretions are difficult to interpret because they do not necessarily reflect events in the lower respiratory tract. Moreover, no direct comparison between these 2 diseases in terms of cytokine pattern has been carried out thus far.

The aim of the present study was to compare acute bronchiolitis and acute asthma in terms of their pattern of cytokine response (T_H1 or T_H2) in the airways. To perform the study, we determined the levels of IFN- γ (T_H1) and IL-4 and IL-5 (T_H2) in the supernatants of BAL fluid from infants during their first episode of RSV-induced bronchiolitis and from children with acute asthma with-

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out identifiable RSV infection. These results were compared with those of control subjects who had no asthma and no identifiable airway infections.

METHODS

Study population

The study subjects consisted of 18 patients with acute asthma who had no identifiable RSV infection, 22 patients with acute RSV-induced bronchiolitis, and 14 control subjects, all of whom underwent flexible bronchoscopy (FB) with BAL from July 1998 through July 2000 either as part of an approved study protocol or for clinical indications. Nasopharyngeal aspirates (NPAs) were screened for viral infections by using indirect immunofluorescence for 6 respiratory viruses (RSV, adenovirus, influenza viruses A and B, and parainfluenza 1 and 3) in all 3 groups at the time of entry. Complete history taking, physical examination, and routine laboratory tests were performed for all subjects.

The asthma group consisted of 11 boys and 7 girls (median age, 5.5 years; range, 2.0-10.5 years) who were hospitalized because of an acute asthma exacerbation. All children in this group had been given a diagnosis of asthma on the basis of recurrent wheezing and dyspnea attacks with proven β_2 -agonist reversible airflow limitation.¹² The patients were atopic, which was defined as having at least one positive skin prick test response to 14 common airborne allergens in Korea, and had been using bronchodilators on demand during symptomatic periods. Ten of them had also received prophylactic therapy (ie, inhaled glucocorticoids or cromoglycate). The patients had no signs of viral or bacterial infections at the time of admission, as evaluated on the basis of clinical examination, chest radiographic films, serum C-reactive protein measurement, serologic testing for *Mycoplasma* species, and viral antigen tests in NPAs. Initially, 22 patients with asthma were enrolled; however, 4 of these patients had positive viral antigen test results and were excluded from this study. None in this group had a positive viral culture result in BAL fluid. During hospitalization, all subjects were medicated with regular nebulized albuterol (0.5 mL of a 0.5% solution diluted with 2 mL of saline every 4 to 6 hours) and systemic corticosteroids (2 mg/kg methylprednisolone or prednisolone).

The RSV-induced bronchiolitis group consisted of 12 boys and 10 girls (median age, 0.8 years; range, 0.4-1.8 years). All of these infants required hospitalization for treatment of their first episode of bronchiolitis. Bronchiolitis was defined as an acute respiratory illness with tachypnea, dyspnea, wheezing, and fine crepitation on auscultation.¹³ Infants with atopic dermatitis or a family background of atopy were excluded. RSV infection was ascertained by means of identification of viral antigens in NPAs,¹⁴ recovery of the virus in cell culture, or both. For 5 infants, BAL culture results were positive for RSV when the results of viral antigen tests had been negative.¹⁵ All infants in this group received conventional therapy with ribavirin (Virazole; ICN Pharmaceuticals, Costa Mesa, Calif) that did not include corticosteroids while they were hospitalized.

Fourteen children who were admitted with indications for FB (ie, lung mass, stridor, or localized emphysema) served as control subjects (median age, 6.4 years; range, 3.8-10.4 years). They had no allergic diseases and had never had asthma. They had no identifiable airway infections within 4 weeks of the study. In all subjects, both NPA antigen test and BAL fluid culture results were negative for the viruses.

Parents of both patients and control subjects provided informed consent, and the study was approved by the hospital ethics committee.

FB with BAL

FB with BAL was performed between 3 and 5 days after admission, with a 3.6-mm pediatric flexible bronchoscope (Olympus BF-

3C30; Olympus, New Hyde Park, NY) for children younger than 8 years and a 4.8-mm flexible bronchoscope (Olympus BF-1T10) for older children. Premedication consisted of intramuscular atropine sulfate (0.01-0.02 mg/kg and less than 1 mg) and intravenous midazolam (0.1-0.2 mg/kg). Subjects were also given an albuterol and lidocaine (titrated by weight) nebulizer treatment to anesthetize the upper airway. During bronchoscopy, oxygen and epinephrine were readily available, and patients had an intravenous infusion to provide venous access. Heart rate and transcutaneous SaO_2 values were monitored throughout the procedure and continued for 1 hour. After examination of the upper and lower airways, the tip of the bronchoscope was wedged into a segmental or subsegmental bronchus of the right middle lobe. Three 1-mL/kg aliquots of sterile nonbacteriostatic saline solution at room temperature were instilled through the instrumentation channel. Each aliquot was immediately aspirated into a sterile specimen container by using a wall suction pressure of 100 to 150 mm Hg. BAL fluid aspirated after each instillation was pooled together into a single specimen and immediately placed on ice.

Processing and analysis of the BAL fluid

The total amount of recovered fluid was measured, and the recovery was calculated as a percentage of the volume instilled. Pooled BAL fluid was placed into 2 aliquots. One aliquot of the pooled BAL fluid was submitted for viral and bacterial cultures to the hospital microbiology department. Standard culture techniques were used to isolate 6 respiratory viruses (RSV, adenovirus, influenza A and B, and parainfluenza 1 and 3) and common respiratory bacteria in BAL fluids from all 3 groups. The remainder of the pooled BAL fluid was taken to the laboratory for analysis of the cellular and fluid fractions. The fluid was centrifuged at 400g for 10 minutes at 4°C to separate fluid from cells. The total cell count was measured with a hemocytometer (Weber, Teddington, United Kingdom). Viability was assessed by using the trypan blue exclusion test. Differentials were obtained from cytospin (Shandon, Pittsburgh, Pa) slide preparations by using a May-Grunwald-Giemsa stain and by figuring a percentage of 400 cells. The cell-free fluid was frozen at -70°C until required for cytokine assay.

Concentration of the BAL fluid

Because of the large dilution effect of the instilled fluid and the expected low concentration of cytokines, BAL fluid was concentrated by using Centricon-10 concentrators (Amicon Co, Beverly, Mass) with a molecular weight cutoff of 10 kd, according to the manufacturer's recommendations. Briefly, three 2-mL aliquots of BAL fluid per patient were centrifuged at 4900g by using the Sorvall ultracentrifuge (Du Pont Co, Wilmington, Del). Several centrifuge cycles were performed. Three 100- μL volumes were retained, which corresponded to a 20-fold concentration.

Cytokine assays

IFN- γ , IL-4, and IL-5 levels were measured in the 20-fold concentrated BAL fluid by using a commercial ELISA kit from Endogen (Woburn, Mass). The kits were able to detect concentrations as low as 2 pg/mL IFN- γ , IL-4, and IL-5. All assays were run in duplicate, and the mean value was used for statistical analysis.

Subgrouping by eosinophils

The infants with RSV-induced bronchiolitis were divided into eosinophil-positive and eosinophil-negative subgroups on the basis of eosinophil percentages in BAL fluid. Eosinophil-positive infants ($n = 6$) were defined as those falling beyond 2 SDs of the normal mean ($0.11\% \pm 0.20\%$)¹⁶ for eosinophil percentages. The remaining infants with RSV-induced bronchiolitis were classified as the eosinophil-negative subgroup ($n = 16$).

TABLE I. Baseline characteristics of the subjects

	Asthma group (n = 18)	Bronchiolitis group			Control group (n = 14)
		Total (n = 22)	Eosinophil positive (n = 6)	Eosinophil negative (n = 16)	
Age (y)*	5.5† (2.0-10.5)	0.8‡ (0.4-1.8)	1.0 (0.4-1.4)	0.8 (0.5-1.8)	6.4 (3.8-10.4)
Sex (M:F)	11:7	12:10	4:2	8:8	9:5
Serum IgE (IU/mL)*	246†‡ (120-3312)	21‡ (5-106)	18 (7-34)	21 (5-106)	106 (30-259)
Blood eosinophils (/mm ³)*	503†‡ (150-1150)	129 (28-388)	210 (28-388)	120 (45-280)	146 (30-321)
Blood neutrophils (10 ³ /mm ³)*	3.2 (1.2-4.4)	3.4 (1.7-6.7)	3.6 (2.0-6.7)	3.3 (1.7-5.2)	2.6 (1.0-4.3)
FEV ₁ (% predicted)*§	80 (66-94)	—	—	—	92 (73-103)

*Data are expressed as median (range).

† $P < .001$ compared with the bronchiolitis group.‡ $P < .001$ compared with the control group.

§Data available in 6 children with asthma and 6 control subjects.

TABLE II. Bronchoalveolar cell profiles of the 3 groups of subjects*

	Asthma group (n = 18)	Bronchiolitis group			Control group (n = 14)
		Total (n = 22)	Eosinophil positive (n = 6)	Eosinophil negative (n = 16)	
Recovery, %	43.5 (35.0-45.0)	40.0 (35.0-50.0)	36.5 (29.8-51.0)	42.5 (36.0-49.5)	45.5 (38.8-52.0)
Viability, %	85.0 (78.8-90.0)	82.0 (76.0-87.3)	80.0 (73.3-83.0)	84.0 (76.0-87.8)	80.5 (87.0-91.3)
Total cells, 10 ⁴ /mL	24.5 (14.0-28.0)	32.0†‡ (20.0-58.0)	29.0 (10.0-54.5)	33.5 (25.8-74.5)	16.0 (9.1-20.5)
Macrophages, %	78.0‡ (73.8-83.3)	55.0‡§ (42.0-62.3)	42.0 (29.5-63.0)	55.0 (48.0-62.8)	86.5 (82.0-89.0)
Lymphocytes, %	10.0 (5.0-14.5)	5.5 (3.0-10.0)	10.0 (4.3-13.5)	5.0 (4.6-7.8)	8.0 (5.8-10.5)
Neutrophils, %	3.3 (2.0-10.0)	37.5‡§ (26.0-48.5)	41.5 (21.1-62.0)	36.8 (27.0-47.8)	2.3 (1.0-4.3)
Eosinophils, %	3.0‡¶ (2.0-8.2)	0 (0-1.6)	2.3 (1.7-3.1)	0 (0-0)	0 (0-0)
Epithelial cells, %	2.8 (1.8-3.6)	2.1 (1.5-3.3)	2.6 (1.9-3.8)	2.1 (1.5-3.0)	2.0 (1.0-3.1)

*Data are expressed as medians and interquartile ranges (25th-75th).

† $P < .01$ compared with the asthma group.‡ $P < .001$ compared with the control group.§ $P < .001$ compared with the asthma group.¶ $P < .001$ compared with the bronchiolitis group.

Statistical analysis

Cytokine levels were expressed per milliliter in concentrated BAL fluid and were presented as means $\pm$ SD. Cellularity data were expressed as the number per milliliter in unconcentrated BAL fluid (total cell count) or as a percentage of the total cell count (cell differential) and were presented as medians with interquartile ranges (25th-75th). Because the majority of the data were not normally distributed, nonparametric analysis was used. Screening of data for differences in cytokine and cell profiles among groups was performed by using the Kruskal-Wallis test. When significant differences were identified, individual groups were compared by using the Mann-Whitney *U* test. Correlation between values was assessed by calculating the Spearman correlation coefficient. A *P* value of less than .05 was considered statistically significant.

RESULTS

Subject characteristics

The characteristics of the subjects studied are shown in Table I. Comparisons of the 3 groups showed that children with RSV-induced bronchiolitis were significantly younger than subjects in the other 2 groups and that those with asthma had significantly higher serum IgE and blood eosinophil counts. The difference ($P < .001$) in serum IgE levels between the whole bronchiolitis group and the control group is likely to be due to age differ-

ences. No differences were found in subject characteristics between the eosinophil-positive and eosinophil-negative bronchiolitis subgroups.

Procedure-associated complications

FB with BAL was relatively well tolerated by all subjects. Minor procedure-associated complications occurred on 9 occasions, comprising transient hypoxia ($n = 5$), mild bronchospasm ($n = 2$), transient bradycardia ($n = 1$), and minor epistaxis ($n = 1$). These complications, however, did not preclude the completion of the procedure. Three children had a transitory hoarse cough, and 4 others had a low-grade fever (temperature up to 38°C) occurring within 12 hours of the procedure. These adverse events resolved spontaneously within 24 hours.

BAL cell profiles

The recovery of BAL fluid, the cell viability, the absolute number of total cells per milliliter, and the percentages for each cell type are shown in Table II. No differences were found in the recovery of BAL fluid or the cell viability between the groups. The total cell number was significantly higher in the whole bronchiolitis group than in the asthma group or the control group. With regard to cell types, there were significant differences in the per-

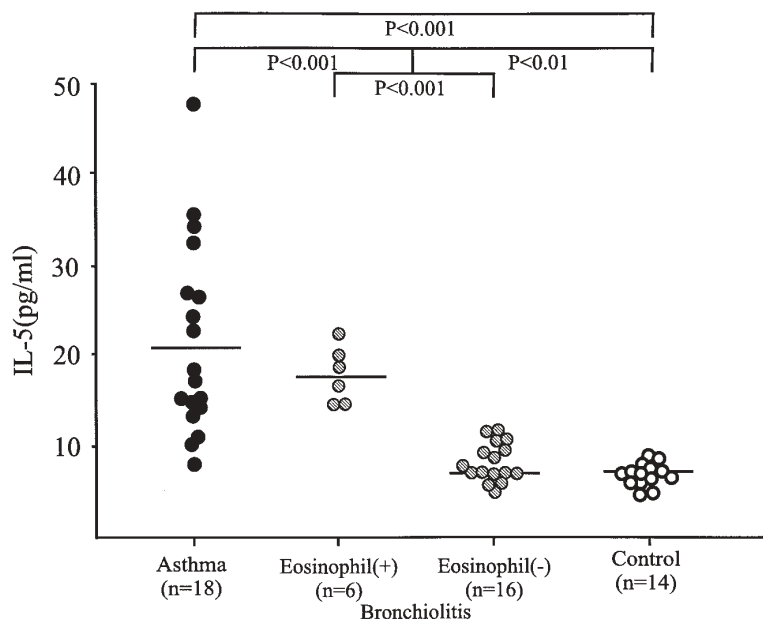


FIG 1. IL-5 levels in concentrated BAL fluid from the 3 groups. The bronchiolitis group was divided into eosinophil-positive and eosinophil-negative subgroups. Mean values are represented as horizontal bars.

centages of macrophages, neutrophils, and eosinophils among the 3 groups. The neutrophil percentages in the whole bronchiolitis group were significantly higher than those in the asthma group or the control group. On the other hand, the eosinophil percentages in the asthma group were significantly greater than those in the other 2 groups. The percentage of macrophages was lower in both the asthma and the whole bronchiolitis groups than in the control group in conjunction with a marked increase in the percentage of eosinophils or neutrophils. There were no differences in the percentages of other cell types beside the eosinophils between the eosinophil-positive and eosinophil-negative bronchiolitis subgroups.

BAL cytokine profiles

The levels of IL-5 in the concentrated BAL fluid of the 3 studied groups are shown in Fig 1. The IL-5 level was significantly higher in the asthma group (mean $\pm$ SD: 20.9 ± 7.9 pg/mL, $P < .001$) than in the control group (6.1 ± 1.3 pg/mL). The whole bronchiolitis group had a level of IL-5 (10.2 ± 5.0 pg/mL) intermediate between that of the asthma group and that of the control group. Its level in the eosinophil-positive subgroup (17.3 ± 3.1 pg/mL), comparable ($P = .69$) with that in the asthma group, was significantly higher ($P < .001$) than that in the control group, whereas the level in the eosinophil-negative subgroup (7.5 ± 2.0 pg/mL, $P = .13$) was not significantly different from that in the control group.

No difference in the IFN- γ level was found among the 3 groups (data not shown). IL-4 levels were at the level of detection in only 3 subjects among the asthma group (data not shown).

The IL-5/IFN- γ ratio in the concentrated BAL fluid of the 3 studied groups are shown in Fig 2, and its profile

was similar to that of the IL-5 level. The IL-5/IFN- γ ratios were significantly higher in both the asthma group (2.84 ± 0.86 , $P < .001$) and the whole bronchiolitis group (1.44 ± 0.83 , $P < .05$) than in the control group (0.84 ± 0.21). The ratio in the eosinophil-positive subgroup (2.52 ± 0.46), comparable ($P = .74$) with that in the asthma group, was significantly higher ($P < .01$) than that in the control group, whereas the ratio in the eosinophil-negative subgroup (1.04 ± 0.52 , $P = .41$) was not significantly different from that in the control group.

We investigated correlations between IL-5 levels and eosinophil percentages in the BAL fluids. The percentage of BAL eosinophils correlated significantly with the level of BAL IL-5 in the asthma group ($r = 0.80$, $P = .000$) and in the whole bronchiolitis group ($r = 0.82$, $P = .000$), respectively (Fig 3).

DISCUSSION

This is the first reported comparative study of T_H1/T_H2 cytokine responses between acute RSV-induced bronchiolitis and acute asthma. We have shown that the cytokine profile suggests a T_H2 -like response in RSV-induced bronchiolitis on the basis of increased IL-5 levels and IL-5/IFN- γ ratios in BAL fluid. We identified 2 different inflammatory subtypes in bronchiolitis: eosinophil-positive and eosinophil-negative subgroups. The former subgroup had increased IL-5 levels and IL-5/IFN- γ ratios. However, similar cytokine responses were not induced in the latter subgroup. Thus these BAL findings demonstrate that there is a heterogeneity of airway inflammation in bronchiolitis and that the eosinophil-positive subgroup and not the RSV-induced bronchiolitis per se results in a T_H2 -like response.

Asthma, rhinitis,
other respiratory
diseases

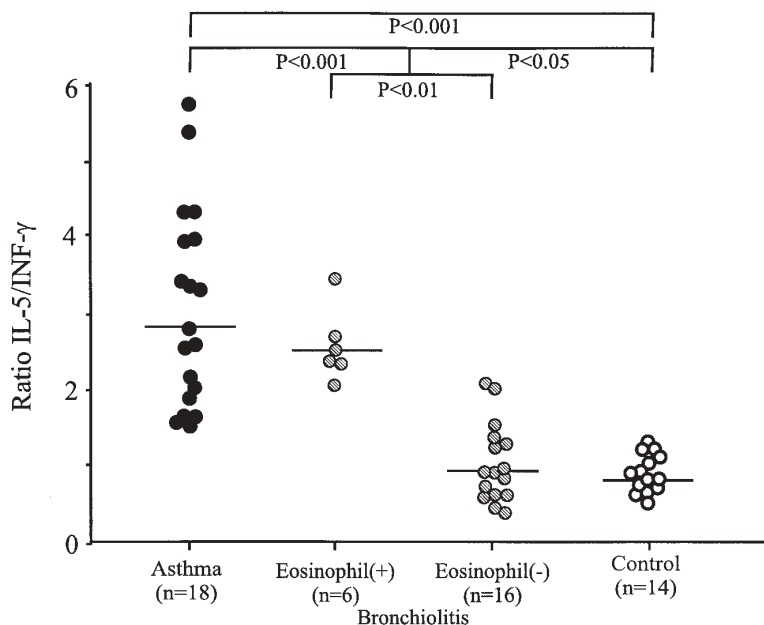


FIG 2. IL-5/INF- γ ratios in concentrated BAL fluid from the 3 groups. The bronchiolitis group was divided into eosinophil-positive and eosinophil-negative subgroups. Mean values are represented as horizontal bars.

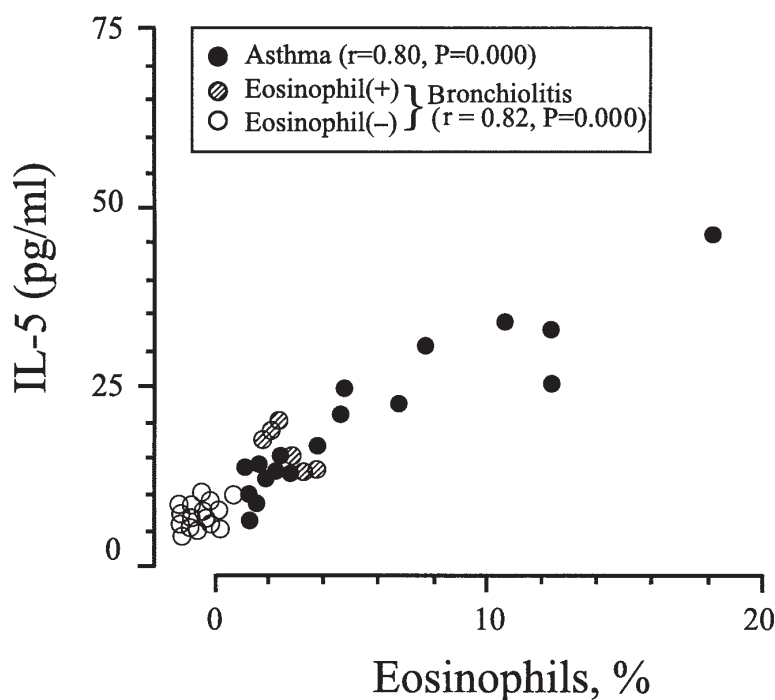


FIG 3. Correlation between eosinophil percentages and IL-5 levels in BAL fluid from the asthma group and the bronchiolitis group.

Technical difficulties and ethical considerations place serious limitations on the use of FB with BAL in the investigation of infants and children with airway diseases.¹⁷ There is currently no consensus among researchers as to whether the first BAL aliquot should be processed separately or be pooled with subsequent

aliquots. Most authors sample the first aliquot separately for cellular studies.^{18,19} However, evidence suggests that the first lavage aspirate would likely contain the highest cytokine concentrations²⁰ because the concentrations of acellular components progressively decrease in sequential aspirates. Hence it was considered acceptable to pool

all specimens, including the first BAL aliquot, for cytokine analysis in the present study. Wheezing children underwent bronchoscopy to identify potential anatomic causes, infectious causes, or both and to rule out aspiration by examining the BAL for lipid-laden macrophages, as well as the presence of a foreign body. Infants with bronchiolitis underwent bronchoscopy for the detection of RSV in infants with severe bronchiolitis before treatment with Ribavirin. However, this procedure posed an increased risk, including hypoxemia, for subjects in the presence of acute asthma and bronchiolitis. Hence it would seem prudent to continue to limit the use of the procedure, both for clinical and research purposes.

In adults with asthma, several studies have used BAL to examine various cytokine profiles.^{8,21} Expressions of mRNA for IL-4 and IL-5⁸ and levels of these cytokines²¹ were increased in BAL fluid from adult patients with atopic asthma. The IFN- γ level and its mRNA expression were generally normal in these BAL studies. Thus atopic asthma appears to be associated with a T_H2-type response. In children with asthma, no BAL cytokine study has been reported. In a peripheral blood study of atopic asthmatic children, Gemou-Engesaeth et al⁷ detected a high percentage of PBMCs expressing mRNA for IL-4 and IL-5 but a normal percentage for IFN- γ compared with that of control subjects. In the present study increased levels of IL-5 (but not of IL-4) and normal levels of IFN- γ were observed in the asthma group compared with in the control group, although IL-5 is subject to underestimation in consequence of systemic corticosteroid administration²² in the asthmatic group. The levels of IL-5 correlated significantly to the percentages of eosinophils. Thus our results suggest that IL-5, with its link to eosinophil inflammation, might play a key role in the pediatric asthmatic airway, although the contribution of IL-4 is less clear. Viral infections frequently cause exacerbations of asthma.²³ Although we attempted to identify viral infection with viral antigen tests and cultures for 6 respiratory viruses, some cases of asthma exacerbation might have been associated with other unexplored respiratory viruses, such as rhinovirus. We cannot therefore rule out a possibility that viral infection and allergy interacted to produce the cytokine response seen in this study.

Although IL-4 is an important regulator of IgE synthesis *in vitro*²⁴ and can actually be measured in BAL fluid,²¹ only 3 of the 18 asthmatic subjects in our study had detectable levels of IL-4 in their BAL fluid. An analogous observation that neither patients with asymptomatic nor those with symptomatic asthma had detectable levels of IL-4 in 10-fold concentrated BAL fluid was made by Broide et al,²⁵ although the assay in their study was sensitive only to 200 pg/mL. Several potential explanations for an inability to detect IL-4 in BAL fluid need to be considered, such as the binding of secreted IL-4 to target receptors as opposed to remaining free to be recovered in BAL fluid,²⁶ the degradation of IL-4 by proteolytic enzymes in BAL fluid during acute exacerbation, and the use of antiasthma medications (including corti-

costeroids), before the possibility that detectable amounts of IL-4 are not produced in the airways of children with symptomatic asthma can be confirmed.

Data on cytokine profiles in terms of T_H1/T_H2 type in RSV infections are controversial. Studies in mice have demonstrated that RSV infection can induce an alteration in the T_H1/T_H2 balance in favor of a T_H2 response with increased IL-5 production.²⁷ However, studies in human subjects have yielded conflicting results. Roman et al²⁸ observed an increased IL-4/IFN- γ ratio in the supernatant of cultured PBMCs and suggested a predominant T_H2-like response in infants with acute lower respiratory tract infection. On the contrary, van Schaik et al²⁹ observed that acute RSV-induced bronchiolitis resulted in a T_H1 response in nasopharyngeal secretions. However, results obtained from these studies do not necessarily reflect events in the lower respiratory tract. Our results indicate that IL-5 and the IL-5/IFN- γ ratio in BAL fluid are higher in the bronchiolitis group than in the control group, although their intensity is not so high as in the asthma group. It should be mentioned that our study lacks an adequate control group for the bronchiolitis group because of the age difference. There are no reference values available for IL-5 or IFN- γ concentrations of BAL fluid in relation to age. One study that examined age-related changes in cytokine release from PBMCs showed that the production of IL-5 was reduced in the 0- to 2-year age group when compared with that in the 5- to 10-year group.³⁰ If this ontogeny is relevant to BAL data, we believe that the increase in IL-5 levels observed in the bronchiolitis group cannot be explained simply by the age difference. No increase in IFN- γ level in the bronchiolitis group might have resulted from the immaturity of infants' immune system. Of particular note is the reduced capacity of neonatal T cells to produce IFN- γ .³¹ Another factor to be considered is the severity of illness. Aberle et al³² observed significantly lower levels of mRNA for IFN- γ in PBMCs in infants with severe RSV-induced disease than in those with a milder clinical course of illness.

Although the cellular origin of the cytokine production was not evaluated in the present study, the predominant cell source of IL-5 merits discussion. It is recognized that IL-5 in patients with atopic asthma is largely generated by CD4 T cells.³³ In contrast, studies on RSV infection of human mononuclear cells *in vitro* indicated that the IL-5-producing cells displayed the CD8 phenotype and showed no concomitant increase in IL-4 production, as would be expected from classical T_H2 cells.³⁴ This is in concordance with results obtained from a mouse model, which suggest a dominant role of CD8 cells in the pathophysiology of RSV-mediated disease.³⁵ However, there are conflicting results from another group demonstrating the predominance of CD4 cells in RSV-infected mice.³⁶

The results presented here support the concept of pathophysiologic heterogeneity of RSV-induced bronchiolitis. The 22 infants with RSV-induced bronchiolitis in the present study represent a superficially homogeneous group with a high ratio of neutrophils. However, 6 (27.3%) of 22 infants also had a higher ratio of eosinophils. Therefore 2

subgroups were defined from these infants: eosinophil-positive bronchiolitis and eosinophil-negative bronchiolitis. The cytokine response appeared to be different in the 2 subgroups. Whereas the eosinophil-positive bronchiolitis group had an increased IL-5 level and IL-5/IFN- γ ratio, similar cytokine responses were not induced in the eosinophil-negative bronchiolitis group. These findings suggest that the eosinophil-positive subgroup is associated with a skewed immune response toward T_H2. The low IL-5 level in the eosinophil-negative bronchiolitis subgroup offset the high IL-5 level in the eosinophil-positive bronchiolitis subgroup and thus led to the relatively low IL-5 level in the whole bronchiolitis group compared with that in the asthma group.

It remains unclear what differentiates the patients who recover completely from bronchiolitis from those who go on to have asthma in the future. The increased IL-5 level and IL-5/IFN- γ ratio in the eosinophil-positive RSV-induced bronchiolitis subgroup suggest a T_H2-type response. Childhood asthma was also found to induce a strong T_H2 immune response with a similar cytokine imbalance. In the present study we were unable to address whether RSV actually induced the altered immune responses or rather merely selected those infants with a predisposition for the T_H2-type response. Differential patterns of cells (eosinophils)^{37,38} and cytokines^{39,40} have been noted in infants with bronchiolitis who go on to have persistent wheezing and asthma. We speculate that the infants with eosinophil-positive bronchiolitis might represent the patients who go on to have asthma in the future. A follow-up study is needed for this subgroup of infants with eosinophil-positive RSV-induced bronchiolitis to determine whether they later have asthma. Although further confirmation is required, these BAL findings suggest that it is a subgroup, indeed a heterogeneity, of RSV-induced bronchiolitis rather than the RSV-induced bronchiolitis per se that results in the T_H2-type response. This hypothesis could provide a valuable framework to explain the link between RSV-induced bronchiolitis and asthma.

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Asthma, rhinitis,
other respiratory
diseases

Correction

With regard to the April 2003 article entitled "Fexofenadine is efficacious and safe in children (aged 6-11 years) with seasonal allergic rhinitis" (2003;111:763-9), the third paragraph of the Discussion section (the second paragraph on page 768) should have appeared as follows:

A number of other studies have examined the effects of antihistamines in children with SAR.¹³⁻¹⁹ However, only one of these studies, which investigated the effects of cetirizine (5 and 10 mg once daily [qd]) in a randomized, double-blind, placebo-controlled trial of children with SAR aged 6-11 years, used symptom scoring similar to that used in adult studies.¹³ This study showed that cetirizine (10 mg qd) is effective in the treatment of SAR (n = 209), as assessed by measurement of the TSS, in 2 out of 4 weeks; the first week of treatment did not reach statistical significance. In this study the TSS corresponded to the sum of the individual symptoms of sneezing, nasal discharge, itchy eyes, itchy nose or mouth, and conjunctivitis but excluded nasal congestion. Assessment of individual symptoms showed that cetirizine (10 mg qd) demonstrated a significant decrease in the symptoms of itchy eye and itchy nose or mouth. There were no statistically significant differences from placebo for nasal discharge, conjunctivitis, sneezing, or nasal congestion with cetirizine (10 mg).