

Allergen challenge primes for IL-5 mRNA production and abrogates β -adrenergic function in peripheral blood T lymphocytes from asthmatics

P. BORGER*†‡, G.-J. JONKER*, E. VELLENGA†, D. S. POSTMA‡, J. G. R. DE MONCHY* and H. F. KAUFFMAN*

Divisions of *Allergology, †Hematology, and ‡Pulmonology, Department of Internal Medicine, University of Groningen, The Netherlands

Summary

Background In previous studies, we have found a dysfunctional adenylyl cyclase (AC) system in patients with asthma after allergen provocation, which resulted in a 40–50% decreased generation of intracellular cAMP. In addition, in activated T helper lymphocyte clones, it has been demonstrated that IFN- γ (TH1-like cytokine) and IL-5 (TH2-like cytokine) are differentially regulated by the AC system. Therefore, we postulate that an increased IL-5/IFN- γ ratio as observed in asthmatics might be due to a dysfunctional AC system.

Objective To assess whether a dysfunctional AC system as observed in asthmatics after allergen provocation, is responsible for an increased IL-5/IFN- γ cytokine ratio.

Methods Peripheral blood T lymphocytes of seven asthma patients were stimulated with anti-CD3 plus anti-CD28 MoAbs in the absence and presence of isoproterenol (ISO) and prostaglandin E₂ (PGE₂) to activate the AC system. Before, 3 h and 24 h after allergen provocation, IFN- γ and IL-5 mRNAs were detected by semiquantitative RT-PCR.

Results Before allergen provocation, ISO (10^{-5} mol/L) significantly downregulated IFN- γ mRNA ($P < 0.03$, $n = 6$), and showed a trend to upregulate IL-5 mRNA ($P = 0.138$, $n = 5$). Three and 24 h after allergen provocation, ISO was not longer able to modulate IFN- γ and IL-5. In contrast with the observations with ISO, PGE₂ still dose-dependently inhibited IFN- γ mRNA, both before, 3 h and 24 h after allergen provocation ($n = 7$). IL-5 mRNA, but not IFN- γ mRNA, was significantly upregulated in anti-CD3 plus anti-CD28-activated T cells ($P < 0.05$, $n = 5$) 24 h after allergen provocation, compared with before allergen provocation.

Conclusion Twenty-four hours after allergen provocation, a significant reduction of β -adrenergic control on IFN- γ and IL-5 mRNA expression was observed in peripheral blood T lymphocytes, which coincides with a selective priming of IL-5 mRNA production.

Keywords: adenylyl cyclase, allergen provocation, asthma, dysfunctional adenylyl cyclase, IFN- γ , IL-5, IL-5/IFN- γ ratio, T lymphocytes

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Introduction

Asthma is characterized by episodic reversible airway obstruction, with associated bronchial hyperresponsiveness

Correspondence: H. F. Kauffman, Division of Allergology, Department of Internal Medicine, University Hospital Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands.

[1]. Although progress has been made in understanding these asthmatic features, the exact underlying mechanism(s) are still elusive. At present, cytokines are regarded as important modulators of the immune system and are thought to play a key role in pathophysiological changes recognized as asthma. In particular, IL-5 has been implicated in the inflammatory features and disease severity of asthma [2,3].

Increased expression of IL-5 in peripheral blood cells and/or bronchoalveolar lavage have been found in asthma [3–5]. Moreover, it has been shown that a low protein secretion level of IFN- γ (TH1-like cytokine) by peripheral blood mononuclear cells (PBMCs) correlated positively with bronchial hyperresponsiveness in asthmatic children [6]. Therefore, the IFN- γ /IL-5 ratio may be an important determinant in asthmatic airway responses.

The PGE₂-induced cAMP-dependent signalling pathway has been reported to have differential effects on the expression of IFN- γ and IL-5. For instance, in TH0 clones it was demonstrated that IFN- γ productions are dose-dependently inhibited by exogenous PGE₂ [7]. In contrast, IL-5 productions are not regulated by the PGE₂-induced signalling pathway or, under certain conditions, may be enhanced in T-cell lines and T-cell clones [7–11]. Thus, in activated T lymphocytes the PGE₂-induced signalling pathway may decrease the IFN- γ /IL-5 ratio, a phenomenon often observed in asthmatic subjects. The ultimate effect of PGE₂ on IL-5 gene expression, however, seems to be dependent on the T-cell stimulus. We have recently shown that in freshly isolated human T lymphocytes that PGE₂ was able to generate a substantial negative control on the expression of IL-4 and IL-5 mRNA and protein [12,13].

In addition, it has been demonstrated that after allergen challenge isoproterenol-induced cAMP levels are lower in activated PBMCs isolated from patients with asthma than in corresponding cells from healthy control subjects [14]. The defect in activation of the adenylyl cyclase (AC) system was associated with an increase in bronchial hyperresponsiveness [14,15]. The reduced cAMP response was already observed in asthmatic patients as early as 3 h after allergen-induced airway obstruction which was not observed in healthy controls [16,17]. Taken together, these results

suggest an impaired function of adenylyl cyclase after allergen-induced airway obstruction, which may also contribute to an imbalanced IFN- γ /IL-5 ratio.

In this study we examined the modulatory effect of the AC system on the expression of IFN- γ and IL-5 mRNA in anti-CD3 plus anti-CD28-activated T lymphocytes isolated from asthmatic patients, before and after an allergen-induced airway obstruction. The results demonstrate that the control of β -adrenoceptors on IFN- γ and IL-5 mRNA expression is significantly reduced after allergen challenge, whereas the PGE₂ control is not affected. Moreover, after allergen challenge the peripheral T lymphocytes become primed to enhance IL-5 mRNA expression.

Methods

Subjects

Seven patients with mild to moderate asthma participated in the study (for clinical characteristics see Table 1). They were selected on the basis of a history of episodic wheeze, positive skin test and/or enhanced specific IgE for house dust mite, birch pollen or cat, and a provocation concentration of histamine causing a 20% fall in FEV₁ (PC₂₀) < 32 mg/mL (30 s method). The FEV₁ was $\geq$ 70% of the predicted FEV₁ value. All antiasthma medication (e.g. inhaled corticosteroids, xanthines, long-acting β -agonists, cromones) was stopped at least 4 weeks before the start of the study; only short-acting bronchodilators were allowed. The patients did not receive oral corticosteroids in the preceding 2 months, and they did not experience respiratory tract infections or acute asthmatic attacks within 4 weeks prior to the start of the study. None of the patients were smokers. The study was approved by the Medical Ethics

Table 1. Clinical data of the patients

Patient	Age (years)	Gender	PC ₂₀ met* (mg/mL)	FEV ₁ † (% pred.)	LAR‡	Specific IgE (PRU/mL)
1	48	M	1.85	78	Pos	ND
2	50	F	0.32	110	Neg	Neg§
3	28	M	0.74	114	Neg	63.5
4	44	F	0.22	72	Pos	43.6
5	30	M	0.80	95	Pos	28.6
6	48	M	3.53	93	Neg	0.92
7	21	M	0.29	105	Pos	> 100

*PC₂₀ methacholine determined as described in methods. †Forced expiratory volume in one second expressed as percentage of the predicted value of FEV₁. ‡LAR = late asthmatic reaction after allergen challenge. All patients had at least 20% fall in FEV₁ within the first hour after allergen challenge (early obstructive reaction). §but skin test positive. ND = not determined.

Committee of the University Hospital of Groningen, and all patients gave their written informed consent.

Study design

After a 4-week run-in period (to assess the stability of the patients' asthmatic state), every patient underwent 2 consecutive examination days. During the first day the allergen provocation took place as described by Aalbers *et al.* [18]. Blood samples were collected before allergen provocation ($t = 0$ h), three hours after ($t = 3$ h) and 24 h after allergen provocation ($t = 24$ h). For safety reasons, the patients spent the night in the hospital after allergen challenge.

Methacholine challenge

After measuring a baseline FEV₁ with a spirometer, PC₂₀ methacholine was assessed using the 2-min tidal breathing method adapted from Cockcroft *et al.* [19]. Doubling concentrations of methacholinebromide ranging from 0.038 to 78.5 mg/mL were administered by a DeVilbiss 646 nebulizer with an output of 0.13 mL/min. FEV₁ was measured 30 and 90 s after each inhalation until the fall of FEV₁ was $\geq 20\%$ compared with baseline. PC₂₀ methacholine was derived by linear interpolation between the last two points on the logarithmic concentration–response curve.

Allergen provocation

The extracts for allergen provocation were prepared from stock solutions of *Dermatophagoides pteronyssinus* (house dust mite), and of cat dander, diluted in PBS with 0.03% human serum albumin and with 0.5% phenol (ALK Benelux, Groningen, The Netherlands), producing fivefold increasing concentrations ranging from 80 to 10 000 BU/mL [18]. These concentrations were inhaled, using the same equipment as during the methacholine provocation, at 15-min intervals, measuring FEV₁ immediately and 15 min after each inhalation. The provocation started with the lowest concentration and was terminated at the concentration which evoked a 20% fall of FEV₁. After the last inhalation, FEV₁ was measured every 10 min for the first hour and then hourly for 10 h.

Preparation of cells

Mononuclear cell suspensions were prepared by Ficoll-Hypaque (Lymphoprep, Nycomed, Oslo, Norway) density-gradient centrifugation of peripheral blood. T lymphocytes were isolated by 2-aminoethyl-isothiuronium bromide- (AET) treated sheep red blood cell (SRBC) rosetting. The SRBCs were lysed with 155 mmol/L NH₄Cl, 10 mmol/L KHCO₃,

0.1 mmol/L EDTA and the remaining cell preparations contained more than 98% T lymphocytes as assessed by flow cytometric analysis after staining with an anti-CD2 monoclonal antibody (Becton Dickinson, Mountain View CA, USA) and less than 1% CD14-positive cells (Becton Dickinson). T lymphocytes were cultured at 37 °C in RPMI 1640 media (Flow, Rockville MD, USA) containing 5% fetal bovine serum (FBS; Hyclone, Logan, UT) supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mmol/L L-glutamine and 6 ng/mL colistine.

Stimulation of cells

T lymphocytes (10^6 cells/mL) were incubated for 6 h in RPMI 1640 medium with anti-CD3 and anti-CD28 antibodies in a 5% hybridoma supernatant solution (gift from Dr B. J. Kroesen, Department of Immunology, University of Groningen, The Netherlands) in the presence and absence of prostaglandin E₂ (PGE₂; Sigma, St Louis MO, USA) and isoproterenol (Sigma). Sodiumbisulfite (10^{-5} mol/L) was added to the medium as antioxidant.

Functional control of the AC system on IFN- γ and IL-5 mRNA was studied by β -adrenergic stimulation. T lymphocytes (10^6) from asthmatic patients were stimulated with antibodies against CD3 and CD28 (anti-CD3/anti-CD28). Expression of IFN- γ mRNA ($n = 6$) and IL-5 mRNA ($n = 5$) was assessed in the absence and presence of the β -adrenergic agonist isoproterenol (10^{-5} mol/L), before ($t = 0$ h), 3 h after ($t = 3$ h) and 24 h after ($t = 24$ h) allergen challenge. The effect of PGE₂-induced AC activation on IFN- γ mRNA expression was assessed before ($t = 0$ h), 3 h after ($t = 3$ h) and 24 h after ($t = 24$ h) allergen-induced pulmonary obstructive reactions. Concentrations of PGE₂ ranged from 10^{-4} to 10^{-7} mol/L.

Finally, we analysed the expression of IFN- γ and IL-5 mRNA levels in anti-CD3/anti-CD28-stimulated T lymphocytes before, 3 h after and 24 h after the allergen challenge. IFN- γ and IL-5 mRNA levels measured at $t = 3$ h and $t = 24$ h are expressed as the percentage of initial mRNA levels as measured before allergen challenge.

Extraction of mRNA, reverse transcription (RT) and polymerase chain reaction (PCR)

The semiquantitative RT-PCR was performed as described previously [13]. In short, total cellular RNA was isolated from 10^6 T lymphocytes by the single-step method as described by Chomczynski and Sacchi [20]. RNA (2 µg) was resuspended in 15 µL diethyl-pyrocabonate- (DEPC; Sigma) treated H₂O and incubated at 68 °C for 10 min. After cooling on ice the samples were spun briefly in a micro-centrifuge. Next, 10 µL of 10 X RT mix (135 mM TRIS-HCl pH 8.3, 204 mmol/L KCl, 27 mmol/L MgCl₂, 0.24 mg/mL

BSA, 5.4 mmol/L of each dNTP, 14.3% glycerol) and 3 units of reverse transcriptase (Promega Corporation, Madison WI, USA) was added to the samples in the presence of random hexanucleotide primers. The samples were spun briefly and incubated at 37 °C for 60 min.

The following specific primer pairs for β -2-microglobulin (β 2 μ G), IL-5 and IFN- γ were obtained from Eurogentec (Seraing, Belgium).

β 2 μ G: 5'-CCAGCAGAGAATGGAAAGTC-3' (sense) and 5'-GATGCTGCTTACATGTCTCG-3' (antisense);

IL-5: 5'-CTTGGCACTGCTTTCTACTC-3' (sense) and 5'-GCAGGTAGTCTAGGAATTGG-3' (antisense);

IFN- γ : 5'-GCAGAGCCAAATTGTCTCCT-3' (sense) and 5'-ATGCTCTTCGACCTCGAAAC-3' (antisense).

One microlitre of 50 μ mol/L sense and antisense of β 2 μ G were added to each RT-reaction sample and DEPC-treated H₂O was added to a final volume of 100 μ L. Next, the samples were heated for 3 min at 94 °C, spun briefly in a microcentrifuge and placed on ice. *Taq* DNA polymerase 0.5 μ L of 5 U/ μ L (Promega) was added to each sample. The tubes were placed in a thermal cycler (Perkin-Elmer, Foster City CA, USA). PCR was performed with β 2 μ G, IFN- γ and IL-5 primers: 1 min denaturation at 94 °C, 0.5 min annealing at 55 °C, and 0.5 min extension at 72 °C. With these primers' fragments spanning 259 base pairs (bp), 290 bp and 268 bp were amplified for IL-5, IFN- γ and β 2 μ G, respectively. After 20 PCR cycles with primers for β 2 μ G, 10 μ L of the reactionmixture was run on a 2% agarose gel, containing 0.2 μ g/mL ethidium bromide in 1 $\times$ TAE buffer. To be sure that equal amounts of cDNA were used in each PCR reaction, bands were quantified with respect to β 2 μ G. Then, equal amounts of RNA were amplified with primers for IFN- γ (20 cycles), IL-5 (30 cycles) and β 2 μ G. PCR products were analysed in the exponential phase of amplification. Relative mRNA levels were quantified using the gelscan program Diversity One (Pharmacia, LKB, Uppsala, Sweden) and normalized with respect to the β 2 μ G signal.

Statistics

Statistical analysis was performed using the SPSS/PC + Statistical Package (SPSS Inc. Chicago IL, USA). The Wilcoxon nonparametric test was used for statistical analysis. For regression analysis the Pearson correlation coefficient was calculated.

Results

Isoproterenol effects on IFN- γ and IL-5 mRNA are modified after allergen challenge

As shown in Table 2, isoproterenol had differential effects on accumulation of IFN- γ and IL-5 mRNA in anti-CD3/anti-CD28-stimulated T cells. Before allergen challenge ($t = 0$ h) isoproterenol significantly suppressed the expression of IFN- γ mRNA in 6/6 patients (66% compared with anti-CD3/anti-CD28-induced IFN- γ mRNA levels, range 53–76%; $P < 0.03$; Table 2). Three hours ($t = 3$ h) after allergen challenge (i.e. in the recovery phase between the early and late bronchoconstrictive reactions) IFN- γ mRNA expression was less or not susceptible to the inhibitory effect of isoproterenol in 4/6 patients (patients 3–6). Moreover, in 2/6 patients (patients 1 and 2) IFN- γ mRNA levels were upregulated. As a consequence, the effect of isoproterenol on IFN- γ mRNA observed at 3 h did not significantly differ from anti-CD3/anti-CD28-stimulated cells in the absence of isoproterenol. In contrast, isoproterenol-induced inhibition was significantly reduced at 3 h after allergen challenge (118%, range 83–179%; $P < 0.03$) compared with the effect of isoproterenol at $t = 0$ h. Twenty-four hours after allergen challenge ($t = 24$ h), we observed complete loss of the modulatory effect of isoproterenol compared with $t = 0$ h (109%, range 75–132%; $P < 0.03$; Table 2).

In contrast with IFN- γ mRNA, IL-5 mRNA accumulation was upregulated by isoproterenol in 4/5 patients studied (patients 1, 3, 4 and 5) before the allergen challenge

Patient	IFN- γ			IL-5		
	$t = 0$ h	$t = 3$ h	$t = 24$ h	$t = 0$ h	$t = 3$ h	$t = 24$ h
1	71	179	132	152	154	113
2	75	145	101	75	162	105
3	53	83	113	124	103	103
4	65	107	104	236	104	93
5	76	93	127	266	171	119
6	57	100	75	ND	ND	ND
Mean	66*	118†	109†	171	139	107‡

* $P < 0.03$ vs in absence of isoproterenol, † $P < 0.03$ vs $t = 0$ h, ‡ $P < 0.05$, ND = not determined.

Table 2. Modulatory effects of isoproterenol (10^{-5} mol/L) on the expression of IFN- γ ($n = 6$) and IL-5 ($n = 5$) mRNA in patients with asthma, before ($t = 0$ h), 3 h after ($t = 3$ h) and 24 h after ($t = 24$ h) an allergen challenge in T lymphocytes stimulated with anti-CD3 plus anti-CD28. The effect of isoproterenol on IFN- γ and IL-5 mRNA accumulation is expressed as the percentage of anti-CD3 plus anti-CD28-induced mRNA levels measured in the absence of isoproterenol (= 100%)

($t = 0$ h), whereas IL-5 mRNA was downregulated in patient 2 (Table 2). Because of the considerable variability of IL-5 mRNA levels in the patients studied, no statistical significance for this isoproterenol induced upregulatory trend could be demonstrated (171% compared with anti-CD3/anti-CD28-induced IL-5 mRNA levels, range 75–266%; $P = 0.138$). Three hours after allergen challenge ($t = 3$ h), IL-5 mRNA accumulation was less responsive to isoproterenol compared with $t = 0$ h in 3/5 patients (patients 3–5), whereas it was upregulated in patient 2 and comparable in patient 1. As observed with IFN- γ mRNA, the effect of isoproterenol on IL-5 mRNA accumulation was significantly diminished 24 h after allergen challenge ($t = 24$ h) compared with $t = 3$ h (107%, range 93–144%; $P < 0.05$; Table 2).

PGE₂-induced inhibition of IFN- γ mRNA is not changed after allergen challenge

Since the AC system can also be activated by PGE₂, we examined IFN- γ mRNA accumulation in response to increasing concentrations of PGE₂, ranging from 10^{-7} to 10^{-4} mol/L. Before allergen challenge ($t = 0$ h) anti-CD3/anti-CD28-induced IFN- γ mRNA accumulation (Fig. 1) was dose-dependently inhibited by PGE₂ in all patients ($n = 7$). Three hours after ($t = 3$ h) and 24 h after ($t = 24$ h) allergen challenge, PGE₂ inhibited IFN- γ mRNA accumulation to the same extent as before allergen challenge ($t = 0$ h).

Allergen challenge primes T lymphocytes to enhance IL-5 mRNA accumulation

There was considerable variation between patients with respect to IFN- γ and IL-5 mRNA levels before allergen challenge ($t = 0$ h). As shown in Table 3, IFN- γ mRNA levels did not significantly change 3 h after (94%, range 58–114%; $n = 6$) and 24 h after the allergen challenge (84%,

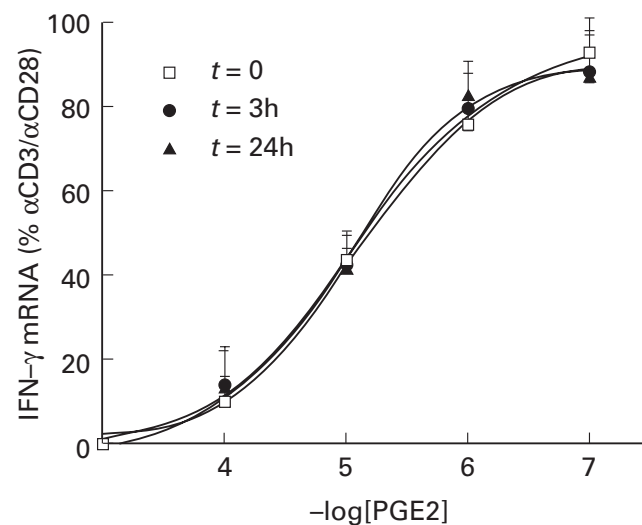


Fig. 1. Effect of prostaglandin E₂ (PGE₂) on IFN- γ mRNA accumulation in T lymphocytes obtained from peripheral blood of patients with asthma. T lymphocytes were stimulated with anti-CD3 plus anti-CD28 in the presence of increasing concentrations of PGE₂ (10^{-7} – 10^{-4} mol/L) before allergen challenge, 3 h after allergen challenge, and 24 h after allergen challenge. Results are expressed as percentages ($\pm$ SEM) of mRNA levels measured in the absence of PGE₂ (=100%). Percentages were determined as described in material and methods.

range 71–118%; $n = 6$) in comparison with $t = 0$ h (100%). In contrast, IL-5 mRNA expression was upregulated in three out of five patients 3 h after allergen provocation, whereas 24 h after allergen provocation IL-5 mRNA expression was upregulated in all five patients studied (293%, range 120–671%; $n = 5$) compared with values before challenge (100%). The upregulation of IL-5 mRNA expression 24 h after the allergen challenge was statistically significant ($P < 0.05$). Figure 2 shows a representative experiment of absolute IL-5 and IFN- γ mRNA levels before and after allergen challenge (patient 4).

Table 3. Relative expressions of IFN- γ ($n = 6$) and IL-5 ($n = 5$) mRNA in patients with asthma, before ($t = 0$ h), 3 h after ($t = 3$ h) and 24 h after ($t = 24$ h) an allergen challenge in T lymphocytes stimulated with anti-CD3 plus anti-CD28. IFN- γ and IL-5 mRNA accumulation is expressed as the percentage of mRNA levels measured before the allergen challenge (=100%)

Patient	IFN- γ			IL-5		
	$t = 0$ h	$t = 3$ h	$t = 24$ h	$t = 0$ h	$t = 3$ h	$t = 24$ h
1	100	69	74	100	92	151
3	100	120	88	100	237	288
4	100	114	78	100	428	671
5	100	104	118	100	118	228
6	100	100	75	ND	ND	ND
Mean	100	94	84	100	191	293*

* $P < 0.05$ vs $t = 0$ h, ND = not determined.

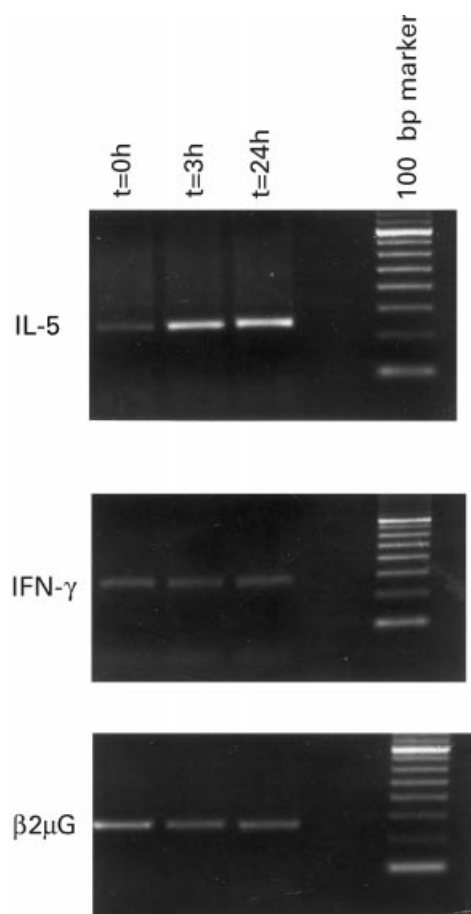


Fig. 2. Representative experiment of absolute IL-5 and IFN- γ mRNA levels in peripheral blood T lymphocytes of patient no. 4. T lymphocytes were activated with anti-CD3 plus anti-CD28 antibodies, and mRNA levels were measured before allergen challenge ($t=0$ h), 3 h after ($t=3$ h) and 24 h after allergen challenge. RT-PCR products with the expected size are demonstrated by a 100-bp marker. β -2-microglobulin ($\beta 2\mu$ G) mRNA signals show comparable amounts of amplified initial cDNA in each lane.

Correlations of laboratory data with clinical parameters

Analysis of the variations in β -adrenergic control by isoproterenol or mRNA levels before, 3 and 24 h after allergen challenge did not show statistically significant correlations with any of the clinical parameters (FEV_1 and PC_{20} methacholine) of the patients measured.

Discussion

In this study we investigated the expression of IL-5 and IFN- γ mRNA in activated T lymphocytes from asthmatic subjects before, and 3 and 24 h after allergen-induced pulmonary obstructive reactions, as well as the regulation of the expression of these cytokines by the cAMP-dependent

signalling pathway. Our results demonstrate that before allergen challenge, the β -adrenoceptor agonist isoproterenol (10^{-5} mol/L) had a significant inhibitory effect on the expression of IFN- γ mRNA (34% inhibition, range 47–24%). In anti-CD3/anti-CD28-activated T lymphocytes isolated from healthy blood donors, the inhibition induced by isoproterenol (10^{-5} mol/L) was shown to be $77 \pm 6\%$ [21]. This discrepancy may result from an impaired cAMP production, since it has been demonstrated that PBMCs from asthma patients generate less cAMP in response to isoproterenol [15–17]. Surprisingly, after allergen challenge the inhibitory control of isoproterenol on IFN- γ gene expression was completely abrogated at 3 and 24 h after allergen challenge. A similar loss of control of the modulatory effect of isoproterenol was observed on the expression of IL-5 mRNA. Before and 3 h after allergen challenge, β -adrenoceptor stimulation showed a trend to upregulate IL-5 mRNA, which was significantly reduced 24 h after allergen challenge.

It has been shown that the β -adrenoceptor numbers on PBMCs isolated from asthmatic subjects are decreased after allergen provocation, which was not observed in healthy control subjects [15]. It has also been reported that after an allergen challenge a 40–50% impairment of adenylyl cyclase activity is observed in intact cells and isolated cell membranes from asthmatic subjects. Moreover, this impairment was located distal from the β -adrenoceptor [14,15]. Reduced numbers of β -adrenoceptors and impairment of adenylyl cyclase activity together result in diminished production of intracellular cAMP. As a consequence, cytokines which are regulated by the cAMP-dependent signalling pathway initiated by catecholamines become dysregulated in asthmatic subjects after an allergen challenge and may thus contribute to subsequent chronic inflammatory responses. This is supported by *in vivo* observations that metabolic responses to catecholamines are reduced in asthmatic patients [22–24]. Although partial loss of β -adrenoceptor control on cytokine gene expression would have been expected with respect to the above described observations, our finding of a complete loss of control is difficult to explain with the assumed 50–60% residual adenylyl cyclase activity. It is conceivable that Ca^{2+} - β -adrenergic-dependent adenylyl cyclases (type I and III), which are responsible for the potentiated adenylyl cyclase activity [16], are coupled to the Ca^{2+} -dependent signal transduction pathways involved in cytokine production, whereas the Ca^{2+} -independent adenylyl cyclase are not. In this way the total loss of beta-adrenergic control on cytokine gene expression may be explained. In contrast with the observations with β -adrenoceptor stimulation, anti-CD3/anti-CD28-induced IFN- γ mRNA was dose-dependently inhibited by PGE_2 , both before and after allergen-induced bronchoconstrictive reaction(s). After allergen challenge

PGE₂-induced inhibition on IFN- γ mRNA accumulation did not significantly differ from results before allergen challenge (Fig. 1). The observed discrepancy between isoproterenol and PGE₂ may be explained by the activation of additional signalling pathways by PGE₂, that cannot be activated through β -adrenoceptor stimulation. In this respect, it has been shown that PGE₂ is able to inhibit anti-CD3-induced tyrosine phosphorylation by a yet unidentified mechanism but independent of cAMP [25].

In contrast with IFN- γ mRNA, IL-5 mRNA levels were significantly enhanced by anti-CD3 plus anti-CD28-stimulated T lymphocytes 24 h after allergen challenge compared with levels before allergen challenge. It has been well established that IL-5 mRNA and protein are elevated in atopic asthma patients [4,5]. Recently, it has been demonstrated that bronchoprovocation with allergen resulted in increased levels of IL-5 protein in bronchoalveolar lavage (BAL) fluid 48 h after allergen challenge [26]. Similarly, our present data indicate that, in addition to BAL T lymphocytes from patients with asthma, peripheral blood T lymphocytes also become 'primed' to selectively enhance IL-5 mRNA accumulation after allergen challenge. This selective priming for IL-5 mRNA seems to be independent from the β -adrenoceptor-mediated upregulation of IL-5 mRNA, since absolute values of IL-5 mRNA are increased. However, the underlying mechanism responsible for priming of the T lymphocytes resulting in IL-5 mRNA accumulation after allergen challenge is at present not understood. It is tempting to speculate that (a) soluble mediator(s) released during the inflammatory response in the lung are leaking into the circulation and prime the T lymphocytes for enhanced IL-5 production. The character of the putative mediator(s) is (are) at present not known. Enhanced IL-5 production by peripheral blood T lymphocytes may explain increased levels of eosinophils observed in the peripheral blood. A similar mechanism has been proposed for the release of haemopoietic progenitor (CD34⁺) cells into the periphery of atopic patients: release of locally produced mediators into the blood circulation are responsible for the increased levels of progenitors [27,28]. Recently, it was demonstrated that these CD34⁺ progenitors from asthmatic subjects also express higher levels of the IL-5 receptor α -subunit, which makes these cells more prone to IL-5 [29].

Although we are aware of the small number of patients examined in our study, the observed upregulation of IL-5 mRNA — together with the loss of β -adrenergic control — may have important implications for the severity of asthma, since IL-5 has been shown to be a chemoattractive and activating factor for eosinophils [2,30]. Other studies have shown that the recruitment of proinflammatory cells by cell-derived chemotactic factors (like IL-5 for eosinophils) and subsequent activation of these cells contribute to the development of the late asthmatic reaction (LAR). Eosinophils

have been found in increased numbers in peripheral blood and in bronchoalveolar lavage obtained from asthmatic subjects and are positively correlated with the level of bronchial hyperresponsiveness [31]. Moreover, a significant positive correlation between the percentage of eosinophils in BAL and the LAR has been demonstrated [32,33]. In our study, however, we were not able to find an association between IL-5 mRNA levels and any of the clinical parameters of the patients, nor of the occurrence of the LAR. The lack of correlation is most probably due to the small number of patients participating in this study. For instance, only four out of the seven studied asthmatics had a late response on allergen challenge. However, it cannot be excluded that enhanced IL-5 production may have effects on clinical parameters later than 24 h.

In conclusion, in this study we present evidence that in asthmatic patients *two distinct events* take place in freshly isolated peripheral T lymphocytes after an allergen-induced asthma attack. First, there is the abrogation of β -adrenergic control on cytokine gene expression in cells that are still firmly under control of PGE₂-induced adenylyl cyclase. Second, priming of T lymphocytes leads to enhanced IL-5 gene expression. Together these phenomena may contribute the dysregulated balance of TH1/TH2 cytokines observed in asthmatic patients.

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References

- 1 American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; 136:225–44.
- 2 Sanderson CJ. Interleukin 5, eosinophils and disease. *Blood* 1992; 179:3101–9.
- 3 Humbert MC, Corrigan CJ, Kimmitt P, Till SJ, Kay AB, Durham SR. Relationship between IL-4 and IL-5 mRNA expression and disease severity in atopic asthma. *Am J Respir Crit Care Med* 1997; 156:704–8.
- 4 Tang CJ, Rolland M, Ward C, Quan B, Walters EH. IL-5 production by bronchoalveolar lavage and mononuclear cells in asthma and atopy. *Eur Respir J* 1997; 10:624–32.
- 5 Till S, Dickason R, Huston D et al. IL-5 secretion by allergen-stimulated CD4⁺ T cells in primary culture: Relationship to expression of allergic disease. *J Allergy Clin Immunol* 1997; 99:563–9.

- 6 Hoekstra MO, Hoekstra Y, de Reus D, Rutgers B, Gerritsen J, Kauffman HF. Interleukin-4 (IL-4), Interferon- γ and Interleukin-5 (IL-5) in peripheral blood of children with atopic asthma. *Clin Exp Allergy* 1997; 27:1254–60.
- 7 Hilkens MU, Vermeulen H, van Neerven RJJ, Snijdwint FGM, Wierenga EA, Kapsenberg ML. Differential modulation of T helper type 1 (Th1) and Th helper type 2 (Th2) cytokine secretion by prostaglandin E₂ critically depends on Interleukin 2. *Eur J Immunol* 1995; 25:63–9.
- 8 Betz M, Fox B. Prostaglandin E₂ inhibits the production of Th1 lymphokines but not of Th2 lymphokines. *J Immunol* 1991; 146:108–13.
- 9 Muñoz E, Zubiaga AM, Merrow M, Sauter NP, Huber BT. Cholera toxin discriminates between Th1 and Th2 receptor-mediated activation: role of cAMP in T cell proliferation. *J Exp Med* 1990; 172:95–102.
- 10 Lee HJ, Koyano-Nakagawa N, Naito Y et al. cAMP activates the IL-5 promoter synergistically with phorbol ester through the signaling pathway involving protein kinase A in mouse thymoma line EL-4. *J Immunol* 1993; 151:6135–42.
- 11 Snijdwint FGM, Kalinski P, Wierenga EA, Bos JD, Kapsenberg ML. Prostaglandin E₂ differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J Immunol* 1993; 150:5321–9.
- 12 Borger P, Kauffman HF, Postma DS, Vellenga E. Interleukin-4 gene expression in activated human T lymphocytes is regulated by the cyclic adenosine monophosphate-dependent signaling pathway. *Blood* 1996; 87:691–8.
- 13 Borger P, Vellenga E, Gringhuis SI et al. Prostaglandin E₂ differentially modulates interleukin-5 gene expression in activated human T lymphocytes depending on the costimulatory signal. *J Allergy Clin Immunol* 1998; 101:321–240.
- 14 Koëter GH, Meurs H, Kauffman HF, de Vries K. The role of the adrenergic system in allergy and bronchial hyperreactivity. *Eur J Respir Dis* 1982; 63 (S121):72–8.
- 15 Meurs H, Koëter GH, de Vries K, Kauffman HF. The beta-adrenergic system and allergic bronchial asthma: changes in lymphocyte beta-adrenergic receptor number and adenylate cyclase activity after an allergen-induced asthmatic attack. *J Allergy Clin Immunol* 1982; 70:272–80.
- 16 Dooper MWSM, Timmermans A, Weersink EJM, de Monchy JGR, Kauffman HF. Defect in potentiation of adenylyl cyclase correlates with bronchial hyperreactivity. *J Allergy Clin Immunol* 1995; 96:628–34.
- 17 Dooper MWSM, Timmermans A, Aalbers R, de Monchy JGR, Kauffman HF. Desensitization of the adenylyl cyclase system in peripheral blood mononuclear cells from patients with asthma three hours after allergen challenge. *J Allergy Clin Immunol* 1993; 92:559–66.
- 18 Aalbers R, Kauffman HF, Groen H, Koëter GH, de Monchy JGR. The effect of nedocromil on the early and late reaction and allergen induced bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1991; 87:993–1001.
- 19 Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977; 7:235–43.
- 20 Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol chloroform. *Anal Biochem* 1987; 162:156–9.
- 21 Borger P, Hoekstra Y, Esselink MT et al. β -Adrenoceptor-mediated inhibition of IFN- γ , IL-3, and GM-CSF mRNA accumulation in activated human T lymphocytes is solely mediated by the β_2 -adrenoceptor subtype. *Am J Respir Cell Mol Biol* 1998; 19:400–7.
- 22 Cookson DV, Reed CE. A comparison of the effects of isoproterenol in the normal and asthmatic subject. *Am Rev Respir Dis* 1963; 88:636–42.
- 23 Lockey SD, Glennon JA, Reed CE. Comparison of some metabolic responses in normal and asthmatic subjects to epinephrine and glucagon. *J Allergy* 1967; 40:349–54.
- 24 Middleton E, Finke SR. Metabolic response to epinephrine in bronchial asthma. *J Allergy* 1968; 42:288–99.
- 25 Elliott LH, Levay AK, Sparks B, Miller M, Roszman TL. Dexamethasone and prostaglandin E₂ modulate T-cell receptor signaling through a cAMP-independent mechanism. *Cellular Immunol* 1996; 169:117–24.
- 26 Becky Kelly EA, Rodriguez RR, Busse WW, Jarjour NN. The effect of segmental bronchoprovocation with allergen on airway lymphocyte function. *Am J Respir Crit Care Med* 1997; 156:1421–8.
- 27 Sehmi R, Howie K, Sutherland R, Schragge W, O'Byrne PM. Increased levels of CD34⁺ hemopoietic cells in atopic subjects. *Am J Respir Cell Mol Biol* 1996; 15:645.
- 28 Inman MD, Denburg JA, Ellis R, Dahlback M, O'Byrne PM. Allergen-induced increase in bone marrow progenitors in airway hyperresponsive dogs: regulation by a serum hemopoietic factor. *Am J Respir Cell Mol Biol* 1996; 15:305.
- 29 Sehmi R, Wood LJ, Watson R et al. Allergen-induced increases in IL-5 receptor α -subunit expression on bone marrow-derived CD34⁺ cells from atopic asthma. *J Clin Invest* 1997; 100:2466–75.
- 30 Clutterbuck CJ, Hurst EMA, Sanderson CJ. Human interleukin-5 regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and GM-CSF. *Blood* 1989; 73:504–12.
- 31 Wardlaw AJ, Dunette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. *Am Rev Respir Dis* 1988; 137:62–9.
- 32 de Monchy JGR, Kauffman HF, Venge P et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985; 131:373–6.
- 33 Diaz P, Conzalez MC, Galleguillos FR et al. Leukocytes and mediators in bronchoalveolar lavage during allergen-induced late-phase asthmatic reactions. *Am Rev Respir Dis* 1989; 139:1383–9.