

Effects of cytokines on β -adrenoceptor function of human peripheral blood mononuclear cells and guinea pig trachea

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In asthma, a β -adrenoceptor dysfunction may be the consequence of an active disease state rather than a fundamental abnormality. In the present study the possible involvement of T lymphocytes in β -adrenergic impairment was investigated by studying the effects of lymphocyte-derived mediators on β -adrenoceptor function of human peripheral blood mononuclear cells (PBMCs) and guinea pig trachea. Supernatants of phytohemagglutinin- or concanavalin A-activated PBMCs from either persons with asthma or healthy persons inhibited isoprenaline stimulated cyclic adenosine 3',5'-monophosphate (cAMP) production of PBMCs after 20 hours of preincubation. These supernatants also inhibited β -adrenoceptor function of PBMCs from patients with asthma to the same extent. The isoprenaline stimulated cAMP production of PBMCs was not altered after a 2-hour preincubation period with human interleukin-1 (IL-1), IL-2, IL-3, IL-4, granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon (IFN- γ). In contrast, after 20 hours of preincubation, stimulated cAMP production of PBMCs was significantly diminished, with 63% by IL-1 (40 U/ml, $p < 0.01$), with 36% by IL-2 (100 U/ml, $p < 0.05$), with 37% by IFN- γ (1000 U/ml, $p < 0.05$), and with 21% by GM-CSF (100 U/ml, $p < 0.05$). Preincubation of guinea pig tracheal segments with IL-1, IL-2, IL-4, or GM-CSF during 1 or 3 days did not affect the EC_{50} values or the maximal relaxation of isoprenaline dose response curves. (J ALLERGY CLIN IMMUNOL 1992;90:340-8.)

Key words: β -adrenoceptor, cAMP, cytokines, mononuclear cells, trachea

Szentivanyi¹ suggested that the pathophysiology and the symptoms of asthma, such as the characteristic airway hyperresponsiveness, eosinophilia, mucosal edema, and increased release of mast cell-derived mediators could be explained by a fundamental β -adrenergic blockade. Despite extensive studies, the status of β -adrenoceptor function in patients with asthma remains controversial. The β -adrenergic agonist isoprenaline was found to be less potent in human asthmatic bronchi than in nondiseased tissue in

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Abbreviations used

cAMP:	Cyclic adenosine 3',5'-monophosphate
BSA:	Bovine serum albumin
Con A:	Concanavalin A
EC ₅₀ :	Concentration causing 50% of the maximal effect
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IL:	Interleukin
IFN:	Interferon
nh:	Natural human
PI-3:	Parainfluenza-3
PHA:	Phytohemagglutinin
PBMC:	Peripheral blood mononuclear cell
rh:	Recombinant human
BAL:	Bronchoalveolar lavage
PAF:	Platelet-activating factor
FCS:	Fetal calf serum
NEP:	Neutral endopeptidase
SPF:	Specific-pathogen free

vitro.²⁻⁴ In contrast, Svedmyr et al.⁵ showed no difference in bronchial β -adrenoceptor function between persons with asthma and healthy persons in vitro. Peripheral blood mononuclear cells (PBMCs) are often used as an in vitro model to study and characterize β -adrenoceptor function in asthma.^{6, 7} Brooks et al.⁶ and Cerrina et al.³ showed an inverse relationship between the severity of asthma in vivo and β -adrenoceptor function on PBMCs or airway smooth muscle in vitro. The reduced β -adrenoceptor function in patients with asthma might be partially due to β -adrenergic agonist therapy, resulting in tachyphylaxis. However, a β -adrenergic impairment has also been found on lymphocytes and airway smooth muscle of asthmatic subjects who are free of adrenergic therapy.^{3, 8} Probably there are other factors that can inhibit β -adrenoceptor function. It has been shown that lymphocyte β -adrenoceptor number and function are decreased in drug free persons with asthma 24 hours after an allergen challenge as compared with before challenge.⁷ Furthermore, viral respiratory tract infections impair β -adrenoceptor function on leukocytes.⁹ Hence, it appears that β -adrenoceptor dysfunction may be the consequence of the inflammatory sequelae in the airways.¹⁰

In a guinea pig model of atopy, in which the animals were injected with bacterial endotoxin, a decreased airway and lymphocyte β -adrenoceptor function has been observed.¹¹ Furthermore, it was shown that this β -adrenergic impairment could be prevented by splenectomy or cyclosporin A treatment, a T-lymphocyte selective immunosuppressive drug.^{11, 12} These data suggest that the activation of T lymphocytes and the decrease of β -adrenoceptor function in this animal model may be related. Studies concerning allergen challenge of persons with asthma have provided evidence for a selective increase in helper T lymphocytes in bronchoalveolar lavage (BAL) fluid accompanying late asthmatic reactions, with a complementary fall in the number of helper cells in the peripheral blood.¹³⁻¹⁵ Furthermore, increased interleukin (IL)-2 receptor expression has been demonstrated in the bronchial mucosa of patients with stable asthma, indicating infiltration and activation of lymphocytes.¹⁶ In addition, IL-2, 3, 4, 5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNA expression has been demonstrated in biopsies and BAL cells of patients with asthma.^{17, 18} These data suggest an involvement of lymphocytes and the cytokines they elaborate in the pathogenesis of asthma. Cytokines, in addition to other inflammatory mediators such as platelet-activating factor (PAF),¹⁹ may be involved in the development of β -adrenoceptor dysfunction in leukocytes and airways of persons with asthma.

In the present study we investigated whether supernatants of activated lymphocytes or purified recombinant cytokines can influence β -adrenoceptor function on PBMCs or airway smooth muscle.

MATERIAL AND METHODS

Materials

Phytohemagglutinin (PHA) was obtained from Wellcome Foundation Ltd. (Dartford, England). Concanavalin A (Con A) was obtained from Calbiochem Corporation, San Diego, Calif. Isoprenaline was obtained from OPG (Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands). Arecoline, 3-isobutyl-1-methyl-xanthine, and bovine serum albumin (BSA) were purchased from Sigma Chemical Company, St. Louis, Mo. Nembutal, containing 60 mg/ml pentobarbitone sodium, was purchased from Abbot Laboratories (North Chicago, Ill.). Fetal calf serum (FCS) was obtained from Gibco Laboratories (Grand Island, N.Y.). Cytokines were purchased from Genzyme Corporation (Boston, Mass.). Specific activities of the cytokines were for natural human IL-1 (nhIL-1) 10^8 U/mg, for recombinant human IL-1 β (rhIL-1) 10^8 U/mg, for natural human IL-2 (nhIL-2) 7×10^4 U/mg, for recombinant human IL-2 (rhIL-2) 2.5×10^8 U/mg, for recombinant human GM-CSF (rhGM-CSF) 5×10^7 CFU/mg, for recombinant human IL-3 (rhIL-3) 10^8 colony-forming unit (CFU)/mg, for recombinant human IL-4 (rhIL-4) 10^8 U/mg, and for natural human interferon (IFN)- γ 1×10^7 U/mg. All cytokines had a purity level of greater than 95%.

Preparation of PBMC supernatants

PBMCs were isolated from the blood of healthy volunteers or from the blood of patients with allergic asthma. Isolation was performed by centrifugation on a Ficoll paque gradient (20 minutes, 1000 g) according to the method of Böyum.²⁰ Cells at the interface were removed and washed three times with RPMI 1640 supplemented with 50 μ g/ml gentamicin (10 minutes, 500 g). Finally, PBMCs were suspended in RPMI 1640 containing 5% FCS at a concentration of 5×10^6 cells/ml. Next PBMCs were incubated for 4 hours in the presence of Con A (10 μ g/ml), PHA (100 μ g/ml), or in the absence of these T-cell mitogenic stimuli. After a 4-hour incubation period, cells were washed three times (5 minutes, 500 g) and suspended in RPMI 1640 without FCS and mitogenic stimuli, and cultured for another 20 hours. Finally, the supernatants were harvested, sterile filtered over a 22 μ m filter, and stored at -20° C.

Measurement of β -adrenoceptor function

PBMCs were isolated from the blood of healthy volunteers, or from buffy coats (Bloodbank Utrecht, The Netherlands) as described above. After the freshly isolated PBMCs had been washed, the cells were suspended in RPMI 1640 at 3×10^7 cells/ml in a volume of 250 mm³ and preincubated in the presence or absence of cytokines or previously prepared supernatants of PBMCs (1:4 dilution) for 2 hours or 20 hours. The cytokines tested were nhIL-1, nhIL-2, rhIL-3, rhIL-4, rhGM-CSF, nhIFN- γ . Preincuba-

tions were carried out at 37° C in a humidified atmosphere of 5% CO₂/95% air. In an additional series of experiments, PBMCs isolated from the blood of patients with allergic asthma were incubated for 20 hours with previously prepared PHA supernatants (from healthy persons). After preincubation of PBMCs for 2 hours or 20 hours with or without cytokines or supernatants, PBMCs (3×10^6 cells/ml) were subsequently incubated for 5 minutes with or without 10^{-6} mol/L isoprenaline. This incubation was carried out in a shaking water bath at 37° C. Incubations were carried out in the presence of 0.5 mmol/L 3-isobutyl-1-methyl-xanthine in a final volume of 200 mm³. The reaction was stopped by means of heating the cell suspensions in a boiling water bath for 5 minutes. Precipitated protein was centrifugated for 15 minutes at 1500 g, and 50 mm³ of the supernatant was used to measure cyclic adenosine 3',5'-monophosphate (cAMP) content. cAMP was determined by a competitive protein binding method with use of a cAMP assay kit (Amersham, Great Britain). cAMP values of PBMCs were expressed as a percentage of the amount of cAMP produced by PBMCs incubated with buffer. The significance of differences between PBMCs incubated with buffer and PBMCs incubated with the different cytokines or supernatants were tested with a Student's paired *t* test and considered significant at $p < 0.05$. The results are expressed as the mean $\pm$ SEM.

Trachea incubations

Male Dunkin-Hartley guinea pigs weighing 300 to 350 gm (Ola Ltd., Bicester, England) were killed by an intraperitoneal injection of approximately 180 mg pentobarbitone sodium (Nembutal). The trachea was removed, placed in oxygenated (95% O₂, 5% CO₂) Krebs buffer, and dissected free from connective tissue and blood vessels. The composition of the Krebs buffer was (in mmol/L): NaCl, 118.1; KCl, 4.7; CaCl₂ $\times$ 2H₂O, 2.5; MgSO₄ $\times$ 7H₂O, 1.2; NaHCO₃, 25.0; KH₂PO₄, 1.2; and glucose, 8.3. The trachea was transversely cut in parts of two cartilage rings. Accordingly, tracheal rings were preincubated for 24 hours or 72 hours in the presence or absence of cytokines in a volume of 3 ml RPMI 1640 supplemented with 0.01% BSA and 50 μ g/ml gentamicin. Preincubations were carried out at 37° C in a humidified atmosphere of 5% CO₂, 95% air. After preincubation, tracheal rings were mounted in 12 ml organ baths filled with oxygenated Krebs buffer (pH 7.4, 37° C). The Krebs buffer was continuously gassed with a 5% CO₂, 95% air mixture. Changes in tonus of the tracheal rings were directly recorded by an isometric transducer (Harvard Apparatus, Ltd., Edenbridge, England) that was integrated in a semiautomatic organbath setup, comprising a Philips NMS 9100, Burr Brown PCI 20.000 interface and OBA-NSP software (InForMed. B.V. IJmuiden, The Netherlands). This setup enabled continuous sampling and real-time display of the response on the computer screen of up to 12 organ baths. In a second series, epithelium denudation of the tracheas was performed with a cotton swab as described previously.²¹ In a third series of experiments the animals were infected with parainfluenza-3 (PI-3) virus as described previously 4 days before isolation of the tracheas.²² Before the experiments tracheal rings were equilibrated for approximately 30 minutes at a tonus of 2.0 gm.

After this equilibration period, tracheal rings were submaximally precontracted with the cholinergic muscarinic receptor agonist arecoline (10^{-5} mol/L). β -adrenergic receptor function of the tracheal rings was determined by a cumulative concentration response curve to the β -adrenergic agonist isoprenaline. In the results, only maximal relaxation and EC₅₀ values of the mean isoprenaline concentration response curves are presented. From each trachea, maximal relaxation was calculated as the percentage of the precontraction to arecoline. EC₅₀ values, and maximal relaxation values were tested with a Student's two-tailed, paired *t* test and considered significant if $p < 0.05$.

RESULTS

Effects of supernatants on PBMC β -adrenoceptor function

In preliminary studies it was observed that isoprenaline induced a concentration-dependent increase of cAMP production in PBMCs (data not shown). In further experiments a submaximal concentration of isoprenaline (10^{-6} mol/L) was used to test the influence of preincubation with supernatants or various cytokines on PBMC β -adrenoceptor function. In Fig. 1, A the influence of different supernatants on isoprenaline (10^{-6} mol/L) stimulated cAMP production of PBMCs from healthy donors after a 20-hour preincubation period are presented. No significant inhibition of cAMP production was observed in PBMCs incubated with supernatants of nonactivated PBMCs derived from either healthy persons or persons with asthma (five and six supernatants, respectively). A significant inhibition occurred in cells incubated with supernatants of PHA- or Con A-activated PBMCs of healthy persons (five and six supernatants, respectively) as compared with PBMCs incubated with medium or with nonactivated supernatants. This inhibition amounted $35.5\% \pm 8.6\%$ ($p < 0.01$) and $30.8\% \pm 6.7\%$ ($p < 0.01$), respectively. In addition, supernatants of PHA- or Con A-activated PBMCs obtained from persons with asthma (five and five supernatants, respectively) inhibited isoprenaline stimulated cAMP production with $40.2\% \pm 4.1\%$ ($p < 0.01$) and $36.1\% \pm 7.1\%$ ($p < 0.01$), respectively, compared with medium incubated PBMCs (Fig. 1, B). PHA supernatants (three supernatants) significantly ($p < 0.05$) decreased isoprenaline stimulated cAMP production in PBMCs derived from donors with asthma ($n = 3$) with $39\% \pm 12\%$ (Fig. 1, C). None of the supernatants affected basal cAMP content of the PBMCs.

Effects of cytokines on PBMC β -adrenoceptor function

Table I presents the isoprenaline (10^{-6} mol/L) stimulated cAMP production of the PBMCs after a 2-hour preincubation period with different cytokines at various concentrations. None of the tested cytokines af-

affected the stimulated cAMP production of PBMCs after a 2-hour preincubation. Moreover, none of the tested cytokines altered basal cAMP content of PBMCs after 2 hours of preincubation. As shown in Fig. 2, 20-hour preincubation of PBMCs with IL-1, IL-2, IFN- γ , and GM-CSF resulted in a dose-dependent inhibition of the isoprenaline stimulated cAMP production. The inhibition of cAMP production by human PBMCs incubated with IL-1 was significant at 10 U/ml ($p < 0.05$) and 40 U/ml ($p < 0.01$) and amounted to $28.7\% \pm 6.7\%$ and $63.1\% \pm 6.4\%$, respectively. IL-2 and GM-CSF both induced an inhibition of cAMP production by PBMCs that became significant at 100 U/ml ($p < 0.05$) and amounted to $36.2\% \pm 8.5\%$ and $36.9 \pm 10.7\%$, respectively. The inhibition of cAMP production by IFN- γ became significant at 1000 U/ml ($p < 0.05$) and amounted to $21.0\% \pm 6.8\%$. No changes in isoprenaline stimulated cAMP production were observed in PBMCs incubated with rhIL-3 or rhIL-4. Basal cAMP content was only reduced significantly ($p < 0.05$) by $15.5\% \pm 5.6\%$ in PBMCs incubated with IFN- γ (1000 U/ml).

Effects of cytokines on tracheal β -adrenoceptor function

Twenty-four-hour and 72-hour preincubation of guinea pig tracheal rings with IL-1 β (100 and 1000 U/ml), IL-2 (100 U/ml), GM-CSF (100 and 1000 U/ml), or IL-4 (100 U/ml) did not induce any change in the EC₅₀ values for isoprenaline (Table II) after a submaximal precontraction with arecoline (10^{-5} mol/L). In addition, the maximal relaxation of the tracheal rings was not affected (Table II). The precontraction to arecoline did not differ between experimental groups (data not shown). Table III shows that IL-1 β , IL-2, or GM-CSF did not affect β -adrenoceptor function of virus infected tracheas after 24 hours of preincubation. Furthermore, tracheal β -adrenoceptor function was not affected by IL-1 β , IL-2, IL-4, or GM-CSF after 1 day of preincubation with epithelium denuded tracheas (Table IV). The precontractions to arecoline did not differ between experimental groups in any series of experiments (data not shown).

DISCUSSION

Several studies have suggested that β -adrenoceptor number and function of persons with asthma are dependent on the state of the disease.^{3,6,7} We previously showed that β -adrenoceptor number and function were decreased in airways and lymphocytes of guinea pigs after endotoxin administration.¹¹ It is interesting to note that it was also demonstrated that splenectomy before endotoxin administration prevented this decrease of β -adrenoceptor number in peripheral lung

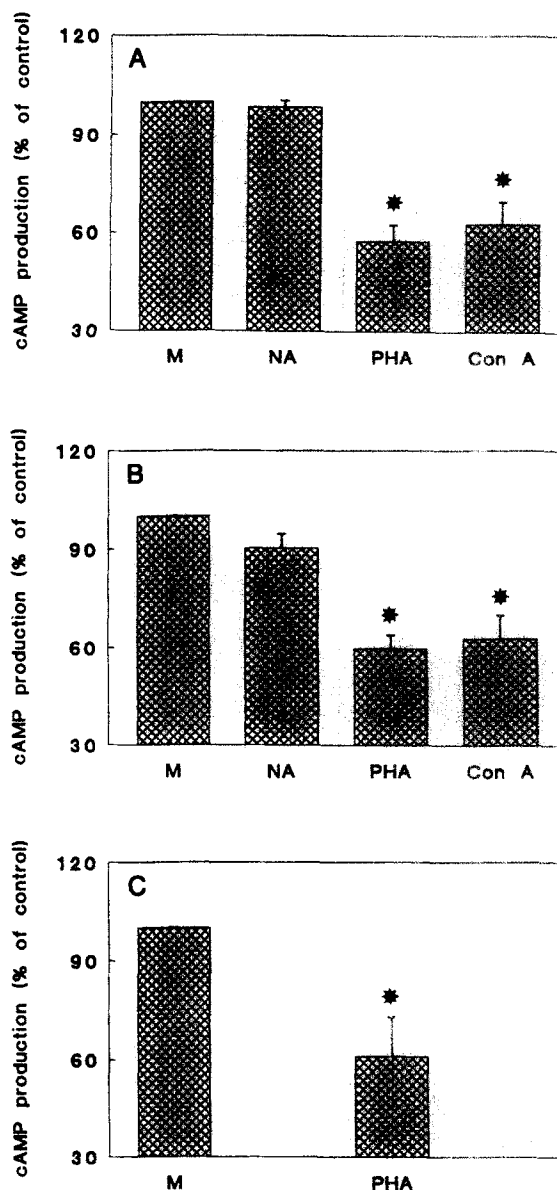


FIG. 1. Effects of supernatants on the isoprenaline (10^{-6} mol/L) stimulated cAMP production (above basal cAMP content) of freshly isolated PBMCs obtained from healthy volunteers (**A**, **B**) or patients with asthma (**C**) after a 20-hour preincubation period. Supernatants were derived from PBMCs of (**A**) healthy persons or (**B**) persons with asthma either activated with PHA, Con A, or nonactivated (NA). Data in (**A**) and (**B**) are the mean of one to three independent experiments and are expressed as the percentage of cAMP production of medium incubated PBMCs (M), and presented as mean $\pm$ SEM. **C**, The effect of three PHA-supernatants on the isoprenaline stimulated cAMP production of PBMCs derived from three persons with asthma. * $p < 0.05$ as determined with Student's paired t test.

tissue and function of tracheal spirals.¹¹ Furthermore, the endotoxin-induced decrease of β -adrenoceptor function on lymphocytes was prevented by treatment of the guinea pigs with the T-lymphocyte selective

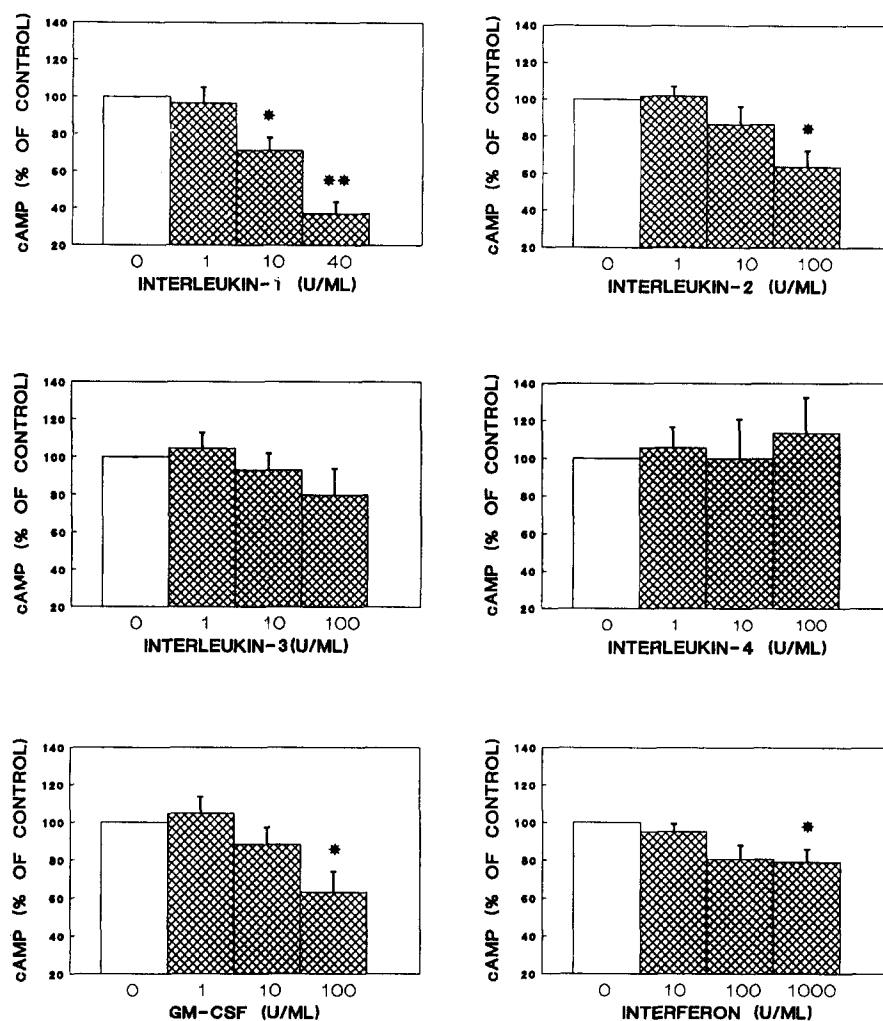


FIG. 2. Effects of cytokines on the isoprenaline (10^{-6} mol/L) stimulated cAMP production (above basal cAMP content) of freshly isolated PBMCs obtained from healthy volunteers after a 20-hour preincubation period. Data are expressed as the percentage of cAMP production of medium incubated PBMCs (open column), and presented as mean $\pm$ SEM of four to six separate experiments. * $p < 0.05$, ** $p < 0.01$ as determined with Student's paired t test.

immunosuppressive drug cyclosporin A.¹² These data indicate that activated T lymphocytes or lymphocyte-derived mediators may affect either directly or indirectly β -adrenoceptor function of both lymphocytes and airway smooth muscle. This postulate is further substantiated in the present study in which an inhibition of PBMC β -adrenoceptor function is observed after 20 hours of preincubation of these cells with supernatants of Con A- or PHA-activated PBMCs obtained from healthy persons or persons with asthma. These observations demonstrate that on stimulation lymphocytes produce factors that can inhibit β -adrenoceptor function of PBMCs. PBMC derived from donors with asthma do not spontaneously produce sufficient amounts of factor(s) that inhibit

β -adrenoceptor function. At the time of study the donors with asthma were not in acute respiratory distress. Helper T lymphocytes isolated from blood of patients with acute severe asthma show increased expression of surface proteins associated with lymphocyte activation compared with those from normal control subjects.²³ Although not studied, it can be suggested that PBMCs secrete factors that inhibit β -adrenoceptor function after allergen challenge or during acute asthma. In accordance herewith, it has been shown that lymphocyte β -adrenoceptor function is decreased 24 hours after allergen challenge of patients with asthma.⁷ PBMCs derived from healthy persons are also capable of producing factors that inhibit β -adrenoceptor function when activated by mitogens

TABLE I. Effects of different cytokines on the isoprenaline (10^{-6} mol/L) stimulated cAMP production (above basal cAMP content) of freshly isolated PBMCs obtained from healthy volunteers after a 2-hour preincubation period

Cytokine (U/ml)	cAMP production*	Cytokine (U/ml)	cAMP production*
IL-1 (1)	113.6 $\pm$ 8.9	IL-4 (1)	103.0 $\pm$ 15.7
IL-1 (10)	90.6 $\pm$ 6.5	IL-4 (10)	100.3 $\pm$ 7.1
IL-1 (40)	90.5 $\pm$ 6.1	IL-4 (100)	100.3 $\pm$ 8.2
IL-2 (1)	103.0 $\pm$ 13.8	IFN- γ (10)	110.7 $\pm$ 13.0
IL-2 (10)	107.0 $\pm$ 20.3	IFN- γ (100)	124.0 $\pm$ 16.0
IL-2 (100)	110.0 $\pm$ 16.2	IFN- γ (1000)	103.7 $\pm$ 9.0
IL-3 (1)	102.8 $\pm$ 16.2	GM-CSF (1)	106.3 $\pm$ 4.1
IL-3 (10)	114.3 $\pm$ 15.6	GM-CSF (10)	108.7 $\pm$ 6.2
IL-3 (100)	99.5 $\pm$ 22.3	GM-CSF (100)	106.7 $\pm$ 6.3

*Data expressed as percentage of cAMP production of medium incubated PBMCs and presented as mean $\pm$ SEM of four to six separate experiments.

TABLE II. Effects of IL-1 β , IL-2, IL-4, and GM-CSF on isoprenaline concentration response curves from guinea pig tracheal rings after (left) 1 day of preincubation and (right) 3 days of preincubation

Cytokine (U/ml)	1 day		3 days	
	EC ₅₀	Maximal relaxation (% of precontraction)	EC ₅₀	Maximal relaxation (% of precontraction)
Control	6.80 $\pm$ 0.09	60.7 $\pm$ 6.4	6.99 $\pm$ 0.12	65.8 $\pm$ 10.0
IL-1 β (100)	6.63 $\pm$ 0.09	57.7 $\pm$ 8.1	6.93 $\pm$ 0.14	66.0 $\pm$ 10.5
Control	6.80 $\pm$ 0.09	60.7 $\pm$ 6.4	6.99 $\pm$ 0.12	65.8 $\pm$ 10.0
IL-2 (100)	6.74 $\pm$ 0.09	60.7 $\pm$ 7.3	7.04 $\pm$ 0.09	67.1 $\pm$ 7.3
Control	6.80 $\pm$ 0.09	60.7 $\pm$ 6.4	6.89 $\pm$ 0.17	63.3 $\pm$ 9.7
IL-4 (100)	6.72 $\pm$ 0.10	58.5 $\pm$ 7.9	6.97 $\pm$ 0.14	69.1 $\pm$ 7.4
Control	6.80 $\pm$ 0.09	60.7 $\pm$ 6.4	6.78 $\pm$ 0.22	62.8 $\pm$ 14.0
GM-CSF (100)	6.69 $\pm$ 0.09	55.7 $\pm$ 7.6	6.76 $\pm$ 0.20	60.8 $\pm$ 13.8
Control	6.68 $\pm$ 0.06	63.6 $\pm$ 5.0		
IL-1 β (1000)	6.75 $\pm$ 0.08	62.3 $\pm$ 6.4	ND	
Control	6.68 $\pm$ 0.06	63.6 $\pm$ 5.0		
GM-CSF (1000)	6.71 $\pm$ 0.07	66.1 $\pm$ 9.6	ND	

Results expressed as mean $\pm$ SEM of four animals.

ND, Not determined.

in vitro. However, it can be assumed that T lymphocytes from healthy persons do not become activated by allergen inhalation and do not infiltrate the airways.

Approximately 90% of the isolated PBMCs are lymphocytes, whereas the remaining 10% are monocytes.⁷ However, the factors in the supernatants of PHA- and Con A-activated PBMCs that inhibit PBMC β -adrenoceptor function are most likely derived from T lymphocytes, because PHA and Con A act as polyclonal mitogens for T lymphocytes. This activation leads to lymphocyte proliferation, and a scale of

cytokines is produced, which becomes detectable 4 to 6 hours after activation.²⁴⁻²⁶ To determine which factors, present in the supernatants, may be responsible for the β -adrenergic impairment in PBMCs, various purified cytokines were studied for their effects on PBMC β -adrenoceptor function. These experiments show that IL-1, IL-2, GM-CSF and IFN- γ inhibit isoprenaline stimulated cAMP production of PBMCs in a dose-dependent manner after 20 hours of preincubation. No inhibition occurred when PBMCs were incubated for 2 hours with the same cytokines. In

TABLE III. Effects of 1 day of preincubation with IL-1 β , IL-2, and GM-CSF on isoprenaline concentration response curves of tracheal rings obtained from virus infected guinea pigs

Cytokine (U/ml)	EC ₅₀	Maximal relaxation (% of precontraction)
Control	6.80 $\pm$ 0.10	61.7 $\pm$ 3.5
IL-1 β (100)	6.76 $\pm$ 0.15	67.7 $\pm$ 8.7
IL-2 (100)	6.85 $\pm$ 0.16	59.0 $\pm$ 4.0
GM-CSF (100)	6.64 $\pm$ 0.06	55.0 $\pm$ 10.6

Results expressed as mean $\pm$ SEM of three animals.

agreement with our study, Beckner et al.²⁷ demonstrated that IL-2 inhibits isoprenaline stimulated cAMP production of T lymphocytes. However, this inhibition occurred rapidly and was significant at an IL-2 activity of 0.1 U/ml. Beckner et al.²⁸ used IL-2 receptor bearing lymphocytes, whereas these receptors are absent on most freshly isolated lymphocytes. The absence of IL-2 receptors on the lymphocytes used in our study might explain why the inhibition of β -adrenoceptor function by IL-2 was observed after 20 hours of preincubation and required a higher IL-2 activity.

Since PBMCs are a heterogenous population of cells a role for monocytes in the inhibition of PBMC β -adrenoceptor function cannot be excluded. It has been shown that inhibition of β -adrenoceptor function on T lymphocytes by the phorbol ester PMA was mediated by activated monocytes.²⁹ Furthermore, Engels et al.³⁰ showed that macrophages attached to tracheal spirals can diminish tracheal β -adrenoceptor function when stimulated appropriately. Both studies showed that the attenuation of β -adrenoceptor function was mediated by the production of reactive oxygen species by the macrophages. Since GM-CSF³¹⁻³⁴ and IFN- γ ³²⁻³⁶ increase the production of reactive oxygen species by monocytes, it is possible that these cytokines affect PBMCs β -adrenoceptor function in this way. In accordance herewith, it has recently been demonstrated that oxygen radicals are involved in β -adrenoceptor dysfunction in an allergic animal model.³⁷ PAF has been shown to downregulate β -adrenoceptors in the human lung.¹⁹ Monocytes, among other inflammatory cells, can release PAF. Although not studied, the diminished β -adrenoceptor function after incubation of PBMCs with cytokines might be related to PAF production. Nevertheless, regardless of the mechanism(s), the observations in this study indicate cytokines as potential inhibitors of β -adrenoceptor function.

Bronchial inflammation in asthma may depend in

TABLE IV. Effects of 1 day of preincubation with IL-1 β , IL-2, IL-4, and GM-CSF on the EC₅₀ value and maximal relaxation of isoprenaline concentration response relationship from epithelium denuded tracheal rings

Cytokine (U/ml)	EC ₅₀	Maximal relaxation (% of precontraction)
Control	6.94 $\pm$ 0.04	67.7 $\pm$ 1.7
IL-1 β (100)	6.96 $\pm$ 0.13	69.3 $\pm$ 4.7
IL-2 (100)	6.93 $\pm$ 0.05	67.7 $\pm$ 9.8
IL-4 (100)	6.91 $\pm$ 0.06	64.7 $\pm$ 6.9
GM-CSF (100)	6.87 $\pm$ 0.08	74.7 $\pm$ 4.9

Results expressed as mean $\pm$ SEM of three animals.

part on the activation of helper T lymphocytes that elaborate proinflammatory cytokines.¹⁷ After allergen inhalation, a selective recruitment of helper T lymphocytes into the airways has been demonstrated in patients with asthma.^{13, 14, 38} These T lymphocytes are activated as demonstrated by increased IL-2 receptor expression.¹⁶ Recently, it has been demonstrated that the number of BAL cells that express mRNA for IL-2, 3, 4, 5, and GM-CSF were enhanced in patients with asthma.¹⁷ Another source of cytokines in the airways could be mast cells that on IgE mediated activation can produce cytokines such as IL-3, 4, 5, 6, and GM-CSF.^{39, 40} It can be speculated that these cytokines may inhibit β -adrenoceptor function of mononuclear cells and possibly other inflammatory cells. β -adrenoceptor stimulation inhibits the release of inflammatory mediators from mast cells, eosinophils, and neutrophils and inhibits eosinophil chemotaxis and lymphocyte activation.^{10, 41, 42} An impairment of feedback control as a result of diminished β -adrenoceptor stimulation by catecholamines might lead to increased tissue inflammation and thereby worsen or prolong or both the active asthmatic state.

Since cytokines are produced locally in the airways of patients with asthma, we hypothesized that cytokines might also affect β -adrenoceptor function of airway smooth muscle. It is interesting to note that Gulick et al.⁴³ showed that IL-1 and tumor necrosis factor inhibit β -adrenoceptor function of cultured cardiac myocytes. On the other hand, it has been demonstrated that IL-1 increased isoprenaline stimulated cAMP production in human A549 lung adenocarcinoma cells (comparable to alveolar type II cells).^{44, 45} In our study, however, we did not observe an inhibition of airway smooth muscle β -adrenoceptor function by the cytokines IL-1 β , IL-2, IL-4, or GM-CSF after 1 or 3 days preincubation.

Wills-Karp et al.⁴⁶ showed that isoprenaline induced relaxation of guinea pig trachea was significantly inhibited after 18 hours of preincubation with IL-1 β (4 U/ml). However, in contrast with our study non-specific-pathogen free (SPF) animals were used (personal communication). Possibly this microbial state of the animals might be related to the influx of inflammatory cells in the airways. The number of inflammatory cells are clearly increased in BAL fluid of non-SPF animals as compared with SPF animals (unpublished observation). These inflammatory cells might be required for an effect of IL-1 β and possibly of other cytokines on airway β -adrenoceptor function. Folkerts et al. (unpublished observation) showed that at day 4 after intratracheal inoculation of PI-3 virus in guinea pigs, increased numbers of alveolar macrophages, monocytes, lymphocytes, and eosinophils were detected in BAL. It can be expected that the tracheas from these animals are also infiltrated by these inflammatory cells. Therefore tracheas from animals infected with virus were incubated during 1 day with IL-1 β , IL-2, and GM-CSF. However the cytokines did not cause a β -adrenergic impairment in tracheas from virus infected animals.

Another explanation for the absence of cytokine-induced inhibition of airway β -adrenoceptor function could be that the cytokines are cleaved into nonactive fragments by peptidases during the incubation period. It is interesting to note that the cell membrane bound enzyme neutral endopeptidase (NEP) is largely present in epithelial cells of the airways of many species, including guinea pigs.⁴⁷ A number of substrates is known to be cleaved by NEP, including IL-1 β .⁴⁸ Epithelium denudation of tracheas decreased NEP activity sufficiently to observe a potentiation of airway smooth muscle responses in ferret tracheas to tachykinins, which is an example of an NEP substrate.⁴⁹ In our study epithelium removal did not result in an alteration of β -adrenoceptor function by IL-1 β , IL-2, IL-4, and GM-CSF after 1 day of preincubation.

As discussed above, more than one cytokine is produced in the airways of persons with asthma, and the effects of different cytokines can be additional or synergistic. However, no impairment of β -adrenoceptor function is observed when tracheal rings are incubated simultaneously with IL-1 β , GM-CSF, and IL-2 (33 U/ml) for 1 day (data not shown). The dissociation in the effect of cytokines on PBMCs and trachea indicates that PBMCs may not always be a good model to study β -adrenoceptor function in lungs or other tissues. Other problems with the use of PBMCs to assess β -adrenoceptors are the differences in the expression of these receptors on leukocyte subpopulations and the influence of β -agonists on the distribution of leukocytes in the body as well as on the

expression of β -adrenoceptors.¹⁰ Therefore PBMCs should be used with caution as a model to study lung β -adrenoceptors.

β -adrenoceptors are widespread throughout the lung, including the airway epithelium, pneumocytes, cholinergic ganglia, submucosal glands, and resident and nonresident inflammatory cells.^{10, 41} Stimulation of airway β -adrenoceptors leads to relaxation of smooth muscle, inhibition of cholinergic neurotransmission, increased secretion of mucus, lipid, water, and surfactant, reduced microvascular leakage, increased ion and fluid transport, and increased ciliary activity.^{10, 41} All these effects are beneficial for airway function in asthma and are the basis for the use of β -agonist therapy. A decreased β -adrenoceptor function in one or more of these cell types could therefore exaggerate asthma. So far, we have only investigated the influence of cytokines on PBMC and smooth muscle β -adrenoceptor function. More studies are necessary to evaluate the influence of cytokines on β -adrenoceptors in other airway cells.

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