

Seasonal variations in cyclic AMP production by peripheral blood mononuclear cells in allergic asthmatics

Y. HOEKSTRA, E. J. M. WEERSINK*, D. S. POSTMA* and H. F. KAUFFMAN

Departments of Allergology and *Pulmonology, Clinic for Internal Medicine, University Hospital, Groningen, The Netherlands

Summary

Background Dysfunction of the β -adrenoceptor (β AR)/adenylyl cyclase (AC) system can impair the response of different cell types, including lymphocytes. In asthma, impairment of this system as well as changes in cytokine production by lymphocytes have been described. Because the severity of asthma can change over the year, a circannual pattern of the β AR/AC system activity may also exist.

Objectives We set out to examine the activity of this β AR/AC signal transduction system in peripheral blood mononuclear cells (PBMCs) of allergic asthmatics to assess whether differences existed between seasons. We investigated whether changes were associated with asthma severity and circannual changes in serum cortisol levels.

Methods During 19 months, 41 allergic asthmatics (mean age 28 years) with nocturnal airway obstruction were enrolled in the study. AC activity was measured by cyclic AMP production. Resting, stimulated and potentiated AC activities and their relationships with clinical parameters, seasonal influences and serum cortisol levels were assessed.

Results The AC activity in resting, stimulated and potentiated cells varied during the year. AC activity was relatively low in the periods June–August and September–November, and higher in December–February and March–May. Receptor-mediated and potentiated responses expressed as percentage of the resting response were equivalent throughout the year. Serum cortisol levels were positively related to AC activity. No relationships were found between clinical parameters and AC activity or serum cortisol levels.

Conclusions These results indicate that AC activity in PBMCs of allergic asthmatics shows a seasonal variation. However, seasonal differences in AC activity seems to be unrelated with clinical parameters. Other factors such as serum cortisol levels may have a modulating influence on AC activity. Future studies of AC systems in blood cells of asthmatic patients need to take into account these seasonal influences.

Keywords: cyclic AMP, allergic asthma, potentiation, cortisol, circannual variation, PBMCs

Clinical and Experimental Allergy, Vol. 28, pp. 271–277. Submitted 31 December 1996; revised 18 July 1997; accepted 24 July 1997.

Introduction

Szentivanyi postulated in 1968 that an impaired β -adrenergic system was involved in the aetiology of asthma [1]. Since then, many studies have been undertaken to delineate the possible β -adrenergic defects in asthmatic patients. The

β -adrenergic signal transduction system consists of three parts: the β -adrenoceptor, a G-protein $\alpha\beta\gamma$ -complex and the adenylyl cyclase enzyme (AC), converting ATP into adenosine 3'-5' cyclic monophosphate (cAMP) [2]. The second messenger cAMP can activate protein kinase A, which in turn phosphorylates a range of proteins, thereby modulating responses of different cell types. In leucocytes, cAMP is involved in processes like proliferation, cytotoxicity and regulation of cytokine production [3–5]. Therefore, cAMP

Correspondence: Dr H. F. Kauffman, Department of Allergology, University Hospital, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.

has become a parameter of increasing importance in studies on the pathophysiology of asthma. A defect or modulation in one or more constituents of the β -adrenergic system may be of clinical relevance since reduced cAMP production results in concurrent changes in cell responses.

Studies in asthmatic patients have shown that both the number and agonist affinity of the β -adrenoceptors on peripheral blood mononuclear cells (PBMCs) are decreased [6,7]. Diminished cAMP production due to changes in signal transduction distal to the receptor have been reported also [8,9]. Furthermore, the crosstalk, i.e. the interaction between different signal transduction systems is impaired in PBMCs of allergic asthmatic patients [10,11]. After allergen challenge the β -adrenergic system in asthmatic patients can be dramatically affected [12,13]. It is well known that allergen exposure enhance airway hyperresponsiveness as well [14]. Van der Heide *et al.* [15] found a seasonal variation in the exposure of house dust mite, which was correlated with the level of airway hyperresponsiveness in asthmatic patients who were mono-sensitized to house dust mite. Therefore, long-term exposure to a relatively low allergen concentration may influence the severity of airway hyperresponsiveness as well as the β AR/AC system of blood cells in allergic asthmatics. However, non-allergic factors may also play a role in changing the β AR/AC activity. Endogenous glucocorticosteroid levels have been shown to change the β -adrenergic system activity in lymphocytes [16]. Another study revealed that cortisol enhanced AC activity in cultured human lung tumour cells [17]. Because variations in serum cortisol levels during the year have been reported in healthy subjects [18], we hypothesized that this might also influence AC activity in asthmatic subjects.

In this study we investigated the β AR/AC system by monitoring cAMP production of PBMCs from allergic patients with nocturnal airway obstruction. These patients were enrolled during a 19-month period, enabling us to investigate seasonal variations in the β AR/AC system in relation to clinical parameters and serum cortisol levels. Since previous studies have shown reduced potentiation of AC activity in the presence of cell-activating stimuli [19], we studied receptor mediated AC activity in the absence or presence of phytohaemagglutinin and calcium ionophore.

Materials and methods

Subjects

Forty-one non-smoking allergic asthmatics with nocturnal airway obstruction (mean age 28, SD 7 years) entered the study over a period of 19 months. All patients gave written informed consent. The study was approved by the hospital medical ethics committee.

Patients were included if they had a positive skin test to house dust mite and/or two other common allergens with a weal size ratio of allergen to histamine ≥ 0.7 or elevated specific IgE to these allergens (RAST class ≥ 2) [20] and airway hyperresponsiveness to methacholine (Mch) ($PC_{20}Mch \leq 9.6$ mg/mL) and to adenosine 5'-mono-phosphate (AMP) ($PC_{20}AMP \leq 80$ mg/mL). None of the patients had a respiratory tract infection nor an acute asthma exacerbation during 2 months prior to the study. All patients had a diurnal variation in peak flow of $> 15\%$.

Blood for PBMC stimulation was also drawn from eight healthy non-allergic subjects with no history of asthma (four female and four male; mean age: 29 years). Controls entered the study in the period from December to May.

Study design

The study consisted of three visits within one week. Airway reversibility was measured during the first visit. During the second visit at 08.00 h, peripheral blood was collected for cAMP measurements, cell differentiation and to obtain serum for cortisol determinations. Subsequently, FEV₁ and $PC_{20}Mch$ were measured. During the third visit, 1 day later, $PC_{20}AMP$ was measured. Prior to the study corticosteroids were withheld for 4 weeks and sodium cromoglycate, nedocromil sodium and histamine-antagonists were withheld for 2 weeks. Before every visit, inhaled long-acting and short-acting β_2 -agonists were withheld for 24 h and 8 h, respectively.

Lung function measurements

FEV₁ was measured using a dry spirometer (Vicat5, Mijndhardt, The Netherlands). Methacholine and AMP challenges were performed as described previously [21]. Doubling concentrations were used ranging from 0.038 to 19.6 mg/mL methacholine bromide and from 0.02 to 320 mg/mL AMP. The concentrations of methacholine and AMP causing 20% reduction of baseline FEV₁ were calculated by linear interpolation of the log-dose response curve. Airway reversibility was assessed with FEV₁ measured before and 15 min after inhalation of 400 μ g dry powder salbutamol, and expressed as percentage of the predicted FEV₁ [22].

Blood cell count and differential

The total number and differential of blood cells was determined using a Coulter Counter STKS (Coulter Electronics, Mijdrecht, The Netherlands).

PBMC isolation

Blood samples (40 mL) were collected in tubes with EDTA

as anticoagulant. PBMCs were isolated by density-gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway), and washed three times (2x 590 g; 1x 200 g) in Tris buffer (4 °C) containing 120 mM NaCl, 1 mM MgCl₂, 5 mM KCl, 0.6 mM CaCl₂, 25 mM Tris, 5 mM glucose and 0.1% human albumin, adjusted to pH 7.4 with HCl. The final cell suspension contained 2.5×10^6 cells per mL.

PBMC stimulation

Immediately after isolation, the cell suspension was divided into samples of 800 µL for stimulation. Ten minutes before stimulation the cell samples were preincubated at 37 °C in the presence of 0.5 mM 3-isobutyl-1-methyl-xanthine (Sigma chemicals Co, St Louis Mo, USA) to inhibit phosphodiesterase activity. After preincubation, the cell samples were stimulated in duplicate for 10 min by adding 100 µL of a stimulus or Tris buffer for determining the basal cAMP production. (-)Isoprenaline (ISO; Sigma, final concentration 10^{-6} M) was used for stimulating the β_2 -receptors; prostaglandin E₂ (PGE₂; Sigma, final concentration 10^{-6} M) was used to stimulate the AC pathway via the PGE₂-receptor. Phytohaemagglutinin (PHA) (Wellcome Diagnostics, Dartford UK; final concentration 4.5×10^{-5} g/mL) was used for indirect stimulation of the AC via other signal transduction pathways. Finally, calcium-ionophore A23187 (CaI; Sigma, final concentration 10^{-5} M) was used to stimulate Ca²⁺-dependent AC. To study the potentiated responses, by stimulating the AC via different routes, PHA or CaI were used in combination with ISO, at the same concentration as used for the single stimuli experiments.

Stimulation was stopped by adding 100 µL 2 M HCl/0.1 M EDTA followed by incubation at 80 °C for 10 min. After centrifugation, the supernatants were collected and neutralized with excess CaCO₃. After removing the CaCO₃ the supernatants were frozen at -20 °C pending cAMP determination.

cAMP measurements

Cyclic AMP concentration was measured by using an enzyme immunoassay (Biotrak, Amersham UK) with a detection range of 12.5–3200 femtomol/well. cAMP concentrations were expressed as pmol/10⁶ cells.

Cortisol measurements

Cortisol levels in sera were measured using a radioimmunoassay utilizing tritium labelled cortisol and an antiserum raised against a bovine serum albumin conjugate with cortisol-21-acetate-3-(o-carboxymethyl)-oxine. The results of this assay satisfy the usual criteria of analytical accuracy. The assay results conform to internal- and external quality

control procedures. The results agree with those of gas chromatography and mass spectrometry. This assay has a sensitivity of 10 pmol/mL.

Data analysis

The potentiated cAMP responses evoked by stimulation with PHA + ISO and CaI + ISO were also expressed as percentage potentiation compared with the cells stimulated by ISO alone. A correction was made for basal production with the formula:

$$\text{Potentiation} = \frac{[\text{X} + \text{ISO}] \text{ induced cAMP production} - \text{X induced cAMP production}}{\text{ISO induced cAMP production} - \text{basal cAMP production}} \times 100\%$$

X = PHA or CaI.

Normal distribution was checked by the Kolmogorov–Smirnov test. PC₂₀Mch and PC₂₀AMP were log transformed for normal distribution. Seasons were chosen as 3-month periods starting from December [15]. One-way analysis of variance was used for comparisons of both clinical data and cAMP productions between the seasons. Student's *t*-test for unpaired observations was used for comparisons between patients and controls. Correlations were calculated using Pearson's correlation coefficient. Statistical analyses were performed using the statistical package SPSS/PC version 4.01 for personal computers (SPSS Inc. Chicago Ill. USA). *P*-values < 0.05 were considered to be significant. Data are presented as mean values $\pm$ SEM, unless otherwise stated.

Results

Patient characteristics

The clinical data of the 41 patients, grouped by season, are summarized in Table 1. No significant differences were observed between the seasons for all variables listed.

Seasonal variation in cAMP production in PBMCs

The cAMP production of isolated PBMC from the patients showed a seasonal variation (Fig. 1). This was observed for the single stimuli PGE₂ and ISO and for the potentiated responses. PGE₂ stimulated values in the periods June–August (31.0 ± 3.3 pmol/10⁶ cells) and September–November (20.4 ± 4.3) were significantly lower compared with values in December–February (52.3 ± 6.9). For ISO significant differences were observed in September–November compared with December–February (9.6 ± 1.9 vs 23.2 ± 3.3). The basal (= unstimulated) cAMP production and cAMP production induced by PHA or CaI (data not shown) showed the same trends without reaching statistical significance. The cAMP production in potentiated responses induced by PHA + ISO and CaI + ISO were significantly

Table 1. Patient characteristics

	Season			
	December–February	March–May	June–August	September–November
Age (years)	27.3 (1.6)	26.0 (1.9)	29.5 (2.5)	31.6 (3.4)
Gender (m/f)	7/5	5/7	7/5	1/4
FEV ₁ (% pred.)	85.0 (4.5)	80.5 (6.9)	83.7 (6.8)	78.5 (8.3)
PC ₂₀ Mch (mg/mL)	0.14 (0.05–0.74)	0.10 (0.015–0.71)	0.09 (0.015–0.76)	0.24 (0.05–0.99)
PC ₂₀ AMP (mg/mL)	0.53 (0.03–11.89)	0.45 (0.03–3.13)	0.62 (0.03–15.00)	0.34 (0.08–1.42)
Peakflow variation (%)	26.9 (3.3)	20.4 (2.5)	24.2 (3.0)	26.9 (17.6)
Reversibility (% of pred.)	16.9 (2.9)	10.0 (2.6)	12.5 (2.2)	10.9 (5.7)
Eosinophils (no/mm ³)	449 (49)	459 (49)	587 (103)	568 (175)

Data are expressed as mean (SEM) except for PC₂₀Mch and PC₂₀AMP, which are geometric means with ranges between parentheses.

lower in September–November (14.8 ± 3.0 and 15.9 ± 3.2) than December–February (41.9 ± 6.6 and 49.1 ± 6.2) and March–May (40.5 ± 4.7 and 47.0 ± 6.3), $P < 0.05$. When stimulated cAMP production was expressed as percentage of the basal production, no significant differences were seen between the seasons. Results for PGE₂ and ISO are shown in Fig. 2. No differences in blood cell count and differential were observed between the seasons. The isolated PBMCs consisted of $75.0 \pm 1.19\%$ lymphocytes and $25.0 \pm 1.19\%$ monocytes.

Positive correlations ($r = 0.47$ – 0.96 , $P < 0.005$) were

found between the basal and stimuli-induced cAMP productions in all 41 patients studied. There were also significant positive correlations between the different stimulated responses when compared with each other (data not shown).

The percentage potentiation calculated for PHA + ISO and CaI + ISO responses was not different between the seasons. For all seasons together, these values were $189 \pm 10\%$ for PHA + ISO and $210 \pm 17\%$ for CaI + ISO. No significant relationships were found between cAMP production or percentage potentiation of PBMCs and any clinical parameter tested in and between the different seasons.

The cAMP production of isolated PBMCs from healthy subjects, measured in the period from December until May

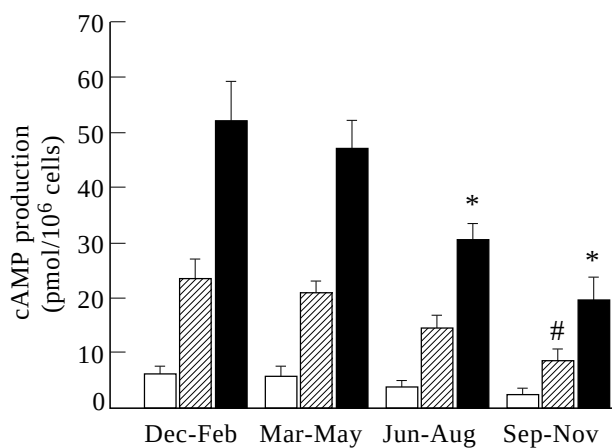


Fig. 1. Seasonal cyclic AMP production in PBMCs of allergic asthmatics. Basal production (□) and production after stimulation with ISO (▨) and PGE₂ (■). * PGE₂ induced cAMP production was decreased in June–August and September–November compared with December–February and March–May ($P < 0.05$). # ISO induced cAMP production was decreased in September–November compared with December–February ($P < 0.05$). Error bars represent SEM.

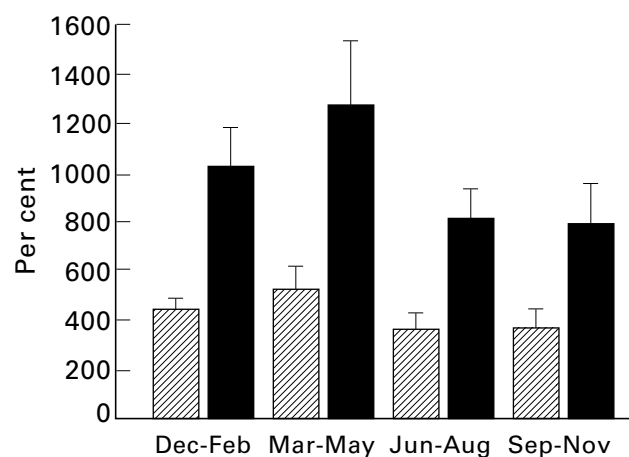


Fig. 2. Seasonal cyclic AMP production in PBMCs after stimulation by ISO (▨) and PGE₂ (■) expressed as percentage of basal (unstimulated) production. No significant differences were observed within the groups. Error bars represent SEM.

Table 2. cAMP production of PBMCs from patients and healthy controls

	Patients	Healthy controls
<i>n</i>	24	8
Basal	6.3 (0.9)	5.8 (1.4)
PGE ₂	49.7 (4.1)	40.4 (5.0)
ISO	22.5 (1.9)	21.0 (3.0)

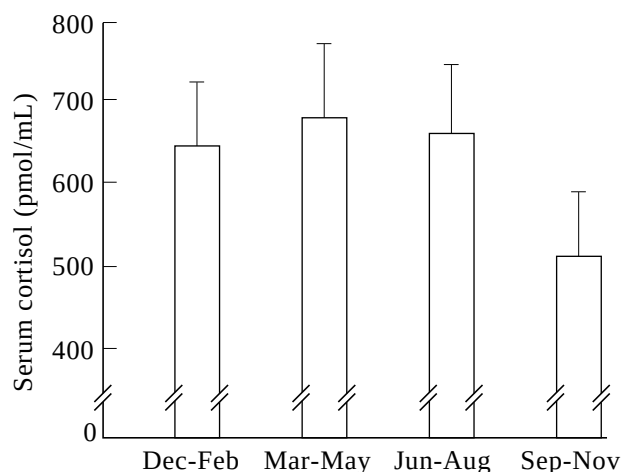
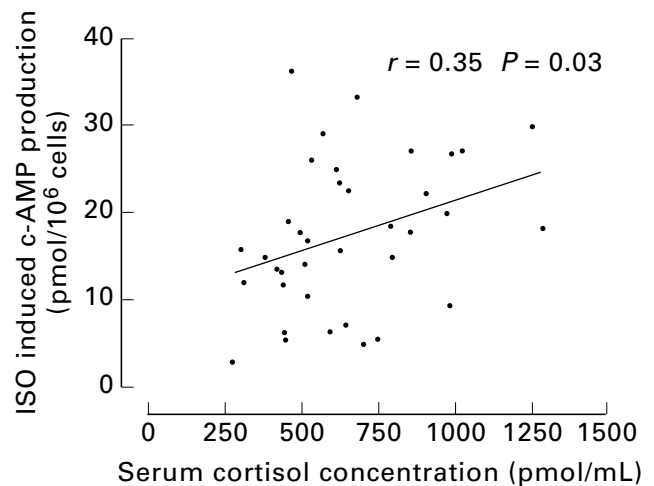
cAMP productions are expressed in pmol/10⁶ cells. The values refer to the period December–May. No significant differences were observed between the two groups. Values are expressed as mean (SEM).

were not different from the patient's values in the same period (Table 2). In the healthy control group there were also no differences in cAMP production between the periods December–February and March–May (data not shown).

Cortisol levels in serum showed a seasonal variation with lowest levels in September–November and highest levels in March–May (Fig. 3), although this did not reach statistical significance. Comparison of the cortisol levels with cAMP production showed a positive correlation with the ISO stimulated cAMP production ($P < 0.05$, Fig. 4). The basal and other stimulated cAMP responses also showed positively related trends with cortisol levels but this did not reach statistical significance. No significant relationships were found between cortisol levels and any clinical parameter.

Discussion

Cyclic AMP is an important second messenger in the

**Fig. 3.** Seasonal variation in serum cortisol levels. Error bars represent SEM.**Fig. 4.** Correlation between serum cortisol levels and ISO induced cAMP production in PBMCs.

regulation of cell responses, such as cytokine production in leucocytes. Since studies on the effects of treatment by drugs that influence the AC system are performed during long lasting periods it is important to know whether the changes observed can be ascribed to drug treatment or to seasonal variations. Therefore we assessed possible seasonal variations in β AR/AC activity in PBMCs of asthmatic patients. Lower AC activity was found in the periods June–August and September–November and higher AC activity in the periods December–February and March–May. There may be several explanations for this observation. Seasonal changes in exposure to allergens may stimulate inflammatory processes in the airways. Released mediators may attenuate the AC activity in peripheral blood cells [23,24]. Van der Heide *et al.* [15] reported variations in house dust mite allergen exposure throughout the year with highest exposure in the period June–October. In the present study, AC activity was least during this period of high house dust mite exposure. However, the patients we studied had multiple allergies, most being allergic to house dust mite and some also to pollen. Although decreased AC activity in the period March–May for pollen allergic patients might be expected, no decrease in AC activity was observed in this subgroup (data not shown), suggesting that other factors are involved.

Gamboa *et al.* [25] showed a decrease in the numbers of β -adrenoceptors on PBMCs in pollen allergic subjects in periods of high exposure to pollen. Meurs *et al.* [13] also found a decrease in β -adrenoceptor density in PBMCs in allergic asthmatics after allergen challenge. Additionally, they showed a diminished cAMP production mainly due to post receptor changes at the level of G-protein–AC interaction. In the present study, both PGE₂ and ISO stimulated

cells showed a seasonal variation in cAMP production, indicating that the changes are not only β -adrenoceptor specific. When expressing the ISO and PGE₂ responses as percentage of the basal productions (Fig. 2) no significant differences could be observed between the seasons. This suggests that the changes are due to changes in signal transduction at the post receptor level. Therefore, these data do not support the hypothesis that a specific β -adrenoceptor dysfunction, as postulated by Szentivanyi [1], exists in this group of patients with severe airway hyperresponsiveness.

In contrast to the findings with AC activity, we did not find any statistically significant seasonal differences in the clinical parameters tested. This might result from the fact that the patients in this study were allergic to more than one allergen with different exposure periods over the year. Studies with mono sensitized subjects [15] showed worsening of the clinical parameters in the period with high specific allergen exposure.

Because no relationships were found between clinical parameters and AC activity, we investigated whether the observed seasonal changes in AC activities might be associated to seasonal variation of other factors. Endogenous serum cortisol is one factor that may also change AC activities [17,26]. Agrimonti *et al.* [18] found a circannual variation in plasma cortisol levels in healthy subjects, with a peak in the winter and lower levels during spring and summer. Del Ponte *et al.* [27] have shown similar plasma cortisol variations. Our study confirmed the same trend in circannual variation of serum cortisol levels in asthmatic patients as well. The AC activity of PBMCs was positively correlated with the serum cortisol levels reaching statistical significance for the ISO-stimulated AC responses. Therefore, we suggest that AC activity in PBMCs can be partly influenced by the serum cortisol levels. It is known that glucocorticosteroids exerts a positive influence on the β AR-cAMP cascade. β -adrenoceptor gene transcription is under control of glucocorticosteroids, which enhances the transcription rate and upregulates the number of β -adrenoceptors [17,28,29]. In asthmatic patients glucocorticosteroids can prevent or reverse downregulation of β -adrenoceptors during β_2 -agonist therapy [30,31]. According to our data we have no evidence of a sole enhancement of β -adrenoceptor numbers in PBMC's, since basal and prostaglandin E₂ induced cAMP production were also positively related with serum cortisol levels. Michel *et al.* [32] found that glucocorticosteroids enhance the activity of the post receptor pathways by altering the adenylyl cyclase catalyst in human lymphocytes. Our data are in agreement with their observations, while the cAMP productions evoked by basal and nonreceptor mediated stimuli (CaI) were also, however, not statistically significant, positively related with serum cortisol levels. Furthermore, all tested cAMP responses

showed a strong correlation with each other, suggesting that a common postreceptor constituent, e.g. the adenylyl cyclase enzyme is involved.

Changes in intracellular crosstalk between different signal transduction systems seems to be related with asthma [10]. Recently, Dooper *et al.* [19] found a relation between the percentage potentiation and airway responsiveness to methacholine, suggesting that changes in crosstalk are involved. Our patients, however, did not show a (seasonal) decrease in percentage potentiation. Therefore, a modulation in AC activity by crosstalk seems unlikely in this group of patients.

The circannual variation in AC activity and serum cortisol levels is not reflected in seasonal changes of clinical parameters in this group of patients. In asthma, reduced lung function and bronchial hyperreactivity are the result of an inflammatory process in the airways. Infiltrating T-lymphocytes are playing an important role in this process by producing proinflammatory cytokines. Elevation of cAMP in these cells can decrease their activation state and cytokine production and hence the degree of inflammation [4,5]. The changes in cAMP production found in our study seems not to have an influence on the inflammatory process in the airways, as measured by clinical parameters. Although we measured cAMP in peripheral cells, it seems reasonable that infiltrating and perhaps resident mononuclear cells in the airways display a similar cAMP producing activity.

In summary, AC activity in PBMCs changes over the year in allergic asthmatic patients. This seems not to be related with clinical parameters. Our data suggest that the observed changes in AC activity are, in part, due to circannual variations of serum cortisol levels. Therefore, studies of AC activity in blood cells of asthmatic patients needs to take serum cortisol levels into account.

Acknowledgements

We thank Glaxo Wellcome BV, the Netherlands for financial support, Miss Marlies Koning for assistance in collecting the data, and Dr A. E. J. Dubois, MD, PhD for critical reading and correction of the manuscript.

References

- 1 Szentivanyi A. The beta adrenergic theory of the atopic abnormality in bronchial asthma. *J Allergy* 1968; 42:203–32.
- 2 Nijkamp FP, Engels F, Henricks PAJ, Van Oosterhout AJM. Mechanisms of beta-adrenergic receptor regulation in lungs and its implications for physiological responses. *Physiological Rev* 1992; 2:323–67.
- 3 Novak TJ, Rothenberg EV. cAMP inhibits induction of interleukin 2 but not of interleukin 4 in T cells. *Proc Natl Acad Sci U S A* 1990; 87:9353–7.

- 4 Borger P, Kauffman HF, Vijgen JIJ. Activation of the cAMP-dependent signaling pathway downregulates the expression of interleukin-3 and granulocyte macrophage colony-stimulating factor in activated human T lymphocytes. *Exp Haematol* 1996; 24:108–15.
- 5 Borger P, Kauffman HF, Postma DS. IL-4 gene expression in activated human T-lymphocytes is regulated by the cAMP-dependent signaling pathway. *Blood* 1996; 87:691–8.
- 6 Gamboa PM, Sanz ML, de la Cuesta CG et al. Number of beta-receptors in rhinitic pollinic patients. *J Invest Allergol Clin Immunol* 1991; 2:113–21.
- 7 Connolly MJ, Crowley JJ, Nielson CP et al. Peripheral mononuclear leucocyte beta adrenoceptors and non-specific bronchial responsiveness to methacholine in young and elderly normal subjects and asthmatic patients. *Thorax* 1994; 49:26–32.
- 8 Meurs H, Kauffman HF, Koëter GH et al. Regulation of the beta-receptor-adenylate cyclase system in lymphocytes of allergic patients with asthma: possible role for protein kinase C in allergen-induced nonspecific refractoriness of adenylate cyclase. *J Allergy Clin Immunol* 1987; 80 (3):326–39.
- 9 Dooper MWSM, Timmermans A, Aalbers R et al. Desensitization of the adenylate cyclase system in peripheral blood mononuclear cells from patients with asthma three hours after allergen challenge. *J Allergy Clin Immunol* 1993; 92:559–66.
- 10 Meurs H, Hoiting B, Roffel A et al. eds. *Bronchitis V, Proceedings of the fifth international symposium on advances in understanding of asthma and COPD*, Groningen, The Netherlands, 9–11 June, 1993. Assen, The Netherlands: Van Gorcum, 1994; Cross-talk between receptor transduction mechanisms in relation to airways obstruction. p. 135–48.
- 11 Dooper MWSM, Timmermans A, Weersink EJM et al. Defect in potentiation of adenylate cyclase correlates with bronchial hyperreactivity. *J Allergy Clin Immunol* 1995; 96:628–34.
- 12 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 attack. *J Allergy Clin Immunol* 1982; 70:272–80.
- 13 Meurs H, Kauffman HF, Timmermans A et al. Specific immunological modulation of lymphocyte adenylate cyclase in asthmatic patients after allergenic bronchial provocation. *Int Arch Allergy Appl Immunol* 1986; 81:224–9.
- 14 Ihre E, Zetterstrom O. Increase in non-specific bronchial responsiveness after repeated inhalation of low doses of allergen. *Clin Exp Allergy* 1993; 23:298–305.
- 15 van der Heide S, de Monchy JGR, De Vries K et al. Seasonal variation in airway hyperresponsiveness and natural exposure to house dust mite allergens in patients with asthma. *J Allergy Clin Immunol* 1994; 93:470–5.
- 16 Allolio B, Ehses W, Steffen HM, Muller R. Reduced lymphocyte beta 2-adrenoceptor density and impaired diastolic left ventricular function in patients with glucocorticoid deficiency. *Clin Endocrinol (Oxf)* 1994; 40:769–75.
- 17 Nakane T, Szentendrei T, Stern L et al. Effects of IL-1 and cortisol on beta-adrenergic receptors, cell proliferation, and differentiation in cultured human A549 lung tumor cells. *J Immunol* 1990; 1:260–6.
- 18 Agrimonti F, Angeli A, Frairia R et al. Circannual rhythmicities of cortisol levels in the peripheral plasma of healthy subjects. *Chronobiologia* 1982; 9:107–14.
- 19 Dooper MWSM, Hoekstra Y, Timmermans A et al. Potentiation of adenylate cyclase in human peripheral blood mononuclear cells by cell-activation stimuli. *Biochem Pharmacol* 1994; 47:289–94.
- 20 Brand PLP, Kerstjens HAM, Jansen HM et al. Interpretation of skin tests to house dust mite and relationship to other allergic parameters in patients with asthma and COPD. *J Allergy Clin Immunol* 1993; 91:560–70.
- 21 Oosterhoff Y, Koëter GH, de Monchy JGR, Postma DS. Circadian variation in airway responsiveness to methacholine, propranolol, and AMP in atopic asthmatic subjects. *Am Rev Respir Dis* 1993; 147:512–7.
- 22 Brand PL, Quanjer PH, Postma DS et al. Interpretation of bronchodilator response in patients with obstructive airways disease. The Dutch Chronic Non-Specific Lung Disease (CNSLD) Study Group. *Thorax* 1992; 47:429–36.
- 23 Barnes PJ. Beta-adrenoceptors and asthma. *Clin Exp Allergy* 1993; 23:165–7.
- 24 Van Oosterhout AJ, Stam WB, Nijkamp FP. Effect of lymphokines on beta-adrenoceptor stimulation of human PBMC. *Agents Actions Suppl* 1990; 31:163–7.
- 25 Gamboa PM, Sanz ML, de la Cuesta CG et al. Number of beta-receptors in rhinitic pollinic patients. *J Invest Allergol Clin Immunol* 1991; 1:113–21.
- 26 Skinner SJ, Somervell CE, Lowe C. Interaction between prostacyclin and cortisol in fetal lung cells: effects on cAMP production. *Prostaglandins* 1991; 4:331–44.
- 27 Del Ponte A, Guagnano MT, Sensi S. Time-related behaviour of endocrine secretion: circannual variations of FT3, cortisol, HGH and serum basal insulin in healthy subjects. *Chronobiol Int* 1984; 4:297–300.
- 28 Hadcock JR, Wang HY, Malbon CC. Agonist-induced destabilization of beta-adrenergic receptor mRNA. Attenuation of glucocorticoid-induced up-regulation of beta-adrenergic receptors. *J Biol Chem* 1989; 264:19928–33.
- 29 Mak JC, Nishikawa M, Shirasaki H et al. Protective effects of a glucocorticoid on downregulation of pulmonary beta 2-adrenergic receptors in vivo. *J Clin Invest* 1995; 96:99–106.
- 30 Samuelson WM, Davies AO. Hydrocortisone-induced reversal of beta-adrenergic receptor uncoupling. *Am Rev Respir Dis* 1984; 130:1023–6.
- 31 Svedmyr N. Action of corticosteroids on beta-adrenergic receptors. Clinical aspects. *Am Rev Respir Dis* 1990; 141:S31–8.
- 32 Michel MC, Knapp J, Ratjen H. Sensitization by dexamethasone of lymphocyte cyclic AMP formation: evidence for increased function of the adenylate cyclase catalyst. *Br J Pharmacol* 1994; 113:240–6.