

Original article

Allergen inhalation decreases adenosine receptor expression in sputum and blood of asthma patients

Background: Adenosine is a signalling nucleoside that has been proposed to contribute to the pathogenesis of asthma. Adenosine is produced in inflammatory environments and acts *via* adenosine receptors (A_1R , $A_{2A}R$, $A_{2B}R$, and A_3R) expressed by a wide variety of cells, resulting in pro- and anti-inflammatory effects.

Objective: To compare AR expression in asthma patients and healthy subjects, and to assess the effect of allergen challenge on AR expression of inflammatory cells and on cytokines in peripheral blood and sputum in asthma.

Methods: Asthma patients underwent an allergen challenge, and blood and induced sputum samples were taken before and 24 h after allergen challenge to study inflammatory cells numbers, AR expression and cytokine production. Blood and sputum were investigated at one time point in healthy subjects. AR expression was measured by flow cytometry (blood) or on cytopins using immunocytochemistry (sputum). Cytokines (luminex, ELISA) and adenosine (HPLC) were measured in sputum supernatant.

Results: The percentage of $A_{2B}R$ expressing neutrophils in sputum was lower in asthma patients than in healthy subjects ($P = 0.016$). Allergen challenge decreased A_1R and $A_{2A}R$ expression on neutrophils and A_1R expression on T cells in peripheral blood (all $P < 0.05$). Allergen challenge increased IL-8 levels and eosinophil numbers ($P < 0.05$), whereas it decreased thymic stromal lymphopoietin levels and the percentage of A_1R expressing macrophages in induced sputum ($P < 0.05$).

Conclusions: Allergen challenge has a down-regulatory effect on AR expression in asthma, suggesting a contribution of adenosine-related effector mechanisms in the pathophysiology.

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Asthma is a chronic inflammatory disease of the airways characterized by episodes of wheezing, breathlessness, cough and chest tightness (1). Several studies have shown a link between adenosine and the pathophysiology of asthma. Adenosine levels are elevated in bronchoalveolar fluid and exhaled breath condensate of asthma patients (2). In addition, adenosine 5'-monophosphate is able to induce bronchoconstriction in asthma patients, and this stimulus for bronchial hyperresponsiveness (BHR) has been shown to be superior to methacholine in demonstrating the presence of bronchial inflammation (3).

Adenosine is a purine nucleoside that is released during tissue hypoxia and inflammation. Adenosine exerts its effects by activation of four different G protein coupled receptors, A_1 (G_i coupled), A_{2A} (G_s coupled), A_{2B} (G_s and G_{q11} coupled) and A_3 (G_s and G_{q11} coupled). Activation of the adenosine receptors (ARs) on different cell types can have different functions, and each receptor can be implicated either beneficially and/or detrimentally in the development or persistence of inflammation (4). In

short, both A_2 receptors act mainly anti-inflammatory, whereas A_1R and A_3R act pro-inflammatory upon activation.

There are no studies available in the literature investigating AR expression on inflammatory cells in asthma, or after allergen challenge. However, the effect of the adenosine A_{2A} agonist GW328267X on the early and late airway response was investigated in two studies, one in asthma patients (5) and one in allergic rhinitis patients (6). In asthmatics, treatment with the inhaled adenosine A_{2A} agonist GW328267X did not protect against the late asthmatic response, expressed as the fall in forced expiratory volume in 1 s (FEV₁) 5–8 h after allergen challenge, or the accompanying increase in airway inflammation, whereas the same drug had limited effects on the early- and late response after nasal allergen challenge and clinical symptoms in allergic rhinitis patients, but showed a reduction in nasal tryptase levels.

In this study, the aim was to compare asthma patients and healthy individuals with respect to AR expression on

inflammatory cells in blood and sputum. Furthermore, we investigated the effect of allergen challenge on inflammatory cells, cytokine production and AR expression in blood and sputum cells of asthma patients. Knowledge on AR expression in stable disease and after allergen exposure is important as ARs may be useful therapeutic targets in the treatment of allergic airway diseases.

Methods

Subjects

Subjects were recruited from the pulmonary outpatient clinic of the University Medical Center Groningen (UMCG) and the University Medical Center (UMC) Utrecht and by advertisements in local newspapers. We included eight asthma patients and 10 asymptomatic healthy subjects. In addition, we used sputum material of nine asthma patients derived from a previous study (5). For both studies participants were included according to the following criteria: Asthma: FEV₁ ≥ 70% predicted, provocative concentration of methacholine or histamine inducing a 20% fall in FEV₁ (PC₂₀) ≤ 8 mg/ml, and at least one positive skin prick test for house dust mite, timothee grass or cat dander. Healthy: FEV₁ > 90% predicted during screening, PC₂₀ methacholine > 8 mg/ml, and negative skin prick test for inhalation allergens. All participants met the following criteria: age between 18 and 55 years, no current smoking, no use of inhaled corticosteroids in the previous 4 weeks, no use of oral corticosteroids in the previous 6 months, and no use of long-acting beta agonists in the previous 2 weeks. Asthma patients were allowed to use a short-acting beta agonist if necessary.

The local medical ethics committees (UMCG and UMC Utrecht) approved the study protocols and all subjects gave their written informed consent.

Study design

For asthma subjects, the study consisted of three visits to the outpatient clinic. During the first visit sputum was induced, at the second visit (3–4 days later) a blood sample was obtained and an allergen challenge was performed, and during the third and final visit (24 h after start of the allergen inhalation) peripheral blood was withdrawn and sputum was induced. Patients were asked not to use short-acting beta agonists and caffeine containing drinks or food during and 8 h before allergen challenge. For healthy control subjects, the study implied one visit to the outpatient clinic to obtain blood and to perform a sputum induction.

Histamine, methacholine and allergen challenge

We applied the standardized guidelines for histamine, methacholine, and allergen challenge, employing the 2-min tidal breathing method (3). For the allergen challenge either house dust mite, cat or timothee grass allergen (ALK Abelló, Hørsholm, Denmark) was used depending on the individual sensitivity of the subject as determined by skin prick tests at screening (5).

Blood processing and FACS staining

To isolate the leucocytes from peripheral blood, heparin blood was diluted 1 : 2 with phosphate-buffered saline (PBS, pH 7.4) and

centrifuged for 20 min at 1400 *g* at room temperature. The buffy coat was withdrawn from the tube and erythrocytes were lysed using 10 ml of a lysingbuffer (150 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM NA₂EDTA·2H₂O). Isolated leucocytes were stained for (i) CD3^{FITC} (T cells), (ii) CD20^{FITC} (B cells) and (iii) CD14^{PerCP} (monocytes), CD11b^{PE} and CD16^{FITC} (eosinophils and neutrophils), all from BD biosciences (Becton Dickinson, Franklin Lakes, NJ, USA). In the same samples, cells were also stained for the expression of three different ARs, A₁, A_{2A}, and A_{2B} (Alpha Diagnostic International, San Antonio, TX, USA), secondary antibody used was allophycocyanin (APC) conjugated goat anti-rabbit IgG (Molecular Probes Europe BV, Leiden, the Netherlands). Cells were resuspended in FACS lysing solution (Becton Dickinson) and analyzed (4 × 10⁴ events) using a FACS Calibur (Becton Dickinson). Raw data analysis was performed with FLOWJO (TreeStar, San Carlos, CA, USA). Cells were gated as follows: T cells CD3⁺, B cells CD20⁺, monocytes CD14⁺, eosinophils CD14^{low}, CD11b^{high}, CD16^{low}, neutrophils CD14^{low}, CD11b^{high} and CD16^{high}. In this specified populations, the mean fluorescence intensity of APC was calculated for AR expression. The antibody directed against A₃R was not available at the start of the study, therefore, A₃R was not included in the above analysis.

Sputum induction, processing and AR immunostaining

Sputum was induced by inhalation of hypertonic saline aerosols and processed as described previously (7). Sputum cytospin slides were stained with May–Grünwald–Giemsa for differential cell counts. Sputum samples containing > 80% of squamous cells were excluded from analysis. For immunostaining, cytospin slides were dried and fixed in acetone for 10 min, dried for 30 min and washed with PBS. Hydrogen peroxide in methanol was used for blocking of endogenous peroxidase. The following polyclonal antibodies were used: rabbit anti-rat adenosine A₁ receptor, rabbit anti-canine adenosine A_{2A} receptor, rabbit anti-human adenosine A_{2B} receptor (all from Alpha Diagnostic International), and rabbit anti-human adenosine A₃ receptor (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Antibodies directed against adenosine A₁ and A_{2A} receptors were raised against rat and canine, respectively, but cross-reacted with human. Rat and canine ARs show 100% and 80% homology with human ARs respectively. Secondary antibody used was goat anti-rabbit biotin conjugated (SBA, Birmingham, AL, USA) and tertiary antibody StreptABComplex peroxidase conjugated (DAKO, Glostrup, Denmark). As chromogen, 3-amino-9-ethyl-carbazol (AEC) substrate was used; tissue was counterstained with Mayers' haematoxylin. Three hundred neutrophilic granulocytes and 300 macrophages were counted in each cytospin, and scored as negative or positive; the percentage of cells expressing ARs was calculated. Because of the small numbers of lymphocytes present in sputum we could not reliably quantify AR expression on this cell type. As only few patients showed more than 10% of eosinophils in sputum, we did not quantify AR expression on eosinophils.

Measurement of adenosine in sputum supernatant using HPLC-MS

Adenosine was measured using a Waters 2690 Alliance Separation Module HPLC system (Waters, Milford, MA, USA) coupled on-line to a Finnigan TSQ Quantum Triple Quadrupole Mass Spectrometer (Finnigan Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was performed with a C18, 3 µm, Atlantis column (Waters) 2.1 mm I.D. × 100 mm length using a gradient of mobile phase A (ammonium acetate 20 mM pH 7.0 in water) and B (ammonium acetate 20 mM, pH 7.0, in aceto-

nitrile) at 0.2 ml/min flow rate. Before measurement samples were pretreated as follows: 100 µl of sputum supernatant were acidified with 20 µl of HCl 120 mM, then 200 µl of chloroform was added to precipitate proteins and eliminate lipids. After vortexing and subsequent centrifugation, 80 µl of the aqueous supernatant was transferred to a glass injection vial and 5 µl of this solution was injected into the HPLC-MS system. MS detection was performed using ESI ionization in positive ion mode. To increase the sensitivity multiple reaction monitoring mode was used. Adenosine was quantified using the transition m/z 260 > m/z 136 corresponding to the loss of the ribofuranose ring. Samples were measured in a randomized way, twice independently at two different days; results were in good agreement within a maximum of 10% variation. The calibration line consisted of eight points in the range of 5–1000 ng/ml adenosine in a mixture of PBS and water (pretreated in the same way as the study samples).

Measurement of cytokines in sputum supernatant

In sputum supernatant levels (sensitivity level) of IL-4 (5 pg/ml), IL-5 (3 pg/ml), IL-8 (3 pg/ml), IL-13 (10 pg/ml), IL-17 (10 pg/ml), MCP-1 (10 pg/ml), RANTES (15 pg/ml), eotaxin (5 pg/ml), IFN- γ (5 pg/ml) and tumour necrosis factor (TNF) (10 pg/ml) were measured using Human Custom Ten-plex Antibody Bead Kits from Biosource International, Inc. (Camarillo, CA, USA) in accordance with the manufacturers' instructions. Standard solutions were diluted with sputolysin reagent (Calbiochem®, Darmstadt, Germany) to mimic the effect it might have had on sputum content during processing.

Thymic stromal lymphopoietin (TSLP) levels in induced sputum supernatant were measured using the TSLP ELISA kit from R&D Systems, Inc. (Minneapolis, MN, USA) according to the manufacturers' instructions. Standard solutions were diluted with sputolysin reagent (Calbiochem®) to copy the effect it might have had on sputum content during processing.

Statistical analysis

Statistic analyses were performed using a Wilcoxon signed rank test, a nonparametric paired test to test for differences within the groups and a nonparametric Mann-Whitney *U*-test to test for differences between both groups at baseline. A value of $P < 0.05$ was considered significant. Correlations between parameters were investigated using Pearson's Rho test.

Results

Patient characteristics are shown in Table 1. Patients with asthma exhibited BHR to methacholine or histamine whereas healthy control subjects did not. In addition, asthma patients had a lower FEV₁ (% predicted) compared with healthy controls ($P = 0.008$), as well as higher percentages of blood eosinophils ($P = 0.002$), numbers of sputum leucocytes ($P = 0.033$, Table 2), and percentages of sputum eosinophils ($P = 0.001$). Allergen challenge did not affect the absolute number of sputum cells, but increased the percentage of sputum eosinophils ($P = 0.018$). Allergen challenge had no effect on peripheral blood cell numbers or cell differentials.

Table 1. Patient characteristics

	Age (years)	FEV ₁ (l)	FEV ₁ (% pred)	PC ₂₀ Methacholine (mg/ml)	PC ₂₀ Histamine (mg/ml)	Allergen	LAR
Asthma							
1	27	4.32	88	0.84		HDM	–
2	24	4.27	92	2.50		HDM	+
3	46	2.41	85	0.60		HDM	+
4	52	2.56	89	0.16		HDM	+
5	53	2.28	80	0.19		HDM	–
6	41	3.60	124	0.68		Cat	–
7	41	4.69	113	6.13		Grass	+
8	47	3.27	116	1.06		Cat	–
9*	54	3.48	104		4.84	Grass	+
10*	19	3.95	105		1.84	HDM	+
11*	21	3.99	81		0.57	HDM	+
12*	27	5.14	100		1.67	HDM	+
13*	18	4.12	115		1.81	HDM	+
14*	21	3.50	75		2.67	Grass	+
15*	20	4.82	98		0.29	Grass	+
16*	20	4.85	94		4.43	Grass	+
17*	18	4.10	94		0.94	HDM	+
Healthy							
1	25	4.86	129	–			
2	37	3.98	122	–			
3†	31	5.05	114	–			
4	25	4.05	111	–			
5†	44	3.80	105	–			
6	21	3.89	97	–			
7	29	4.50	124	–			
8	33	3.56	114	–			
9	29	4.02	118	–			
10‡	20	4.50	99	–			

*AR expression was only determined on sputum cells; in sputum supernatant adenosine was measured only.

†No sputum cytopins of sufficient quality.

‡No sputum induced.

FEV₁, forced expiratory volume in 1 s; PC₂₀, provocative concentration of methacholine or histamine inducing a 20% fall in FEV₁; HDM, house dust mite; LAR, late asthmatic response; –, PC₂₀ methacholine >8 mg/ml.

Adenosine receptor expression on peripheral blood cells

In peripheral blood, all five cell types studied (eosinophils, T cells, neutrophils, monocytes, and B cells) expressed A₁R, A_{2A}R, and A_{2B}R. Asthma patients tended to have a higher expression of A₁R ($P = 0.06$) on T cells and A_{2A}R ($P = 0.06$) on monocytes than healthy controls (Fig. 1). Allergen challenge decreased A₁R expression on T cells ($P = 0.04$) and A₁R and A_{2A}R expression on neutrophils ($P = 0.012$). There were no significant differences in AR expression on B cells (data not shown) and eosinophils (Fig. 1).

Adenosine receptor expression on sputum cells

In sputum, both neutrophils and macrophages expressed all four described ARs. Investigating the percentage of cells expressing ARs, asthma patients had a lower

Table 2. Peripheral blood and sputum cell counts

	Healthy	Asthma	
		Before	After
Peripheral blood	<i>n</i> = 10	<i>n</i> = 8	<i>n</i> = 8
Cell concentration ($\times 10^6/\text{ml}$)	5.4 (4.3–7.3)	4.8 (4.5–5.9)	5.0 (4.3–7.7)
Eosinophils (%)	1.7 (0.5–3.4)	5.9 (1.7–10.1)*	6.3 (2.3–7.9)
Lymphocytes (%)	38.1 (27.7–49.3)	29.8 (20.6–43.1)	29.3 (16.7–43.8)
Neutrophils (%)	50.5 (5.4–62.8)	56.3 (42.7–63.9)	56.2 (41.5–68.2)
Monocytes (%)	6.8 (0.3–12.1)	8.4 (5.4–9.9)	7.9 (5.6–9.9)
Sputum	<i>n</i> = 7	<i>n</i> = 17	<i>n</i> = 17
Total cell count ($\times 10^6$)	1.8 (0.0–4.0)	6.4 (0.0–19.6)*	4.5 (0.5–17.9)
Eosinophils (%)	0.0 (0.0–1.7)	5.4 (0.0–36.8)*	31.3 (0.0–58.0)†
Lymphocytes (%)	2.0 (0.3–3.0)	2.1 (0.0–8.3)	1.2 (0.0–10.5)
Neutrophils (%)	32.7 (4.5–70.5)	44.3 (5.0–74.2)	28.8 (0.0–76.3)
Macrophages (%)	63.0 (24.7–93.5)	39.4 (13.2–90.0)	32.3 (15.2–95.0)

Data presented as median (range). Before, before allergen challenge; after, 24 h after allergen challenge.

* $P < 0.05$ compared with healthy subjects.

† $P < 0.05$ compared with before allergen challenge.

percentage of neutrophils expressing $A_{2B}R$ than healthy subjects ($P = 0.016$, Fig. 2A). There was a positive correlation between the absolute numbers of neutrophils in sputum and numbers of neutrophils expressing $A_{2B}R$ ($\rho = 0.888$, $P = 0.000$, plot not shown).

Allergen challenge decreased the percentage A_1R expressing macrophages in asthma patients ($P = 0.012$, Fig. 2B).

Inflammatory mediators in sputum supernatant

In sputum supernatant, levels of adenosine and various cytokines were measured. IL-8 levels increased in sputum of asthma patients following allergen challenge ($P = 0.028$), whereas levels of TSLP decreased ($P = 0.036$, Fig. 3). Before allergen challenge, there was a significant correlation between IL-8 levels and total number of neutrophils in sputum ($\rho = 0.626$, $P = 0.017$, total group), a correlation that was no longer significant following allergen challenge. Adenosine and cytokine levels did not differ comparing healthy and asthma subjects, nor did allergen challenge alter these levels. Levels of IL-5, IL-13, eotaxin, and TNF were below sensitivity level.

Discussion

We investigated the role of adenosine and ARs in asthma by comparing asthma patients and healthy controls. In addition, we assessed whether allergen-induced airway inflammation in asthma is associated with a change in AR expression on peripheral blood and induced sputum cells.

Our results show that asthma patients had an increased number of eosinophils in peripheral blood and induced sputum, and that these numbers further increased after allergen challenge. These findings support previous studies by our group and others (8–21). However, AR expression on blood eosinophils in asthma patients did not differ from healthy controls nor did it change upon allergen challenge, thus not explaining this increased eosinophil migration.

The increase in eosinophil numbers was accompanied by higher levels of IL-8. Eosinophils can produce IL-8 and could be responsible for enhanced levels of IL-8 upon allergen challenge (22, 23). However, eosinophils do not migrate by IL-8 whereas neutrophils do. A trans-base-membrane migration study has shown that IL-8 attracts eosinophils, only when neutrophils are present and it could be that neutrophils migrated by IL-8 may have led eosinophils to accumulate in the airways of patients with asthma (24). Enhanced levels of IL-8 in induced sputum were also described earlier and support our correlation with neutrophils and IL-8 in asthma before allergen challenge (16).

This study showed that the AR expression on other peripheral blood cell types was not significantly different in asthma and healthy subjects. However, after allergen challenge, A_1R expression was down regulated on T cells and neutrophils, as was $A_{2A}R$ expression on neutrophils. Data of A_1R on T cells are scarce. Takahashi et al. described the anti-inflammatory effects of an $A_{2A}R$ agonist (CGS-21680) on cytokine production and lymphocyte proliferation (25). However, when co-incubated with an A_1R agonist (N6-cyclopentyladenosine, CPA), the anti-inflammatory effects induced by CGS-21680 were reversed, resulting in increased cytokine production and lymphocyte proliferation. These results suggest a pro-inflammatory role for A_1R on lymphocytes. In addition, the A_1R is described to be G_i -protein coupled. Activation of the receptor inhibits adenylyl cyclase activation and subsequent cAMP production, which also points towards a pro-inflammatory role of A_1 receptors. Our observed decreased A_1R expression on T cells following allergen challenge could indicate a compensatory mechanism in peripheral blood of asthma patients in an effort to protect against ongoing inflammation. This could also be the role of the observed decrease in A_1R expression on neutrophils after allergen challenge, as activation of the A_1R on neutrophils induces chemotaxis and increases the adherence to endothelial cells (26–28). Interestingly, $A_{2A}R$ expression on neutrophils was also down regulated after allergen challenge. This is an intriguing observation as activation of both receptors has opposite effects. Activation of $A_{2A}R$ reduces neutrophil adherence to endothelium (26), uncouples chemo attractant receptors from their stimulus-transduction-proteins (29), and inhibits generation of toxic oxygen metabolites and degranulation (30). This indicates that reduced expression of this

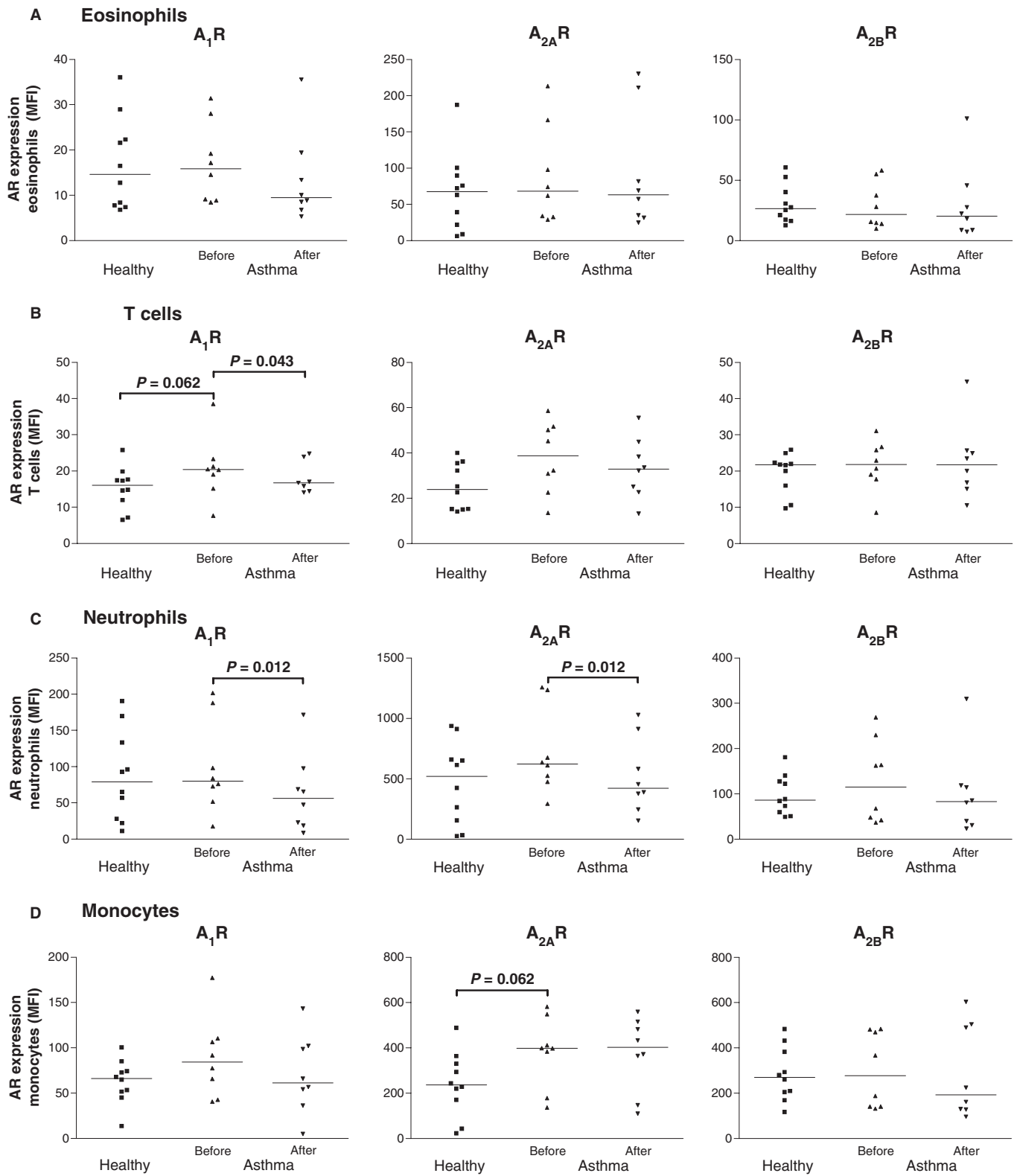


Figure 1. Adenosine receptor expression on eosinophils (A), T cells (B), neutrophils (C), and monocytes (D) in peripheral blood of healthy and asthmatic subjects. Before: before start of allergen challenge; after: 24 h after allergen challenge (asthma patients). MFI: mean fluorescence intensity. Significant differences are indicated in the graphs.

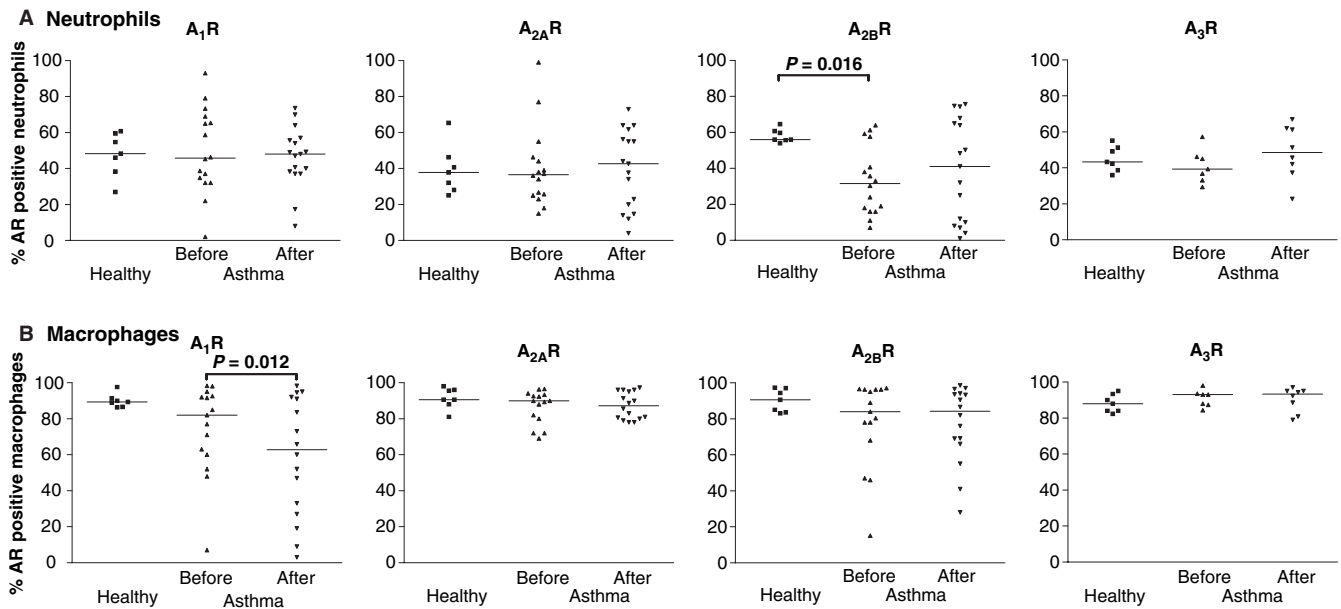


Figure 2. Adenosine receptor expression on neutrophils (A) and macrophages (B) in sputum of healthy and asthmatic subjects. Before: 3–4 days before allergen challenge; after: 24 h after allergen challenge (asthma patients). Significant differences are indicated in the graphs.

receptor could induce neutrophil activation, thereby permitting neutrophils to release greater quantities of free oxygen radicals with consequently increased oxidative damage (27, 31). Together these data suggest that a disturbance of the delicate balance between A₁R and A_{2A}R may ultimately results in a positive or negative effect on neutrophilic inflammation.

We found expression of all four ARs on sputum neutrophils and macrophages in asthma patients and healthy control subjects. The percentage of neutrophils expressing A_{2B}R was lower in asthmatics and allergen challenge decreased the percentage of macrophages expressing A₁R. Adenosine A_{2B} receptors have been described to inhibit trans-endothelial migration of neutrophils upon activation (32). This is of interest as the absolute number of neutrophils was positively correlated to the absolute number of neutrophils expressing A_{2B}R. Similar to A_{2A}R expression, loss of A_{2B}R expression on neutrophils can induce increased production of free oxygen radicals with consequent oxidative damage in the lung (31). For scleroderma, a connective tissue disease of unknown aetiology, a reduced expression and activity of A_{2B}R on blood neutrophils compared with healthy control subjects has been reported as well (31).

We detected a down regulatory effect of allergen challenge on the percentage of sputum macrophages that express A₁R. Activation of A₁R on macrophages has been described to inhibit LPS-induced TNF and IL-10 production (33). Also, occupancy of A₁R on macrophages results in a very rapid and marked enhancement of FcγR function, resulting in enhanced FcγR-mediated

phagocytosis (28). In multiple sclerosis (MS), reduced A₁R protein and RNA levels were detected in cells of the monocyte/macrophage lineage. According to the authors, this decreased A₁R expression by macrophages may contribute to the progression of MS by decreasing the ability of adenosine to regulate the inflammatory response in the brain (34). When translating this to our allergen challenge setting in asthma patients, we found a decreased expression of A₁R on macrophages following allergen challenge which also could prevent adenosine to regulate the inflammatory response in the airways/sputum, and hereby explaining worsening of inflammation and symptoms following an allergen induced exacerbation.

A number of sputum cytokines were measured that are related to the pathogenesis of asthma. Asthma patients and healthy controls had similar levels of cytokines in sputum, but the levels of TSLP decreased after allergen challenge, whereas IL-8 levels increased. TSLP is a cytokine produced by airway epithelium, smooth muscle cells and mast cells and is capable of directing dendritic cells towards a Th2 response, thereby providing a link between epithelial cell activation/injury and an allergic type of inflammation. In this study, levels of TSLP in sputum were not different when comparing asthma patients with healthy subjects. This is the first study in which levels of TSLP were measured in sputum. TSLP expressing cells, measured by immunohistochemistry and mRNA, were described to be increased in bronchial biopsies from asthma patients (35). To our surprise, allergen challenge decreased TSLP levels in sputum of

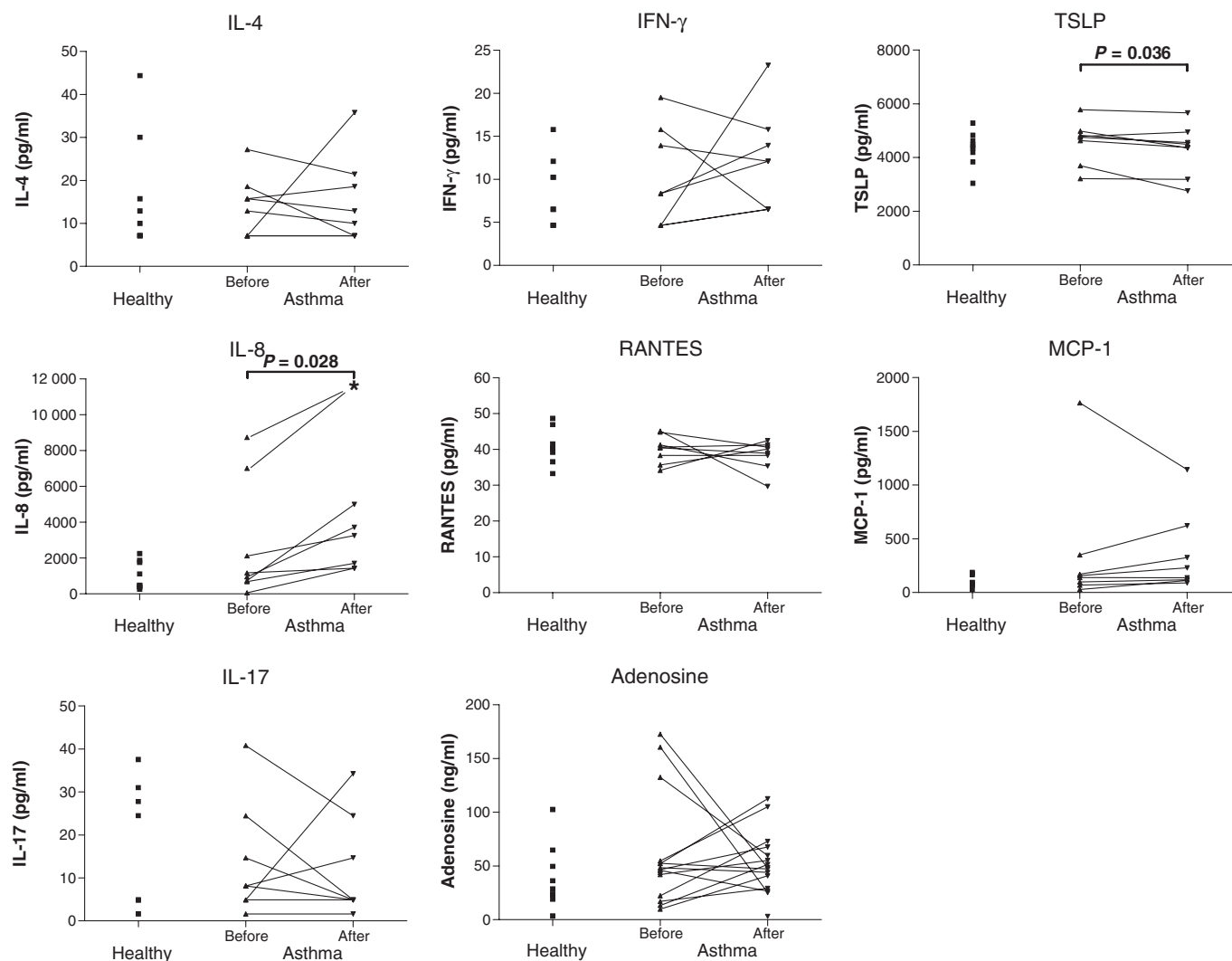


Figure 3. Levels of IL-4, IFN- γ , TSLP, IL-8, RANTES, MCP-1, IL-17, and adenosine in sputum supernatant of healthy and asthmatic subjects. Before: 3–4 days before allergen challenge; after: 24 h after allergen challenge (asthma patients). *: two samples contained > 12 000 pg/ml IL-8. Significant differences are indicated in the graphs.

asthma patients. Epithelial cells are the main producers of TSLP. We speculate that allergen challenge may have altered TSLP production temporarily because of production of other cytokines or growth factors involved in repair. Further studies have to elucidate the role of TSLP in asthma severity or progression.

In summary, this is the first study that reports on AR expression on different cell types in peripheral blood and sputum of healthy controls and asthma patients, and the change with allergen challenge in asthma. Asthma patients had lower A_{2B}R expression on sputum neutrophils than healthy controls. Furthermore, allergen challenge decreased A₁R expression on blood neutrophils and sputum macrophages, and A_{2A}R on blood neutrophils. This may contribute to the enhanced inflammation in asthma after allergen inhalation. This study contributes

to a better insight in the role of adenosine and its receptor in asthma. Further studies should include experiments on intracellular pathways of AR stimulation to get more insight in the downstream effects as well.

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