

Different profiles of T-cell IFN- γ and IL-12 in allergen-induced early and dual responders with asthma

Makoto Yoshida, MD, Richard M. Watson, BSc, Tracy Rerecich, BSc, and Paul M. O'Byrne, MB, FRCP(C) *Hamilton, Ontario, Canada*

Background: IFN- γ and IL-12 are anti-inflammatory cytokines released from various cells, including T cells. Allergen inhalation by atopic subjects with asthma results in 2 bronchoconstrictor phenotypes, termed *isolated early* and *dual responders*. Persistence of allergen-induced airway response and inflammation is a distinctive feature of dual responders.

Objective: To evaluate the roles of IFN- γ and IL-12 in resolving allergen-induced airway inflammation by comparing T lymphocytes (CD4⁺ and CD8⁺ cells) producing these cytokines in isolated early and dual responders.

Methods: Twenty-four subjects with asthma (12 isolated early and 12 dual responders) were challenged with inhaled allergen. Peripheral blood and induced sputum were taken before and 1 day, 3 days, and 7 days after challenge. Frequency of IFN- γ , IL-12, IL-4, and IL-13 producing CD4⁺ and CD8⁺ cells was assessed by using flow cytometry.

Results: After allergen, both CD4⁺ and CD8⁺ IFN- γ positive cells in peripheral blood significantly decreased in dual responders only, whereas CD4⁺ and CD8⁺ IFN- γ positive cells in induced sputum significantly increased in isolated early responders only. By contrast, IL-12 positive cells in peripheral blood significantly increased after allergen challenge only in isolated early responders. The ratio of CD4⁺ and CD8⁺ IL-4/IFN- γ positive cells in peripheral blood significantly decreased in isolated early responders by 3 days and had recovered by 7 days.

Conclusion: These results suggest that contrasting profiles of IFN- γ and IL-12 production may be responsible for different time courses of allergen-induced airway responses between isolated early and dual responders. (*J Allergy Clin Immunol* 2005;115:1004-9.)

Key words: Asthma, allergen challenge, T_H1 and T_H2 cytokines

Allergen inhalation provokes rapid bronchoconstriction in atopic subjects with asthma. This reaction occurs within half an hour, peaks within 2 hours after the exposure, and is called the *allergen-induced early asthmatic response*.¹ In some subjects with asthma, slowly progres-

Abbreviations used

DR: Dual responder

IER: Isolated early responder

sive and persistent bronchoconstriction begins 3 to 7 hours after allergen inhalation,¹ the *allergen-induced late asthmatic response*. The late asthmatic response is associated with airway hyperresponsiveness and eosinophilic airway inflammation,² which can persist for as long as 1 week.

T_H lymphocytes help orchestrate allergen-induced airways inflammation via production of specific cytokines. Among these, T_H2 cytokines such as IL-4, IL-5, and IL-13 are thought to play a pivotal role.³ There is evidence that T_H1 cytokines such as IFN- γ and IL-12 are capable of counteracting T_H2 responses and vice versa.⁴⁻⁶ This has suggested that an imbalance between T_H1 and T_H2 lymphocytes and their respective cytokine production is thought to underlie allergic responses. Furthermore, we have recently reported that treatment with IFN- γ protein reversed ongoing allergic airway responses in IFN- γ -deficient mice.⁷ Although less is known about IFN- γ and IL-12 in asthma, results from one study did not demonstrate any clinical benefit in subjects with severe asthma from treatment with IFN- γ ,⁸ whereas another could not demonstrate attenuation of allergen-induced late responses after treatment with IL-12.⁹

The objective of the current study was to measure T lymphocytes producing IFN- γ and IL-12 in peripheral blood and induced sputum from atopic subjects with asthma by flow cytometry before and after allergen inhalation and to compare the changes between isolated early responders (IERs) and dual responders (DRs) in relation to airway inflammation and airway responsiveness. We hypothesized that the profiles of IFN- γ and IL-12 would be different between IER and DR, which may relate to different time course of allergic responses in these groups of subjects with asthma. We also investigated the profiles of IL-4 and IL-13 as important T_H2 cytokines.

METHODS

Subjects

Twenty-four subjects with mild atopic asthma (12 DR and 12 IER) who fulfilled the American Thoracic Society criteria for asthma¹⁰ were studied (see Table E1 in the Journal's Online Repository at www.mosby.com/jaci). All subjects gave signed consent before

From the Firestone Institute for Respiratory Health, Department of Medicine, McMaster University.

Supported by an operating grant from the Canadian Institutes for Health Research.

Received for publication November 3, 2004; revised February 1, 2005; accepted for publication February 7, 2005.

Available online March 23, 2005.

Reprint requests: Paul M. O'Byrne, MB, Department of Medicine, McMaster University, Health Sciences Centre, Room 3W10, 1200 Main St W, Hamilton, Ontario L8N 3Z5, Canada. E-mail: obynep@mcmaster.ca.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.02.003

participating in the study, which was approved by the Ethics Committee of McMaster University Health Sciences Center. All were nonsmokers, and none had a respiratory infection for at least 4 weeks before the study. Subjects required only intermittent use of inhaled β_2 -agonist, with baseline FEV₁ values >70% predicted. Medication was withheld for at least 8 hours before each study day. Atopic status was defined by positive skin prick test responses. Subjects were classified as DR if they developed both early and late asthmatic responses as defined by a greater than 15% drop in FEV₁ from baseline, or as IER if they developed only an early fall in FEV₁ greater than 15% from baseline.

Study design

Subjects attended the laboratory during 2 study periods. The first period was a screening period to document either an early or a dual airway response to inhaled allergen. The second study period lasted for 9 days. On the first day of this period, preallergen blood samples were obtained. This was followed by spirometry, methacholine inhalation challenge, and induced sputum. On the next day, subjects underwent an allergen challenge. Spirometry was measured until 7 hours after allergen inhalation to evaluate the early and late asthmatic responses. Sputum samples were obtained 7 hours after allergen. The third, fourth, and fifth visits to the laboratory occurred 24 hours, 3 days, and 7 days after allergen inhalation, respectively. At these visits, postallergen blood samples were obtained. This was followed by spirometry, methacholine inhalation challenge, and sputum sampling.

Methacholine inhalation challenge

Methacholine inhalation challenge was performed by using the method described by Cockcroft et al¹¹ (see [Methods](#) in the Online Repository at www.mosby.com/jaci). The concentration of methacholine required to achieve a decrease in FEV₁ of 20% (methacholine PC₂₀) was calculated through linear interpolation of percent fall in FEV₁ against the log-transformed methacholine concentration.

Allergen inhalation challenge

Allergen challenge was performed according to the method described by O'Byrne et al¹ (see [Methods](#) in the Online Repository at www.mosby.com/jaci). The early response was taken to be the largest percent fall in FEV₁ within 2 hours after allergen inhalation, and the late response was taken to be the largest percent fall in FEV₁ in the period beginning 3 hours and ending 7 hours after allergen inhalation.

Sputum analysis

Sputum was induced and processed according to the method described by Pizzichini et al¹² (see [Methods](#) in the Online Repository at www.mosby.com/jaci). Differential cell counts were obtained from the mean of 2 slides with 400 cells counted per slide stained with Diff-Quik (American Scientific Products, McGaw Park, Ill).

mAbs

All mAbs were purchased from PharMingen (San Diego, Calif). Phycoerythrin-conjugated antihuman IFN- γ mAb, B27 (mouse IgG₁), antihuman IL-4 mAb, 8D4-8 (mouse IgG₁), isotype control mouse IgG₁, antihuman IL-12 (p70) mAb, 20C2 (rat IgG₁) antihuman IL-13 mAb, and isotype control rat IgG₁ were used for the study. In nonpermeabilized cells, positive staining for IFN- γ decreased to 20% of the paired sample with permeabilization, showing that approximately 80% of the positive signal was of intracellular origin. Fluorescein isothiocyanate-conjugated anti-CD4 mAb, RPA-T4 (mouse IgG₁, κ), and CyChrome (BD Biosciences, Mississauga, Ontario, Canada)-conjugated anti-CD8 mAb, RPA-T8 (mouse IgG₁, κ) were used for identifying each T-cell subset. Percentages of CD4⁺

and CD8⁺ positive lymphocytes in PBMC was not altered by treatment with 0.1% dithiothreitol compared with control PBMC without treatment (data not shown).

Cell cultures

Isolation and cultures of both peripheral blood mononuclear and sputum cells were performed as described (see [Methods](#) in the Online Repository at www.mosby.com/jaci).

Staining

Cells were washed in simple PBS and resuspended at a density of 1×10^6 cells in 100 μ L PBS with 0.1% sodium azide and 5% normal mouse serum (Sigma, St Louis, Mo), incubated for 10 minutes, followed by surface staining with anti-CD4 mAb and anti-CD8 mAb for 30 minutes in the dark. For intracellular cytokine staining, the cells were washed once in simple PBS and then fixed in 100 μ L fixation buffer (Caltag, Burlingame, Calif) containing 4% paraformaldehyde for 20 minutes. After an additional wash with simple PBS, cells were resuspended in 100 μ L permeabilization buffer containing 0.1% saponin (Caltag) with 5% normal mouse serum or normal rat serum (Sigma) for 10 minutes, and then added with anti-IFN- γ mAb, anti-IL-4 mAb, isotype control mouse IgG₁, anti-IL-12 mAb, anti-IL-13 mAb, or isotype control rat IgG₁ for 30 minutes in the dark. After a final wash in simple PBS, cells were resuspended in PBS with 1% paraformaldehyde and kept in the dark at 4°C until flow-cytometric evaluation.

Flow cytometric analysis

A FACScan flow cytometer (Becton Dickinson, San Jose, Calif) equipped with a 15-mA argon ion laser and filter settings for fluorescein isothiocyanate (530 nm; FL-1), phycoerythrin (585 nm; FL-2), and CyChrome (650 nm; FL-3) was used. The detailed methods are described in the Online Repository (www.mosby.com/jaci). The frequency of true-positive cells was obtained by subtracting the value of isotype control from the value of sample stained with anti-IFN- γ , anti-IL-4, anti-IL-12, or anti-IL-13 mAb (see [Fig E1](#) in the Online Repository at www.mosby.com/jaci).

Statistical analysis

Statistica software, version 5 (StatSoft, Inc, Tulsa, Okla), was used to analyze the data. Data were expressed as the means $\pm$ SEMs. PC₂₀ methacholine measurements were log₂-transformed to normalize the data and are reported as geometric means and geometric SEM. Comparisons between IER and DR were made by using 2-factor repeated-measures ANOVA to analyze the effect of the 2 independent variables, types of asthmatic responses, and time on the outcome variables. Appropriate post hoc testing was performed by using the Duncan test to assess for significant effects while controlling for multiple comparisons. All comparisons were 2-tailed, and *P* values <.05 were considered significant.

RESULTS

Bronchoconstrictor responses

The mean maximal percent falls in FEV₁ during the early response were $27.9\% \pm 7.1\%$ in IER and $29.3\% \pm 6.7\%$ in DR (*P* = .683). The maximal percent falls in FEV₁ during the late response were $7.1\% \pm 5.0\%$ in IER and $22.7\% \pm 6.5\%$ in DR (*P* < .01; [Fig 1, A](#)).

Airway hyperresponsiveness

Methacholine airway hyperresponsiveness developed in both DR and IER 24 hours after allergen challenge.

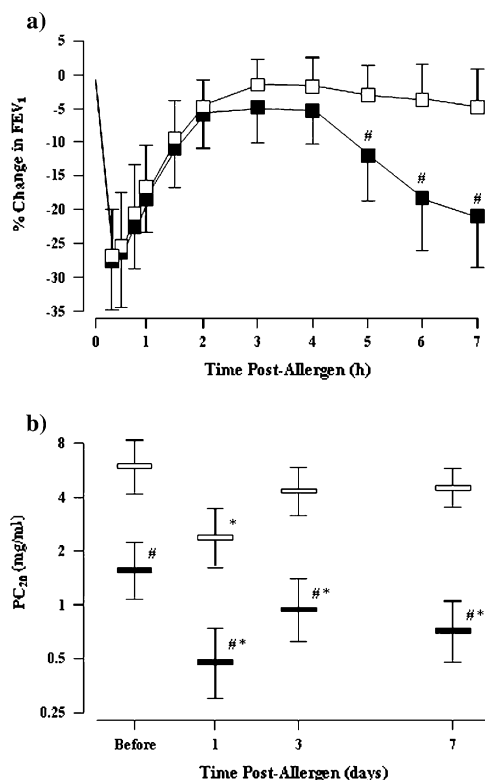


FIG 1. Time course of the decline in FEV₁ after allergen challenge. Methacholine PC₂₀ before and after allergen challenge. Open symbols and solid symbols indicate data obtained from IER and DR, respectively. * $P < .05$ compared with the baseline values before allergen challenge. † $P < .05$ compared with IER.

However, airway responsiveness recovered in IER by 3 days after the challenge, whereas airway hyperresponsiveness persisted at least for 7 days in DR. There were significant differences in PC₂₀ between IER and DR at all time points investigated ($P < .05$ before and 24 hours after the challenge; $P < .01$, 3 days after and 7 days after the challenge; Fig 1, B).

Changes in cell differentials in peripheral blood and induced sputum by allergen challenge

There were no differences in eosinophils in either peripheral blood or induced sputum between the groups before allergen challenge. After the challenge, peripheral blood eosinophils significantly increased in DR by 3 days from $4.31\% \pm 0.33\%$ before to $6.39\% \pm 0.43\%$ ($P < .01$), but not in IER. In induced sputum, eosinophils significantly increased in both groups at 24 hours after allergen from $1.04\% \pm 0.32\%$ to $5.63\% \pm 1.23\%$ in IER ($P < .05$) and from $1.27\% \pm 0.41\%$ to $13.25\% \pm 3.76\%$ in DR ($P < .01$; Table I). However, significant increases in sputum eosinophils at 7 hours and at 3 days were seen only in DR. There were no significant differences in peripheral blood or induced sputum CD4⁺ and CD8⁺ cells in any time points in either group (Table II). Also, the percentages of

CD4⁺ and CD8⁺ cells were not changed by cell stimulation with phorbol 12-myristate 13-acetate and ionomycin compared with unstimulated controls (data not shown).

Changes in IFN- γ -producing cells after allergen challenge

Before the allergen challenge, there were no significant differences in IFN- γ -producing CD4⁺ and CD8⁺ lymphocytes in peripheral blood and induced sputum between IER and DR.

In DR, CD4⁺ and CD8⁺ IFN- γ -positive cells significantly decreased after allergen in peripheral blood ($P < .05$; Fig 2). These decreases persisted for 3 days in CD4⁺ and for 7 days in CD8⁺ lymphocytes. By contrast, in IER, CD4⁺ and CD8⁺ IFN- γ -positive lymphocytes were unchanged or slightly but not significantly increased (Fig 2). The only exception was a small significant decrease in CD8⁺ cells from peripheral blood 3 days after allergen. There were significant differences in IFN- γ -positive cells after allergen between IER and DR at 1 day for both CD4⁺ and CD8⁺ cells and at 3 days for CD4⁺ cells.

In induced sputum, both CD4⁺ and CD8⁺ IFN- γ -positive cells significantly increased 24 hours after allergen challenge in IER, returning to the baseline value by 3 days, whereas no significant differences were seen in DR (Fig 2). There were significant differences in IFN- γ -positive cells after allergen between IER and DR at 1 day for CD4⁺ cells.

Changes in IL-12-producing cells after allergen challenge

There were no differences in CD4⁺ and CD8⁺ IL-12 positive cells between IER and DR before allergen challenge. CD4⁺ IL-12 positive cells significantly increased in IER ($P < .05$), from $0.25\% \pm 0.16\%$ at baseline to $0.56\% \pm 0.19\%$ ($P < .05$) at 1 day after allergen. This increase was significantly different compared with DR and had returned to the baseline value by 3 days. In addition, CD8⁺ IL-12 positive cells were also significantly higher in IER when compared with DR at 1 day after allergen ($P = .04$).

Changes in IL-4-producing cells before and after allergen challenge

There were no differences in IL-4 positive cells between IER and DR before allergen challenge in peripheral blood or induced sputum. After allergen challenge, CD4⁺ IL-4 positive cells significantly decreased in peripheral blood from IER from $2.79\% \pm 0.76\%$ to $1.05\% \pm 0.84\%$ ($P < .05$) and CD8⁺ IL-4 positive cells from $2.60\% \pm 0.63\%$ at baseline to $1.88\% \pm 0.25\%$ ($P < .05$) at 3 days, but not in DR. This change had recovered by 7 days after allergen. There were no significant changes in IL-4 positive cells from induced sputum.

Changes in IL-13-producing cells before and after allergen challenge

CD4⁺ and CD8⁺ IL-13 positive cells were not different between IER and DR before allergen challenge. CD4⁺

TABLE I. Allergen-induced changes in sputum inflammatory cells†

		Baseline	7 h Postallergen	24 h Postallergen	3 d Postallergen	7 d Postallergen
Total sputum cell count (10 ⁶ /mL)	IER	0.79 ± 0.22	1.15 ± 0.39	1.95 ± 0.63*	1.10 ± 0.26	0.76 ± 0.15
	DR	0.78 ± 0.34	1.30 ± 0.33	1.04 ± 0.34	0.61 ± 0.18	0.46 ± 0.12
	IER vs DR	<i>P</i> = .51	<i>P</i> = .63	<i>P</i> = .10	<i>P</i> = .31	<i>P</i> = .60
Eosinophils (%)	IER	1.04 ± 0.32	5.63 ± 1.23	6.67 ± 2.01*	2.30 ± 0.68	1.43 ± 0.34
	DR	1.27 ± 0.41	13.25 ± 3.76*	13.23 ± 4.37*	8.38 ± 2.79*	2.25 ± 0.65
	IER vs DR	<i>P</i> = .85	<i>P</i> = .22	<i>P</i> = .01	<i>P</i> = .01	<i>P</i> = .61
Macrophages (%)	IER	59.74 ± 5.30	35.49 ± 6.75	47.73 ± 5.73	55.78 ± 6.57	58.28 ± 6.10
	DR	52.62 ± 6.58	23.11 ± 3.90	44.05 ± 5.32	52.15 ± 4.59	48.68 ± 6.96
	IER vs DR	<i>P</i> = .89	<i>P</i> = .18	<i>P</i> = .90	<i>P</i> = .79	<i>P</i> = .49
Neutrophils (%)	IER	39.01 ± 5.35	58.54 ± 7.42*	45.46 ± 5.59	41.02 ± 6.77	39.66 ± 6.25
	DR	46.04 ± 6.60	63.61 ± 3.17*	44.49 ± 5.60	40.03 ± 5.59	48.89 ± 7.03
	IER vs DR	<i>P</i> = .92	<i>P</i> = .28	<i>P</i> = .72	<i>P</i> = .62	<i>P</i> = .53
Lymphocytes (%)	IER	0.73 ± 0.43	0.13 ± 0.11*	0.55 ± 0.30	0.40 ± 0.30	0.63 ± 0.23
	DR	0.11 ± 0.10	0.22 ± 0.16*	0.20 ± 0.15	0.21 ± 0.13	0.11 ± 0.09
	IER vs DR	<i>P</i> = .04	<i>P</i> = .74	<i>P</i> = .66	<i>P</i> = .71	<i>P</i> = .12

**P* < .05 allergen-induced change from baseline.

†Values are presented as means ± SEMs.

TABLE II. Allergen-induced changes in T-cell CD4⁺/CD8⁺ subsets*

Sample	Subset	Response	Baseline	24 h Postallergen	3 d Postallergen	7 d Postallergen
PBMC	CD4 ⁺ cells (%)	IER	49.41 ± 2.10	49.76 ± 1.65	49.78 ± 1.64	48.36 ± 1.69
		DR	51.69 ± 2.24	51.18 ± 1.74	50.12 ± 1.56	50.05 ± 2.14
		IER vs DR	<i>P</i> = .48	<i>P</i> = .58	<i>P</i> = .89	<i>P</i> = .56
PBMC	CD8 ⁺ cells (%)	IER	24.67 ± 1.61	24.09 ± 1.86	24.22 ± 1.50	23.49 ± 1.64
		DR	22.79 ± 1.41	22.09 ± 1.57	21.75 ± 1.26	22.73 ± 1.48
		IER vs DR	<i>P</i> = .41	<i>P</i> = .44	<i>P</i> = .24	<i>P</i> = .75
Induced sputum	CD4 ⁺ cells (%)	IER	41.30 ± 3.15	49.45 ± 5.50	40.49 ± 6.55	41.63 ± 3.04
		DR	31.88 ± 4.27	44.57 ± 5.26	33.97 ± 3.69	27.88 ± 5.03
		IER vs DR	<i>P</i> = .17	<i>P</i> = .61	<i>P</i> = .49	<i>P</i> = .08
Induced sputum	CD8 ⁺ cells (%)	IER	15.96 ± 2.15	10.51 ± 1.90	12.57 ± 1.80	16.28 ± 2.60
		DR	16.62 ± 3.07	11.39 ± 1.57	13.68 ± 1.46	13.72 ± 1.86
		IER vs DR	<i>P</i> = .89	<i>P</i> = .78	<i>P</i> = .70	<i>P</i> = .54

*Values are presented as means ± SEMs.

IL-13 positive cells significantly decreased 3 days after allergen in IER and had returned to baseline value by 7 days. By contrast, in DR, CD4⁺ and CD8⁺ IL-13 positive cells slightly but not significantly increased after allergen. There was significant difference in CD8⁺ IL-13 positive cells between IER and DR 1 day after allergen.

Changes in ratios of T_H2/T_H1 cytokine-producing cells after allergen challenge

The ratio of CD4⁺ and CD8⁺ IL-4/IFN-γ positive cells in peripheral blood and in induced sputum were not different between IER and DR before allergen. After allergen challenge, the ratio significantly decreased for both CD4⁺ and CD8⁺ cells in IER by 3 days and had recovered by 7 days (Fig 3). The ratio did not change significantly in DR. There was significant difference in the ratio between IER and DR at 3 days after allergen. There were no significant changes in ratio of IL-4/IFN-γ positive cells in induced sputum (data not shown).

The ratio of CD4⁺ and CD8⁺ IL-13/IFN-γ positive cells in peripheral blood and in induced sputum were again

not different between IER and DR before allergen. After allergen challenge, the ratio significantly decreased for CD4⁺ cells in IER by 3 days and had recovered by 7 days (Fig 3).

DISCUSSION

This study has demonstrated that in peripheral blood, the percentage of IFN-γ positive cells significantly decreases after allergen challenge in DR, whereas it remains unchanged or increases slightly in IER. By contrast, in induced sputum, IFN-γ positive cells significantly increase after allergen only in IER, resulting in a significant difference between IER and DR. These differences in IFN-γ positive cells in peripheral blood and induced sputum were associated with differences in eosinophils in peripheral blood and induced sputum, as well as in methacholine airway hyperresponsiveness. To our knowledge, this is the first study to show that IFN-γ positive cells in the blood and airways are different

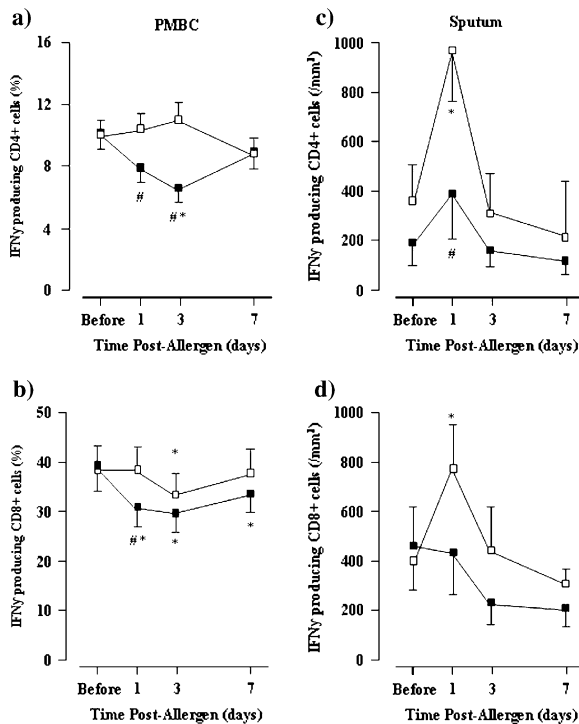


FIG 2. Change in IFN- γ -producing cells before and after allergen challenge. **A**, CD4⁺ cells in peripheral blood. **B**, CD8⁺ cells in peripheral blood. **C**, CD4⁺ cells in induced sputum. **D**, CD8⁺ cells in induced sputum. Open and solid symbols indicate IER and DR, respectively. * $P < .05$ compared with the baseline values. † $P < .05$ compared with IER.

between subjects with asthma with and without late asthmatic responses and the associated differences in airway inflammation.

Other studies have investigated IFN- γ profiles after allergen challenge in subjects with asthma, with discrepant results; some reported decreases in IFN- γ ,^{13,14} whereas another found no change.¹⁵ However, these studies did not discriminate between IER and DR, and in the light of results of the current study, these differences in results could be explained by different proportions of IER and DR evaluated.

There is accumulating evidence that T_H1 lymphocytes counteract the activity of T_H2 lymphocytes and vice versa, and that allergic asthma is based on a cytokine imbalance toward T_H2 predominance. Among the T_H1 cytokines, IFN- γ is known as the most potent in suppressing T_H2-type allergic responses.^{5,6} The mechanisms underlying the pleiotropic action of IFN- γ include suppressing the release of T_H2-type cytokines from activated T cells,^{5,16} suppressing differentiation of naive T cells to T_H2 subtypes,¹⁷ facilitating apoptosis of T cells and eosinophils,¹⁸ suppressing local recruitment of eosinophils,⁶ and inducing nitric oxide production.¹⁹ In addition, we have reported that allergic inflammatory responses persisted in IFN- γ -deficient mice after allergen challenge compared with wild-type control mice, and concluded that IFN- γ has an important role in suppressing the persistence of allergic

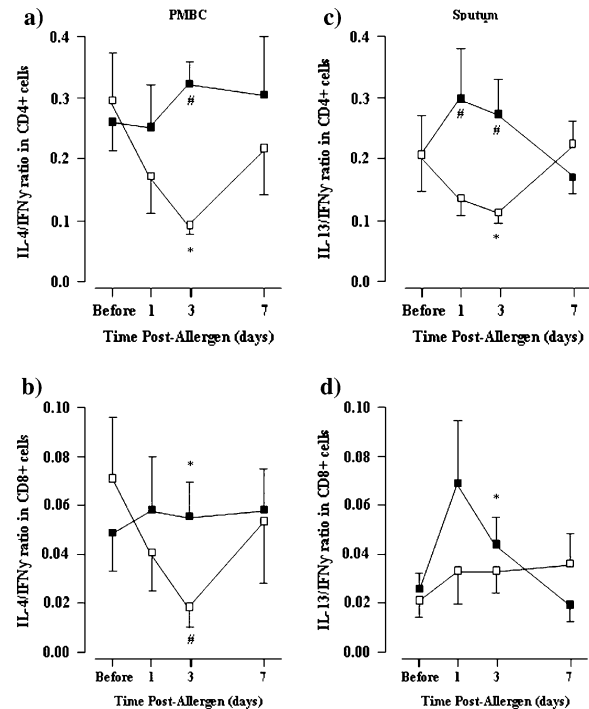


FIG 3. Change in ratio in peripheral blood cells before and after allergen challenge. **A**, IL-4/IFN- γ in CD4⁺ cells. **B**, IL-4/IFN- γ in CD8⁺ cells. **C**, IL-13/IFN- γ in CD4⁺ cells. **D**, IL-13/IFN- γ in CD8⁺ cells. Open and solid symbols indicate IER and DR, respectively. * $P < .05$ compared with baseline values. † $P < .05$ compared with IER.

responses of airways.⁷ The results of the current study in subjects with asthma are consistent with these findings.

A possible explanation for the decrease in peripheral blood IFN- γ positive cells in DR is that these cells accumulated into the local inflammatory site in an effort to resolve the allergic inflammation. Interestingly, IFN- γ positive cells significantly increased in induced sputum, not in DR but in IER. This observation suggests that accumulation of IFN- γ positive cells into the airways is not likely to be the cause of decrease of these cells in peripheral blood. However, this conclusion is predicated on the assumption that induced sputum reflects all of the events occurring in the airway wall. Further studies using airway biopsies are needed to elucidate changes in T-cell IFN- γ production in the airway wall.

Another novel finding of this study was the demonstration of an increase in percentage of peripheral blood IL-12 positive CD4⁺ and CD8⁺ lymphocytes 24 hours after allergen challenge in IER, but not in DR. IL-12 is another potent T_H1 type cytokine known to facilitate IFN- γ production.²⁰ Thus, it is conceivable that increased number of IL-12-producing cells in IER may prevent the reduction in IFN- γ production by lymphocytes after allergen challenge, which we observed in DR but not in IER. However, results of the current study should be interpreted cautiously, because major sources of IL-12 are thought to be monocytes/macrophages.²¹

Unlike previous studies that have investigated IL-4 profiles after allergen challenge,^{15,22} we observed a small

but significant decrease in peripheral blood IL-4-producing cells in IER only. IL-4 is well known to be produced by not only T lymphocytes but also mast cells, eosinophils, and basophils.^{23,24} There are several studies reporting that eosinophils or basophils rather than lymphocytes are the major sources of IL-4 after allergen challenge.^{25,26} Thus, further studies are needed to investigate the entire profile of IL-4 production by cells other than lymphocytes. By contrast, another T_H2 cytokine, IL-13, increased in DR and decreased in IER. This increase of IL-13 after allergen challenge may partly explain the persistence of airway hyperresponsiveness in DR.

To evaluate the T_H2/T_H1 cytokine balances, we calculated the ratios of IL-4/IFN- γ and IL-13/IFN- γ . Both ratios showed similar trends, increase in DR after allergen challenge indicating T_H2 predominance, and decrease in IER showing T_H1 predominance. This suggests that an intervention to change the cytokine balance toward T_H1 predominance may be of value to abrogate allergen-induced airway inflammation. Such strategies include both direct administration of T_H1-type cytokines via various routes^{8,9} and interventions to facilitate production of these cytokines, such as inhaled steroid.²⁷

In conclusion, this study has identified a difference in the production of T_H1-type cytokines IFN- γ and IL-12 as well as T_H2 cytokines IL-4 and IL-13 between subjects with asthma with and without allergen-induced late asthmatic responses and with differences in allergen-induced airway hyperresponsiveness and eosinophilic airway inflammation. These differences may be an important factor in determining the persistence of allergic inflammation and airway responses.

REFERENCES

- O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. *Am Rev Respir Dis* 1987;136:740-51.
- Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. *Am J Respir Crit Care Med* 1999;160:640-7.
- Hogan SP, Matthaie KI, Young JM, Koskinen A, Young GI, Foster PS. A novel T cell-regulated mechanism modulating allergen-induced airways hyperactivity in BALB/c mice independently of IL-4 and IL-5. *J Immunol* 1998;161:1501-9.
- Gavett SH, O'Hearn DJ, Li X, Huang SK, Finkelman FD, Wills-Karp M. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J Exp Med* 1995;182:1527-36.
- Tang C, Inman MD, van Rooijen N, Yang P, Shen H, Matsumoto K, et al. Th type 1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN- γ -dependent mechanism. *J Immunol* 2001;166:1471-81.
- Lack G, Bradley KL, Hamelmann E, Renz H, Loader J, Leung DY, et al. Nebulized IFN- γ inhibits the development of secondary allergic responses in mice. *J Immunol* 1996;157:1432-9.
- Yoshida M, Leigh R, Matsumoto K, Wattie J, Ellis R, O'Byrne PM, et al. Reversing effect of interferon on T_H2 responses after allergen challenge in sensitized interferon-deficient mice. *Am J Respir Crit Care Med* 2002;166:451-6.
- Boguniewicz M, Schneider LC, Milgrom H, Newell D, Kelly N, Tam, et al. Treatment of steroid-dependent asthma with recombinant interferon- γ . *Clin Exp Allergy* 1993;23:785-90.
- Bryan SA, O'Connor BJ, Matti S, Leckie MJ, Kanabar V, Khan J, et al. Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2149-53.
- American Thoracic Society. Definitions, epidemiology, pathology, diagnosis and staging. *Am J Respir Crit Care Med* 1995;152:S78-83.
- Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235-43.
- Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace D, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid phase measurements. *Am J Respir Crit Care Med* 1996;154:308-17.
- Bodey KJ, Semper AE, Redington AE, Madden J, Teran LM, Holgate ST, et al. Cytokine profiles of BAL T cells and T-cell clones obtained from human asthmatic airways after local allergen challenge. *Allergy* 1999;54:1083-93.
- Krug N, Erpenbeck VJ, Balke K, Petschallies J, Tschernig T, Hohlfeld JM, et al. Cytokine profile of bronchoalveolar lavage-derived CD4(+), CD8(+), and gammadelta T cells in people with asthma after segmental allergen challenge. *Am J Respir Cell Mol Biol* 2001;25:125-31.
- Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased Th2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993;92:313-24.
- Swain SL, Bradley LM, Croft M, Tonkonogy S, Atkins G, Weinberg AD, et al. Helper T-cell subsets: phenotype, function and the role of lymphokines in regulating their development. *Immunol Rev* 1991;123:115-44.
- Gajewski TF, Fitch FW. Anti-proliferative effect of IFN- γ in immune regulation, I: IFN- γ inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. *J Immunol* 1988;140:4245-52.
- Morita M, Lamkhioed B, Soussi GA, Aldebert D, Delaporte E, Capron A, et al. Induction by interferons of human eosinophil apoptosis and regulation by interleukin-3, granulocyte/macrophage-colony stimulating factor and interleukin-5. *Eur Cytokine Netw* 1996;7:725-32.
- Barnes PJ, Liew FY. Nitric oxide and asthmatic inflammation. *Immunol Today* 1995;16:128-30.
- Morris SC, Madden KB, Adamovicz JJ, Gause WC, Hubbard BR, Gately MK, et al. Effects of IL-12 on in vivo cytokine gene expression and Ig isotype selection. *J Immunol* 1994;152:1047-56.
- Camporota L, Holloway JW. Interleukin-12 and allergic tissue response. *Clin Exp Allergy* 1999;29:1298-300.
- Virchow JC Jr, Walker C, Hafner D, Kortsik C, Werner P, Matthys H, et al. T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. *Am J Respir Crit Care Med* 1995;151:960-8.
- Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, et al. Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994;10:471-80.
- Moqbel R, Ying S, Barkans J, Newman TM, Kimmitt P, Wakelin M, et al. Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. *J Immunol* 1995;155:4939-47.
- Nouri-Aria KT, O'Brien F, Noble W, Jacobson RM, Rajakulasingam K, Durham SR. Cytokine expression during allergen-induced late nasal responses: IL-4 and IL-5 mRNA is expressed early (at 6 h) predominantly by eosinophils. *Clin Exp Allergy* 2000;30:1709-16.
- Nouri-Aria KT, Irani AM, Jacobson MR, O'Brien F, Varga EM, Till SJ, et al. Basophil recruitment and IL-4 production during human allergen-induced late asthma. *J Allergy Clin Immunol* 2001;108:205-11.
- Purello-D'Ambrosio F, Gangemi S, Merendino RA, Arena A, Guarnieri F, Ricciardi L. Effect of fluticasone propionate on interleukin-12 and interferon- γ production in patients affected by allergic bronchial asthma. *J Invest Allergol Clin Immunol* 1999;9:262-7.