

The role of endogenous and exogenous AMP in asthma and chronic obstructive pulmonary disease

Maarten van den Berge, MD, PhD, Riccardo Polosa, MD, PhD, Huib A.M. Kerstjens, MD, PhD, and Dirkje S. Postma, MD, PhD *Groningen, The Netherlands*

Bronchial hyperresponsiveness is present in virtually all patients with asthma and in more than two thirds of patients with chronic obstructive pulmonary disease. Thus far, methacholine and histamine are usually used to measure bronchial hyperresponsiveness. Both are direct stimuli, because they act directly on airway smooth muscle. Another possible stimulus to measure bronchial hyperresponsiveness is AMP. AMP is an indirect stimulus, because it acts via the release of histamine and other mediators from immunologically primed mast cells. There is increasing interest in the role of AMP as a bronchoconstrictor stimulus because it has been suggested that the concentration of AMP causing the FEV₁ to decrease by 20% (PC₂₀ AMP) may be used as a noninvasive marker of airway inflammation. The aim of this article was to review the literature assessing AMP's value in asthma and chronic obstructive pulmonary disease. (*J Allergy Clin Immunol* 2004;114:737-46.)

Key words: AMP, adenosine, hyperresponsiveness, inflammation

Bronchial hyperresponsiveness (BHR) is present in virtually all patients with asthma and in more than two thirds of patients with chronic obstructive pulmonary disease (COPD).^{1,2} Methacholine and histamine act directly on receptors of airway smooth muscle and are the gold standard for quantifying BHR. An additional level of complexity as to the significance of BHR has been uncovered by using a wide range of indirect stimuli—such as hypertonic saline, ultrasonically nebulized distilled water, exercise performed under various climatic conditions, and AMP—that elicit bronchoconstriction through the release of inflammatory mediators.

Abbreviations used

BHR: Bronchial hyperresponsiveness
COPD: Chronic obstructive pulmonary disease
MCP-3: Monocyte chemotactic protein 3

Once inhaled, AMP is rapidly converted to adenosine by the ubiquitous enzyme 5'-nucleotidase. There is now a considerable amount of evidence both in asthma and COPD that AMP and, by implication, adenosine mainly act via the release of histamine and other mediators from immunologically primed mast cells.³⁻⁶

There is increasing interest in the role of AMP as a bronchoconstrictor stimulus because it has been suggested that the concentration of AMP causing the FEV₁ to decrease by 20% (PC₂₀ AMP) may be used as a noninvasive marker of airway inflammation.^{7,8} The aim of this article was to review the literature assessing its value in asthma and COPD. To this end, we discuss (1) the role of endogenous adenosine in airway inflammation in asthma and COPD, (2) the use of PC₂₀ AMP as a marker of inflammation in asthma and COPD, (3) the potential drawbacks of AMP challenge, and (4) the bronchoprotective effect of β_2 -adrenoceptor agonists against PC₂₀ AMP. We refer to Joos et al⁹ and Martinez-Garcia et al¹⁰ for the methodology of the PC₂₀ AMP and the comparison with other indirect challenges.

ROLE OF ENDOGENOUS ADENOSINE IN ASTHMATIC AIRWAY INFLAMMATION

Adenosine is a signaling nucleotide that acts on extracellular cell-surface receptors.^{11,12} Four different 7-transmembrane-spanning adenosine receptors have been described, namely, adenosine A₁, A_{2A}, A_{2B}, and A₃ receptors.¹³ Human lung mast cells have been shown to express A_{2A} and A_{2B} receptors, but not A₁ or A₃ receptors.^{14,15} In a recent study, only activation of the A_{2B} receptor resulted in mast cell activation.^{16,17} Thus, there is evidence suggesting that AMP-induced bronchoconstriction is mediated by activation of the A_{2B} receptor.

From the Department of Pulmonology, University Hospital Groningen.

The authors declare no conflict of interest.

Received for publication December 15, 2003; revised May 9, 2004; accepted for publication May 13, 2004.

Available online August 31, 2004.

Reprint requests: Dirkje S. Postma, MD, PhD, Department of Pulmonary Diseases, University Hospital Groningen, Hanzeplein 1, 9700 RB, Groningen, The Netherlands. E-mail: D.S.Postma@int.azg.nl.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2004.05.071

It seems unlikely that the cyclic AMP protein kinase pathway is responsible for adenosine-induced mast cell mediator release, because this is not inhibited by treatment of mast cells with KT5720, which completely blocks cyclic AMP-dependent protein kinase activity.¹⁷ Alternatively, activation of phospholipase C and the subsequent increase in intracellular calcium levels may mediate the effects of adenosine.

Adenosine is a naturally occurring purine nucleoside with a ubiquitous presence in human tissue. AMP is formed by the catabolism of high-energy adenosine phosphates such as adenosine diphosphate or ATP during the process of energy generation. When sufficient oxygen and energy are available, AMP is reconverted to high-energy phosphates forming a component of the energy cycle. However, in case of excessive cell stimulation or hypoxia, AMP can not be reconverted and is transported to the exterior of the cell, where it is metabolized to adenosine by the enzyme 5'-nucleotidase. The discovery that adenosine levels are increased in the bronchoalveolar lavage fluid of patients with asthma and increase further after allergen challenge raises the possibility that the adenosine generated in asthmatic airways itself contributes to the pathogenesis of asthma.^{18,19} An explanation for this finding could be that stimulation of inflammatory cells and subsequent smooth muscle contraction during the asthmatic inflammatory process leads to an increase in oxygen and energy demand, thus leading to an increase in the level of adenosine in the airway in asthma.

We have recently reported that inhalation of AMP and, by implication, adenosine induces a rapid (within 1 hour) inflammatory response with an increase of eosinophils in the airway in asthmatic subjects.²⁰ A possible explanation for these findings is the release of mast cell-derived inflammatory mediators. There are various clues that mast cell-derived mediators could account for an influx of eosinophils into the airway. For example, it has been demonstrated that the proteases tryptase and chymase provide a potent stimulus for eosinophil accumulation after injection into the skin of guinea pigs.²¹⁻²³ In addition, inhalation of leukotriene D₄ and leukotriene E₄, which are the major lipoxygenase products of mast cells, is associated with increases of eosinophils in sputum and bronchial biopsy samples.²⁴⁻²⁶ Further, the cytokines tumor necrosis factor- α and IL-4, which are stored preformed in the granules of mast cells, can facilitate eosinophil recruitment by upregulating adhesion molecules (vascular cell adhesion molecule 1 and E-selectin) in the endothelial layer of the bronchial vasculature.^{27,28} Another possible explanation for the AMP-induced influx of eosinophils into the airway might be that adenosine also exerts an effect on A₁, A_{2A}, and A₃ receptors, which have been identified on neutrophils, eosinophils, and macrophages. The exact consequences of their activation are difficult to predict because various proinflammatory and anti-inflammatory actions have been described.^{14,29-31} Thus, although adenosine may well contribute to the presence or extent of airway inflammation, it is not yet known which adenosine receptors are involved.

Animal models provide an opportunity to specifically investigate the role of adenosine in asthma. It has thus been shown that mice that lack the enzyme adenosine deaminase (and are therefore unable to break down endogenously formed adenosine) spontaneously develop features of asthma including BHR, enhanced mucus secretion, airway eosinophilia, and increased IgE synthesis.^{32,33} These asthmatic features were reversed by exogenous administration of adenosine deaminase. Transcript array technology was used to examine which genes in the lung become activated by adenosine when accumulated in adenosine deaminase-deficient mice. Thus, overexpression of the monocyte chemotactic protein 3 (MCP-3) gene in the airway was found and was paralleled by enhanced MCP-3 secretion. MCP-3 is a chemokine with potent eosinophil chemotactic properties. The same study showed greatly enhanced airway expression of molecules involved in tissue remodeling, including vascular endothelial growth factor, osteopontin, and fibronectin, as well as the cathepsin family of neutral proteases. This provides a further important link between adenosine and asthma. Thus, taken together, it could be speculated that adenosine and adenosine receptors play an important role in asthma.

A therapeutic potential of adenosine receptor antagonists and agonists may be anticipated, because it has been shown in animal models that adenosine A₁ inhibitors and adenosine A_{2A} agonists have a protective effect on the late asthmatic response after allergen challenge.^{34,35} In this context, it is of interest that treatment with theophylline, which is also an adenosine A_{2B} antagonist, has been shown to reduce airway inflammation both in asthma and in COPD.^{36,37} Although it was originally hypothesized that theophylline acts via inhibition of phosphodiesterases, it has been observed that the concentration of theophylline required to effectively inhibit phosphodiesterases *in vivo* was much greater than the therapeutic level achieved in humans.³⁸ Because theophylline also inhibits adenosine A_{2B} receptors at concentrations likely to occur *in vivo*, this could well be an alternative explanation for its beneficial effects in asthma and COPD.³⁹⁻⁴²

In conclusion, adenosine is likely to play an important role in asthma and, most likely, in smokers with COPD. The role of adenosine and adenosine receptors deserves further study, because it may have therapeutic implications.

PC₂₀ TO AMP AS A NONINVASIVE MARKER OF AIRWAY INFLAMMATION IN ASTHMA AND COPD

Asthma

Although it is generally accepted that airway inflammation contributes to both the presence and the severity of BHR, the association between airway inflammation and direct BHR is weak.⁴³⁻⁴⁷ Other factors also contribute to the presence and severity of BHR. Indeed, recent morphologic and functional studies have demonstrated that

BHR may be compounded by airway remodeling.⁴⁸ It is interesting to note that it has been demonstrated in patients with asthma that both AMP and methacholine responsiveness are associated with lung function. However, associations were stronger with methacholine than with AMP. Conversely, sputum eosinophilia was more closely associated with AMP responsiveness (Fig 1).⁴⁹ In this study, the PC₂₀ AMP was performed 1 hour after PC₂₀ methacholine, and a sputum induction was performed 1 day later. Furthermore, when these patients were treated with corticosteroids for 2 weeks, it was found that the steroid-induced improvement of PC₂₀ AMP was also more closely related to the concomitant reduction in airway inflammation than was the PC₂₀ methacholine.⁵⁰ In agreement with this, it has been shown that the PC₂₀ AMP is more sensitive for detecting changes in airway inflammation after a stay of 1 month in a hypoallergenic environment at high altitude (Davos, Switzerland).⁵¹ Prosperini et al⁵² studied longitudinal changes in BHR with methacholine and AMP, as well as sputum cell counts before, during, and up to 3 weeks after withdrawal from a 6-week therapy with inhaled budesonide (800 µg daily). When the 3 markers were compared, the PC₂₀ AMP promptly detected inflammatory changes as early as the first week of budesonide treatment, whereas changes in PC₂₀ methacholine and sputum cell counts could be observed only by the fourth week of treatment. After treatment discontinuation, PC₂₀ AMP values returned to near-baseline levels as early as the first week of treatment, when methacholine responsiveness and the percentage of sputum eosinophils were still significantly decreased. That AMP responsiveness more closely reflects reductions in airway inflammation after inhaled corticosteroids than inflammatory markers in induced sputum has also been substantiated in a study by Taylor et al,⁵³ in which the effect of 3 different doses of inhaled ciclesonide on PC₂₀ AMP and sputum eosinophils was assessed. There was a dose-dependent decrease in PC₂₀ AMP, whereas the reduction in the percentage of eosinophils did not differ between doses. These data suggest that the PC₂₀ AMP may be one of the most accurate means of monitoring inhaled corticosteroid requirements in asthma and of establishing the appropriate dose needed to control airway inflammation. These promising data, obtained in a small population of highly selected patients with asthma, now need to be confirmed in larger populations of patients for longer periods of time. Finally, AMP seems to be considerably more specific and sensitive than methacholine at discriminating asthma from pediatric chronic obstructive lung disorders such as cystic fibrosis, bronchiolitis, pulmonary ciliary dyskinesia, and bronchiectasis.⁵⁴ Taken together, there is increasing evidence that airway responsiveness to AMP may have useful clinical applications. However, specific recommendations for AMP challenge testing will require further work.

In this context, the recent findings of Green et al⁵⁵ are of special interest. They compared a treatment strategy directed at normalizing airway inflammation (as reflected by the percentage of sputum eosinophils) with a treatment

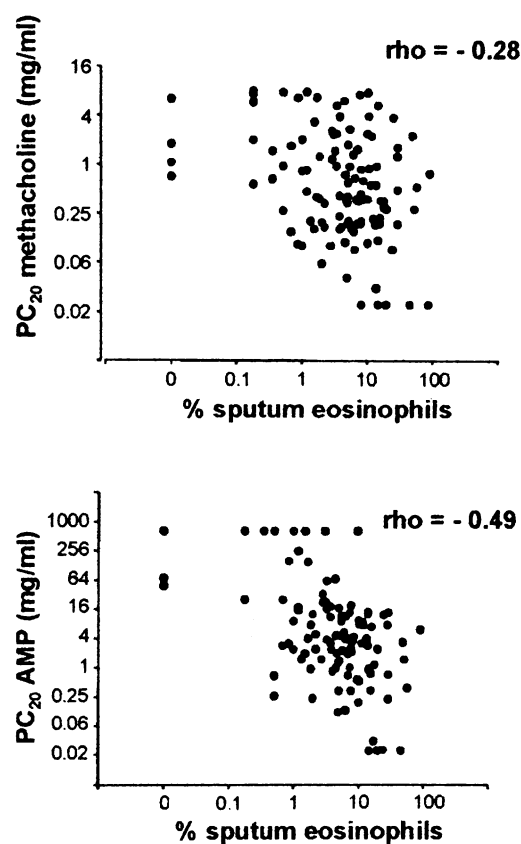


FIG 1. Unadjusted relationship between PC₂₀ methacholine and PC₂₀ AMP with the percentage of sputum eosinophils in patients with asthma. Reprinted with permission from Van Den Berge et al. 2001. PC₂₀ adenosine 5'-monophosphate is more closely associated with airway inflammation in asthma than PC₂₀ methacholine. *American Journal of Respiratory and Critical Care Medicine*. Vol 163, pp. 1546-60. Official Journal of the American Thoracic Society. © American Thoracic Society.

strategy guided by symptoms and lung function according to the British Thoracic Society guidelines. Patients who received adjustment of anti-inflammatory treatment on the basis of the percentage of sputum eosinophils showed a remarkable improvement in asthma control, with less airway inflammation and BHR and fewer asthma exacerbations (Fig 2). In addition, the doses of inhaled corticosteroids did not differ between groups. Thus, this is the first study undisputedly showing that monitoring of airway inflammation may be important for improving the management and outcome of asthma. Despite this positive notion, sputum induction is unlikely to have a place in asthma management in the foreseeable future, given the drawbacks that limit its use in daily clinical practice. Dedicated, highly qualified laboratory technicians are needed, and the technique is time-consuming, preventing rapid help in decision making to change treatment. The sputum induction procedure takes at maximum 1 hour, and processing up to the necessary provision of percentage eosinophils takes a further 3 hours. Therefore, it is now clear that further development and validation of other noninvasive markers of airway

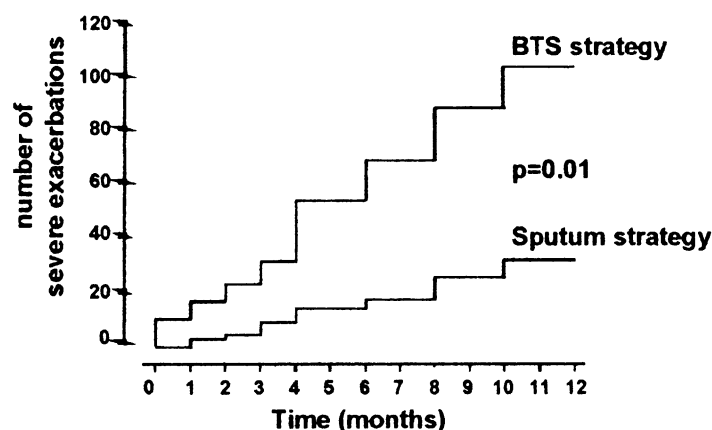


FIG 2. Cumulative asthma exacerbations in the treatment strategy directed at normalizing airway inflammation (as reflected by the percentage of sputum eosinophils; *sputum strategy*) compared with the treatment strategy guided by symptoms and lung function according to the British Thoracic Society guidelines (*BTS strategy*). Reprinted with permission from Elsevier (The Lancet, 2002, Vol 260, pp. 1715-1721).

inflammation is an important research goal. In this context, a provocation test with AMP may well have a role. One also needs a qualified lung function technician, but the value of PC₂₀ AMP is available for decision making within 45 minutes to 1.5 hours and requires fewer personnel. Taken together, it would be interesting to investigate whether a treatment of asthma with inhaled corticosteroids based on the severity of PC₂₀ AMP leads to better control of (eosinophilic) airway inflammation when compared with treatment based on a doctor's decision according to current guidelines (symptoms, β -mimetic rescue treatment, and peak expiratory flow variability).

A possible explanation for the finding that the PC₂₀ AMP is more closely associated with airway inflammation than the PC₂₀ methacholine may be that AMP indirectly acts via the release of inflammatory mediators by mast cells. An overall increased inflammatory cell activity, ie, primed cells, can result in an increased production of cytokines and other mediators that increase the number and activation state of mast cells. As a result, more mast cell mediators will be released after provocation with AMP, but not with methacholine. Alternatively, the number or activation state of eosinophils may be increased as a result of an increased production of inflammatory mediators by mast cells, especially IL-5. It could be speculated that airway challenges with other indirect stimuli (eg, exercise, hypertonic saline, or mannitol) also better reflect airway inflammation than direct stimuli, but this has not yet formally been demonstrated with measures of inflammation, as has been performed in the case of methacholine and AMP responsiveness.⁹ Furthermore, when different direct and indirect stimuli are compared head to head, it seems that responsiveness to AMP always improves more than responsiveness to the direct stimulus methacholine.^{52,56-60} By contrast, when bradykinin, exercise, or ultrasonically nebulized distilled water is assessed, responsiveness is frequently similar with the indirect and direct stimuli (Table I).^{58,61-66} This would suggest that

AMP better reflects airway inflammation than these indirect stimuli.

COPD

AMP can induce bronchoconstriction in subjects with COPD as well. Although mast cells are commonly not associated with the inflammatory process in COPD, higher numbers of mast cells have been found by several groups.^{67,68} Hyperresponsiveness to AMP has a higher prevalence and severity in smokers than in nonsmokers (or ex-smokers) with COPD who have comparable levels of hyperresponsiveness to methacholine.⁶⁹ This is in agreement with findings of increased concentrations of tryptase and histamine in the bronchoalveolar lavage fluid of smokers.^{67,68} Although the underlying inflammatory process is different in asthma and COPD, AMP responsiveness might also reflect airway inflammation in COPD. It has been shown that subjects with COPD who have hyperresponsiveness to AMP have higher percentages of sputum eosinophils than those without hyperresponsiveness.⁷⁰ The identification of sputum eosinophilia by measuring hyperresponsiveness to AMP might create therapeutic possibilities, because it has been found in several studies that subjects with COPD who have higher percentages of eosinophils in induced sputum or bronchoalveolar lavage fluid respond better to therapy with corticosteroids.^{71,72} In support of this, Rutgers et al⁷³ previously showed in 44 subjects with COPD that the improvement in PC₂₀ AMP after 6 weeks of therapy with inhaled budesonide was significantly associated with more peripheral blood eosinophils at baseline.

Longitudinal studies in patients with COPD have shown an association between bronchial responsiveness to methacholine and a subsequent decline in lung function.^{74,75} For this reason, it has been speculated that BHR is an inherent risk factor for the evolution of COPD. It could also be argued, however, that both an increased BHR and an accelerated decline in lung function in COPD

TABLE I. Relative effects of inhaled corticosteroids on indirect versus direct stimuli

Study	Treatment			Relative effect (indirect vs direct stimuli)
	Drug	Dose ($\mu\text{g}/\text{d}$)	Duration (wk)	
O'Connor ⁵⁶	BUD	1600	2	AMP > methacholine
Weersink ⁵⁷	FP	500	6	AMP > methacholine
Doull ⁵⁸	BDP	400	12	AMP > methacholine (no effect)
Meijer ⁵⁹	FP	500	2	AMP > methacholine
		2000	2	AMP > methacholine
Ketchell ⁶⁰	FP	1000	Single dose	AMP > histamine (no effect)
Prosperini ⁵²	BUD	800	6	AMP > methacholine
Fuller ⁶¹	BUD	1200	3	Bradykinin = histamine
Doull ⁵⁸	BDP	400	12	No effect on bradykinin or histamine
Reynolds ⁶²	FP	440	16	Bradykinin > methacholine
Vathenen ⁶³	BUD	1600	6	Exercise = histamine
Hofstra ⁶⁴	FP	200	6	Exercise > methacholine
	FP	500	6	Exercise = methacholine
Groot ⁶⁵	BDP	800	8	UNDW = histamine
Bootsma ⁶⁶	BDP	1500	6	UNDW = histamine
	FP	750	6	UNDW = histamine

BUD, Budesonide; FP, fluticasone propionate; BDP, beclomethasone dipropionate; UNDW, ultrasonically nebulized distilled water.

are the result of smoking-related airway inflammation and remodeling. In agreement with this possibility, several studies have found that smoking cessation is associated with a subsequent similar rate of decline as in never-smokers and considerably less decline than in persistent smokers.^{76,77} Whether this decreased decline in lung function is accompanied by improvement in BHR is unknown. In this context, the findings of Oosterhoff et al⁷⁸ are of special interest. They found that ex-smokers with COPD have a lower level of BHR to AMP and a similar level of hyperresponsiveness to methacholine than smokers with COPD. This may suggest that smoking cessation improves BHR to AMP, but not to methacholine.⁷⁸ Taken together, it now becomes interesting to speculate that the level of BHR to AMP may reflect some degree of smoking-related airway inflammation involved in the accelerated decline in lung function in COPD. Therefore, future studies are needed to assess the value of BHR to AMP as a risk factor for subsequent decline in lung function in COPD and to assess the effect of smoking cessation on BHR to methacholine and AMP, as well as airway inflammation, in patients with COPD.

DRAWBACKS OF AMP CHALLENGE

There are several possible drawbacks to the use of AMP challenge. First, the cutoff point between normal and abnormal for the PC₂₀ AMP is arbitrary, because no population-based data are available. It has been proposed to use a cutoff point of 160 mg/mL because this was discriminatory between patients with asthma and healthy controls in a study of Oosterhoff et al⁷⁹ with a limited number of patients. However, in other studies it was found

that a large percentage of patients with asthma is unresponsive to AMP even after the highest concentration of AMP applied (which is between 320 and 400 mg/mL; above these concentrations, AMP becomes insoluble).^{59,80,81} In a recent study of Fowler et al,⁸⁰ 92 (50%) of 185 patients with a doctor's diagnosis of asthma were unresponsive to AMP (PC₂₀ AMP >200 mg/mL), whereas only 45 (24%) of 185 patients were unresponsive to methacholine. Thus, it seems that a provocation test with AMP may not be useful to establish a diagnosis of asthma. Second, no published safety data are available on the use of AMP, although thousands of adenosine challenges have been performed by different laboratories, with no reported serious side effects. This may become an important item for AMP to be officially approved for use in humans both in Europe and the United States. Third, it has been shown that subjects with allergy but without asthma also exhibit a bronchoconstrictor response after inhalation of AMP.^{3,8} An explanation for this finding could be that subjects with allergy but without asthma also exhibit airway inflammation. Indeed, more eosinophils have been found in the bronchial mucosa of subjects with allergy but without asthma compared with subjects without allergy or asthma.⁸² It is interesting to note that similar to the findings in patients with asthma, it was recently demonstrated that the PC₂₀ AMP, but not the PC₂₀ methacholine, was significantly associated with inflammation in the lower airways in subjects with allergic rhinitis in whom the diagnosis of asthma was specifically excluded.⁸ Fourth, it may be that several medications specifically inhibit the PC₂₀ AMP, whereas they do not seem to have a marked effect on airway inflammation. This can be a pitfall during interpretation of the outcome of a provocation test with AMP in patients with asthma and COPD. AMP induces its bronchoconstrictor effect via the release

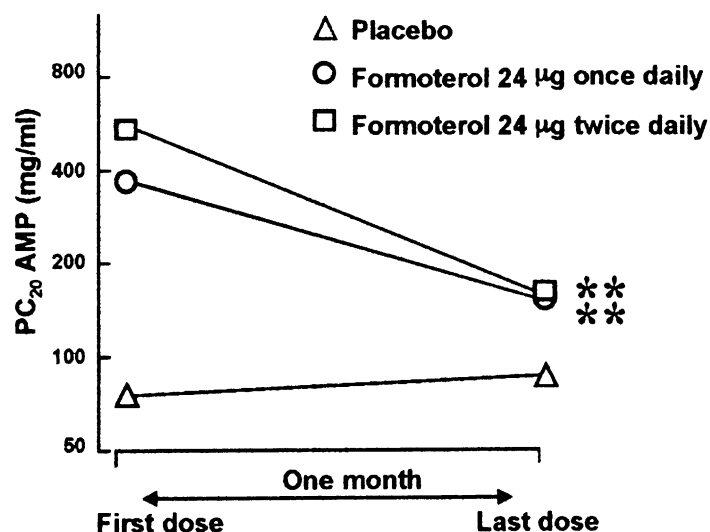


FIG 3. PC₂₀ AMP after 1 month of treatment with placebo, formoterol 24 µg once daily, or formoterol 24 µg twice daily. The PC₂₀ AMP was measured 12 hours after the first and last doses. The bronchoprotective effect of formoterol significantly decreased after 1 month of treatment with both formoterol 24 µg twice daily and formoterol 24 µg once daily. ***P* < .01. Reprinted with permission from Aziz et al. 1998. Subsensitization to bronchoprotection against adenosine monophosphate challenge following regular once daily formoterol. *European Respiratory Journal*. Vol 12. pp. 580-4.

of mediators (histamine, prostaglandins, and leukotrienes) from immunologically primed mast cells. For this reason, the PC₂₀ AMP becomes difficult to interpret in patients who take antihistamines, prostaglandin synthetase inhibitors (nonsteroidal anti-inflammatory drugs), and leukotriene inhibitors and in patients who take mast cell-stabilizing drugs such as nedocromil or sodium cromoglycate. Further, the use of theophylline and enprofylline protects to a large extent against the PC₂₀ AMP, and this could be at least partially explained by a specific inhibiting effect on the adenosine A_{2B} receptor. Thus, the PC₂₀ AMP becomes difficult to interpret in patients who take any of the above-mentioned medications, and this has to be accounted for when the PC₂₀ AMP is used to monitor airway inflammation. This may be also true for β₂-agonists (see below). Fifth, it has been shown that the number of eosinophils increases 1 hour after AMP challenge.²⁰ After allergen challenge, inflammation occurs in the airway of patients with asthma; this is represented by increases in eosinophils and is accompanied by an early and late obstructive response.⁸³ In this context, one might consider AMP challenge to have a negative influence on asthma. However, neither an increase in nonspecific BHR nor a decline in FEV₁ up to 24 hours after AMP challenge could be demonstrated in the study of Phillips and Holgate.⁸⁴ This makes it likely that the increase in eosinophils is induced by mast cell stimulation, yet without the sequel of an allergen challenge. Finally, it has been shown that the PC₂₀ AMP, in contrast to the PC₂₀ methacholine or the concentration of exhaled nitric oxide, significantly improves within 2 hours of a single inhaled dose as low as 100 µg of fluticasone propionate.^{60,85} The mechanism underlying this phenom-

enon is not well established; it has been proposed that it involves an effect on airway mucosal blood flow and microvascular permeability. Although the rapid protective effect by a single dose of an inhaled corticosteroid has never been studied in patients with asthma taking chronic inhaled corticosteroids, the extreme sensitivity of AMP to these drugs could be a relative disadvantage to its potential clinical applications.

MAST CELLS, β₂-ADRENOCEPTOR AGONISTS, AND BRONCHOPROTECTION AGAINST PC₂₀ AMP

In recent years, there has been a tendency to attach less importance to the role of the mast cell as an effector cell in asthma. However, new findings have led to a resurgence of interest in the mast cell and support a re-evaluation of its role. In particular, the discovery that mast cells are a source of cytokines has suggested new ways in which mast cell activation could also participate in more persistent and even chronic inflammatory responses.⁸⁶⁻⁸⁸ These observations have obvious implications for the role of mast cells in both allergic and nonallergic asthma. Provocation with AMP may therefore become a useful tool for further exploration of the role of mast cells in asthma, because there is now convincing evidence that the airway response to this nucleotide is an index of mast cell number or activity.

β₂-Adrenoceptor agonists are, next to inhaled corticosteroids, important in the management of asthma. The main benefit of these drugs is bronchodilation, but additional actions include stabilization of inflammatory

cell activity. In this context, the mast cell may be an important target.^{89,90} In agreement with this, it has been demonstrated that inhaled albuterol, terbutaline, and formoterol provide greater bronchoprotection against AMP-induced bronchoconstriction than against methacholine- or histamine-induced bronchoconstriction.^{91,92} This differential bronchoprotective effect of these β_2 -adrenoceptor agonists has been interpreted as mast cell stabilization. The differential bronchoprotective effect of formoterol seems to be greater than that of albuterol or terbutaline, whereas no difference in bronchoprotective effect with AMP versus methacholine was found with salmeterol either 2 or 14 hours after a single dose.⁹³⁻⁹⁵ The lack of such a differential bronchoprotective effect of salmeterol may reflect the fact that it is a partial agonist compared with formoterol, albuterol, or terbutaline.

The protection provided by β_2 -agonists against mast cell mediator release might be important to prevent acute exacerbations in asthma that are partly mediated by mast cell activation.⁹⁶ This action may contribute to the reduction in severe and mild exacerbations seen when formoterol is added to maintenance treatment with inhaled corticosteroids.⁹⁷ Unfortunately, no studies are available about the additive effect of salmeterol in patients with asthma who are already taking inhaled corticosteroids. Thus far, studies investigating the additive effect of salmeterol have compared this with the additive effect of a higher dose of inhaled corticosteroids.⁹⁸⁻¹⁰⁰ An argument against the explanation for the beneficial effect of combination therapy with formoterol and budesonide could be that subsensitivity to the bronchoprotective effect against AMP develops when patients with asthma take formoterol regularly for 1 month (Fig 3).¹⁰¹ These findings suggest that the β_2 -adrenoceptors present on mast cells desensitize rapidly after daily treatment with formoterol. Although corticosteroids prevent the loss of function of β_2 -agonists with chronic use, this effect occurred despite concomitant inhaled corticosteroid therapy (Fig 3).^{101,102} It has been reported that the extent of desensitization is highly variable between subjects and that these interindividual differences are linked to genetic polymorphisms in the β_2 -adrenoceptor.¹⁰³ Thus, it could be speculated that a subgroup of patients with asthma is resistant to mast cell β_2 -adrenoceptor desensitization. In this subgroup of patients with asthma, adding formoterol to maintenance therapy with inhaled corticosteroids may well have an additional anti-inflammatory effect.

CONCLUSIONS

Measurement of BHR not only seems to be of pathophysiological interest, but may also be of clinical importance. It is interesting to note that the PC₂₀ AMP (1) is more closely associated with airway inflammation in asthma than the PC₂₀ methacholine and (2) better reflects corticosteroid-induced reduction airway inflammation. Moreover, the PC₂₀ AMP seems to offer substantial advantages over other noninvasive markers of inflamma-

tion because of its exquisite sensitivity in probing changes to airway inflammation in response to corticosteroids. This may be important, because it has been shown that a management plan incorporating a noninvasive marker of airway inflammation might improve the treatment of asthma; in this context, the PC₂₀ AMP can be superior to a plan based on lung function and symptom control alone. The PC₂₀ AMP is also associated with airway inflammation in COPD. This might create therapeutic possibilities, because it has been found in several studies that subjects with COPD who have a higher number of eosinophils respond better to therapy with corticosteroids. Information obtained from bronchial challenge tests provides additional and complementary information about different aspects of asthma, ranging from remodeling over diagnosis to guidance in the optimization of anti-inflammatory treatment. In addition, the information obtained by direct and indirect challenges is different, providing us with increasingly more refined information, the applications of which are currently only partially grasped. This intriguing research area is in full expansion and will undoubtedly provide us with interesting new insights into asthma, COPD, and other illnesses.

REFERENCES

- Postma DS, Kerstjens HAM. Characteristics of airway hyperresponsiveness in asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;158:S187-92.
- Kanner RE, Connett JE, Altose MD, Buist AS, Lee WW, Tashkin DP, et al. Gender difference in airway hyperresponsiveness in smokers with mild COPD. The Lung Health Study. *Am J Respir Crit Care Med* 1994;150:956-61.
- Phillips GD, Ng WH, Church MK, Holgate ST. The response of plasma histamine to bronchoprovocation with methacholine, adenosine 5'-monophosphate, and allergen in atopic nonasthmatic subjects. *Am Rev Respir Dis* 1990;141:9-13.
- Polosa R, Ng WH, Crimi N, Vancheri C, Holgate ST, Church MK, et al. Release of mast-cell-derived mediators after endobronchial adenosine challenge in asthma. *Am J Respir Crit Care Med* 1995;151:624-9.
- Rutgers SR, Koeter GH, Van Der Mark TW, Postma DS. Protective effect of oral terfenadine and not inhaled ipratropium on adenosine 5'-monophosphate-induced bronchoconstriction in patients with COPD. *Clin Exp Allergy* 1999;29:1287-92.
- van den Berge M, Kerstjens HAM, Postma DS. Provocation with adenosine 5'-monophosphate as a marker of inflammation in asthma, allergic rhinitis and chronic obstructive pulmonary disease. *Clin Exp Allergy* 2002;32:824-30.
- van den Berge M, Meijer RJ, Kerstjens HAM, de Reus DM, Koeter GH, Kauffman HF, et al. PC(20) adenosine 5'-monophosphate is more closely associated with airway inflammation in asthma than PC(20) methacholine. *Am J Respir Crit Care Med* 2001;163:1546-50.
- Polosa R, Ciamarra I, Mangano G, Prosperini G, Pistorio MP, Vancheri C, et al. Bronchial hyperresponsiveness and airway inflammation markers in nonasthmatics with allergic rhinitis. *Eur Respir J* 2000;15:30-5.
- Joos GF, O'Connor B, Anderson SD, Chung F, Cockcroft DW, Dahlen B, et al. Indirect airway challenges. *Eur Respir J* 2003;21:1050-68.
- Martinez-Garcia MA, Perpina-Tordera M, Vila V, Compte-Torrero L, Diego-Damia A, Macian-Gisbert V. Analysis of the stability of stored adenosine 5'-monophosphate used for bronchoprovocation. *Pulm Pharmacol Ther* 2002;15:157-60.
- Cushley MJ, Tallant N, Holgate ST. The effect of dipyrindamole on histamine- and adenosine-induced bronchoconstriction in normal and asthmatic subjects. *Eur J Respir Dis* 1985;67:185-92.

12. Crimi N, Palermo F, Oliveri R, Maccarrone C, Palermo B, Vancheri C, et al. Enhancing effect of dipyrindamole inhalation on adenosine-induced bronchospasm in asthmatic patients. *Allergy* 1988;43:179-83.
13. Collis MG, Hourani SM. Adenosine receptor subtypes. *Trends Pharmacol Sci* 1993;14:360-6.
14. Walker BA, Jacobson MA, Knight DA, Salvatore CA, Weir T, Zhou D, et al. Adenosine A3 receptor expression and function in eosinophils. *Am J Respir Cell Mol Biol* 1997;16:531-7.
15. Marquardt R, Fischer J, Kustner W. Effect of arterial hypoxia and acidosis on fibrillation threshold in the cat heart in vivo. *Z Kardiol* 1976;65:585-9 [in German].
16. Feoktistov I, Biaggioni I. Adenosine A2b receptors evoke interleukin-8 secretion in human mast cells. An enprofylline-sensitive mechanism with implications for asthma. *J Clin Invest* 1995;96:1979-86.
17. Marquardt DL, Walker LL. Inhibition of protein kinase A fails to alter mast cell adenosine responsiveness. *Agents Actions* 1994;43:7-12.
18. Driver AG, Kukoly CA, Ali S, Mustafa SJ. Adenosine in bronchoalveolar lavage fluid in asthma. *Am Rev Respir Dis* 1993;148:91-7.
19. Mann JS, Holgate ST, Renwick AG, Cushley MJ. Airway effects of purine nucleosides and nucleotides and release with bronchial provocation in asthma. *J Appl Physiol* 1986;61:1667-76.
20. van den Berge M, Kerstjens HAM, de Reus DM, Koeter GH, Kauffman HF, Postma DS. Provocation with adenosine 5'-monophosphate, but not methacholine, induces sputum eosinophilia. *Clin Exp Allergy* 2004;34:71-6.
21. Walls AF, He S, Teran LM, Buckley MG, Jung KS, Holgate ST, et al. Granulocyte recruitment by human mast cell tryptase. *Int Arch Allergy Immunol* 1995;107:372-3.
22. He S, Walls AF. Human mast cell chymase induces the accumulation of neutrophils, eosinophils and other inflammatory cells in vivo. *Br J Pharmacol* 1998;125:1491-500.
23. Compton SJ, Cairns JA, Holgate ST, Walls AF. Interaction of human mast cell tryptase with endothelial cells to stimulate inflammatory cell recruitment. *Int Arch Allergy Immunol* 1999;118:204-5.
24. Gauvreau GM, Parameswaran KN, Watson RM, O'Byrne PM. Inhaled leukotriene E(4), but not leukotriene D(4), increased airway inflammatory cells in subjects with atopic asthma. *Am J Respir Crit Care Med* 2001;164:1495-500.
25. Diamant Z, Hiltermann JT, van Rensen EL, Callenbach PM, Veselic-Charvat M, van der Veen H, et al. The effect of inhaled leukotriene D4 and methacholine on sputum cell differentials in asthma. *Am J Respir Crit Care Med* 1997;155:1247-53.
26. Mulder A, Gauvreau GM, Watson RM, O'Byrne PM. Effect of inhaled leukotriene D4 on airway eosinophilia and airway hyperresponsiveness in asthmatic subjects. *Am J Respir Crit Care Med* 1999;159:1562-7.
27. Hirata N, Kohrogi H, Iwagoe H, Goto E, Hamamoto J, Fujii K, et al. Allergen exposure induces the expression of endothelial adhesion molecules in passively sensitized human bronchus: time course and the role of cytokines. *Am J Respir Cell Mol Biol* 1998;18:12-20.
28. Blease K, Seybold J, Adcock IM, Hellewell PG, Burke-Gaffney A. Interleukin-4 and lipopolysaccharide synergize to induce vascular cell adhesion molecule-1 expression in human lung microvascular endothelial cells. *Am J Respir Cell Mol Biol* 1998;18:620-30.
29. Cronstein BN, Daguma L, Nichols D, Hutchison AJ, Williams M. The adenosine/neutrophil paradox resolved: human neutrophils possess both A1 and A2 receptors that promote chemotaxis and inhibit O₂ generation, respectively. *J Clin Invest* 1990;85:1150-7.
30. Felsch A, Stocker K, Borchard U. Phorbol ester-stimulated adherence of neutrophils to endothelial cells is reduced by adenosine A2 receptor agonists. *J Immunol* 1995;155:333-8.
31. Suzuki H, Takei M, Nakahata T, Fukamachi H. Inhibitory effect of adenosine on degranulation of human cultured mast cells upon cross-linking of Fc epsilon RI. *Biochem Biophys Res Commun* 1998;242:697-702.
32. Chunn JL, Young HW, Banerjee SK, Colasurdo GN, Blackburn MR. Adenosine-dependent airway inflammation and hyperresponsiveness in partially adenosine deaminase-deficient mice. *J Immunol* 2001;167:4676-85.
33. Zhong H, Chunn JL, Volmer JB, Fozard JR, Blackburn MR. Adenosine-mediated mast cell degranulation in adenosine deaminase-deficient mice. *J Pharmacol Exp Ther* 2001;298:433-40.
34. Fozard JR, Ellis KM, Villela Dantas MF, Tigani B, Mazzoni L. Effects of CGS 21680, a selective adenosine A2A receptor agonist, on allergic airways inflammation in the rat. *Eur J Pharmacol* 2002;438:183-8.
35. Nyce JW, Metzger WJ. DNA antisense therapy for asthma in an animal model. *Nature* 1997;385:721-5.
36. Lim S, Tomita K, Carramori G, Jatakanon A, Oliver B, Keller A, et al. Low-dose theophylline reduces eosinophilic inflammation but not exhaled nitric oxide in mild asthma. *Am J Respir Crit Care Med* 2001;164:273-6.
37. Morali T, Yilmaz A, Erkan F, Akkaya E, Ece F, Baran R. Efficacy of inhaled budesonide and oral theophylline in asthmatic subjects. *J Asthma* 2001;38:673-9.
38. Sullivan P, Bekir S, Jaffar Z, Page C, Jeffery P, Costello J. Anti-inflammatory effects of low-dose oral theophylline in atopic asthma. *Lancet* 1994;343:1006-8.
39. Feoktistov I, Polosa R, Holgate ST, Biaggioni I. Adenosine A2B receptors: a novel therapeutic target in asthma? *Trends Pharmacol Sci* 1998;19:148-53.
40. Klotz KN, Hessling J, Hegler J, Owman C, Kull B, Fredholm BB, et al. Comparative pharmacology of human adenosine receptor subtypes—characterization of stably transfected receptors in CHO cells. *Naunyn Schmiedeberg Arch Pharmacol* 1998;357:1-9.
41. Evans DJ, Taylor DA, Zetterstrom O, Chung KF, O'Connor BJ, Barnes PJ. A comparison of low-dose inhaled budesonide plus theophylline and high-dose inhaled budesonide for moderate asthma. *N Engl J Med* 1997;337:1412-8.
42. Barnes PJ. Therapy of chronic obstructive pulmonary disease. *Pharmacol Ther* 2003;97:87-94.
43. Sont JK, Han J, van Krieken JM, Evertse CE, Hooijer R, Willems LN, et al. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. *Thorax* 1996;51:496-502.
44. Aalbers R, de Monchy JG, Kauffman HF, Smith M, Hoekstra Y, Vrugt B, et al. Dynamics of eosinophil infiltration in the bronchial mucosa before and after the late asthmatic reaction. *Eur Respir J* 1993;6:840-7.
45. Alvarez MJ, Olaguibel JM, Garcia BE, Rodriguez A, Tabar AI, Urbiola E. Airway inflammation in asthma and perennial allergic rhinitis. Relationship with nonspecific bronchial responsiveness and maximal airway narrowing. *Allergy* 2000;55:355-62.
46. Crimi E, Spanevello A, Neri M, Ind PW, Rossi GA, Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am J Respir Crit Care Med* 1998;157:4-9.
47. Bradley BL, Azzawi M, Jacobson M, Assoufi B, Collins JV, Irani AM, et al. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1991;88:661-74.
48. Wiggs BR, Bosken C, Pare PD, James A, Hogg JC. A model of airway narrowing in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1992;145:1251-8.
49. Van den Berge M, Meijer RJ, Kerstjens HAM, de Reus DM, Koeter GH, Kauffman HF, et al. PC(20) adenosine 5'-monophosphate is more closely associated with airway inflammation in asthma than PC(20) methacholine. *Am J Respir Crit Care Med* 2001;163:1546-50.
50. Van den Berge M, Kerstjens HAM, Meijer RJ, de Reus DM, Koeter GH, Kauffman HF, et al. Corticosteroid-induced improvement in the PC20 of adenosine monophosphate is more closely associated with reduction in airway inflammation than improvement in the PC20 of methacholine. *Am J Respir Crit Care Med* 2001;164:1127-32.
51. Benckhuijsen J, van den Bos JW, van Velzen E, de Bruijn R, Aalbers R. Differences in the effect of allergen avoidance on bronchial hyperresponsiveness as measured by methacholine, adenosine 5'-monophosphate, and exercise in asthmatic children. *Pediatr Pulmonol* 1996;22:147-53.
52. Prosperini G, Rajakulasingam K, Cacciola RR, Spicuzza L, Rorke S, Holgate ST, et al. Changes in sputum counts and airway hyperresponsiveness after budesonide: monitoring anti-inflammatory response on the basis of surrogate markers of airway inflammation. *J Allergy Clin Immunol* 2002;110:855-61.

53. Taylor DA, Jensen MW, Kanabar V, Engelstatter R, Steinijans VW, Barnes PJ, et al. A dose-dependent effect of the novel inhaled corticosteroid ciclesonide on airway responsiveness to adenosine 5'-monophosphate in asthmatic patients. *Am J Respir Crit Care Med* 1999; 160:237-43.
54. Avital A, Springer C, Bar-Yishay E, Godfrey S. Adenosine, methacholine, and exercise challenges in children with asthma or paediatric chronic obstructive pulmonary disease. *Thorax* 1995;50:511-6.
55. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360:1715-21.
56. O'Connor BJ, Ridge SM, Barnes PJ, Fuller RW. Greater effect of inhaled budesonide on adenosine 5'-monophosphate-induced than on sodium-metabisulfite-induced bronchoconstriction in asthma. *Am Rev Respir Dis* 1992;146:560-4.
57. Weersink EJ, Douma RR, Postma DS, Koeter GH. Fluticasone propionate, salmeterol xinafoate, and their combination in the treatment of nocturnal asthma. *Am J Respir Crit Care Med* 1997;155:1241-6.
58. Doull J, Sandall D, Smith S, Schreiber J, Freezer NJ, Holgate ST. Differential inhibitory effect of regular inhaled corticosteroid on airway responsiveness to adenosine 5' monophosphate, methacholine, and bradykinin in symptomatic children with recurrent wheeze. *Pediatr Pulmonol* 1997;23:404-11.
59. Meijer RJ, Kerstjens HAM, Arends LR, Kauffman HF, Koeter GH, Postma DS. Effects of inhaled fluticasone and oral prednisolone on clinical and inflammatory parameters in patients with asthma. *Thorax* 1999;54:894-9.
60. Ketchell RI, Jensen MW, Lumley P, Wright AM, Allenby MI, O'Connor BJ. Rapid effect of inhaled fluticasone propionate on airway responsiveness to adenosine 5'-monophosphate in mild asthma. *J Allergy Clin Immunol* 2002;110:603-6.
61. Fuller RW, Choudry NB, Eriksson G. Action of budesonide on asthmatic bronchial hyperresponsiveness. Effects on directly and indirectly acting bronchoconstrictors. *Chest* 1991;100:670-4.
62. Reynolds CJ, Togias A, Proud D. Airways hyperresponsiveness to bradykinin and methacholine: effects of inhaled fluticasone. *Clin Exp Allergy* 2002;32:1174-9.
63. Vathenen AS, Knox AJ, Wisniewski A, Tattersfield AE. Effect of inhaled budesonide on bronchial reactivity to histamine, exercise, and eucapnic dry air hyperventilation in patients with asthma. *Thorax* 1991; 46:811-6.
64. Hofstra WB, Neijens HJ, Duiverman EJ, Kouwenberg JM, Mulder PG, Kueth MC, et al. Dose-responses over time to inhaled fluticasone propionate treatment of exercise- and methacholine-induced bronchoconstriction in children with asthma. *Pediatr Pulmonol* 2000;29:415-23.
65. Groot CA, Lammers JW, Molema J, Festen J, van Herwaarden CL. Effect of inhaled beclomethasone and nedocromil sodium on bronchial hyperresponsiveness to histamine and distilled water. *Eur Respir J* 1992;5:1075-82.
66. Bootsma GP, Dekhuijzen PN, Festen J, Mulder PG, van Herwaarden CL. Comparison of fluticasone propionate and beclomethasone dipropionate on direct and indirect measurements of bronchial hyperresponsiveness in patients with stable asthma. *Thorax* 1995;50:1044-50.
67. Grashoff WF, Sont JK, Sterk PJ, Hiemstra PS, de Boer WI, Stolk J, et al. Chronic obstructive pulmonary disease: role of bronchiolar mast cells and macrophages. *Am J Pathol* 1997;151:1785-90.
68. Kalenderian R, Raju L, Roth W, Schwartz LB, Gruber B, Janoff A. Elevated histamine and tryptase levels in smokers' bronchoalveolar lavage fluid. Do lung mast cells contribute to smokers' emphysema? *Chest* 1988;94:119-23.
69. Brown HM, Storey G, George WH. Beclomethasone dipropionate: a new steroid aerosol for the treatment of allergic asthma. *Br Med J* 1972;1:585-90.
70. Rutgers SR, Timens W, Tzanakis N, Kauffman HF, Van Der Mark TW, Koeter GH, et al. Airway inflammation and hyperresponsiveness to adenosine 5'-monophosphate in chronic obstructive pulmonary disease. *Clin Exp Allergy* 2000;30:657-62.
71. Chanez P, Vignola AM, O'Shaughnessy T, Enander I, Li D, Jeffery PK, et al. Corticosteroid reversibility in COPD is related to features of asthma. *Am J Respir Crit Care Med* 1997;155:1529-34.
72. Pizzichini E, Pizzichini MM, Gibson P, Parameswaran K, Gleich GJ, Berman L, et al. Sputum eosinophilia predicts benefit from prednisone in smokers with chronic obstructive bronchitis. *Am J Respir Crit Care Med* 1998;158:1511-7.
73. Rutgers SR, Koeter GH, Van Der Mark TW, Postma DS. Short-term treatment with budesonide does not improve hyperresponsiveness to adenosine 5'-monophosphate in COPD. *Am J Respir Crit Care Med* 1998;157:880-6.
74. Tashkin DP, Altose MD, Connett JE, Kanner RE, Lee WW, Wise RA. Methacholine reactivity predicts changes in lung function over time in smokers with early chronic obstructive pulmonary disease. The Lung Health Study Research Group. *Am J Respir Crit Care Med* 1996;153: 1802-11.
75. Postma DS, Wempe JB, Renkema TE, Van Der Mark TW, Koeter GH. Hyperresponsiveness as a determinant of the outcome in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1991;143:1458-62.
76. Anthonisen NR, Connett JE, Murray RP. Smoking and lung function of Lung Health Study participants after 11 years. *Am J Respir Crit Care Med* 2002;166:675-9.
77. Lange P, Groth S, Nyboe GJ, Mortensen J, Appleyard M, Jensen G, et al. Effects of smoking and changes in smoking habits on the decline of FEV1. *Eur Respir J* 1989;2:811-6.
78. Oosterhoff Y, de Jong JW, Jansen MA, Koeter GH, Postma DS. Airway responsiveness to adenosine 5'-monophosphate in chronic obstructive pulmonary disease is determined by smoking. *Am Rev Respir Dis* 1993; 147:553-8.
79. Oosterhoff Y, Jansen MA, Postma DS, Koeter GH. Airway responsiveness to adenosine 5'-monophosphate in smokers and nonsmokers with atopic asthma. *J Allergy Clin Immunol* 1993;92:773-6.
80. Fowler SJ, Dempsey OJ, Sims EJ, Lipworth BJ. Screening for bronchial hyperresponsiveness using methacholine and adenosine monophosphate. Relationship to asthma severity and beta(2)-receptor genotype. *Am J Respir Crit Care Med* 2000;162:1318-22.
81. Cockcroft DW. How best to measure airway responsiveness. *Am J Respir Crit Care Med* 2001;163:1514-5.
82. Gibson PG, Allen CJ, Yang JP, Wong BJ, Dolovich J, Denburg J, et al. Intraepithelial mast cells in allergic and nonallergic asthma. Assessment using bronchial brushings. *Am Rev Respir Dis* 1993;148:80-6.
83. Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993;92:313-24.
84. Phillips GD, Holgate ST. Absence of a late-phase response or increase in histamine responsiveness after bronchial provocation with adenosine 5'-monophosphate in atopic and non-atopic asthma. *Clin Sci (Lond)* 1988;75:429-36.
85. Luijk B, Kampsford RD, Wright AM, Zanen P, Lammers JJ. Duration of effect of single-dose inhaled fluticasone propionate on AMP-induced bronchoconstriction. *Eur Respir J* 2004;23:559-64.
86. Bradding P, Holgate ST. The mast cell as a source of cytokines in asthma. *Ann N Y Acad Sci* 1996;796:272-81.
87. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, et al. Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994; 10:471-80.
88. Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993;151:3853-65.
89. Barnes PJ. Beta-adrenergic receptors and their regulation. *Am J Respir Crit Care Med* 1995;152:838-60.
90. Peachell PT, MacGlashan Jr DW, Lichtenstein LM, Schleimer RP. Regulation of human basophil and lung mast cell function by cyclic adenosine monophosphate. *J Immunol* 1988;140:571-9.
91. O'Connor BJ, Fuller RW, Barnes PJ. Nonbronchodilator effects of inhaled beta 2 agonists. Greater protection against adenosine monophosphate- than methacholine-induced bronchoconstriction in asthma. *Am J Respir Crit Care Med* 1994;150:381-7.
92. Egabagbe E, Pavord ID, Wilding P, Thompson-Coon J, Tattersfield AE. Adenosine monophosphate and histamine induced bronchoconstriction: repeatability and protection by terbutaline. *Thorax* 1997;52: 239-43.

93. Ketchell RI, Jensen MW, Spina D, O'Connor BJ. Dose-related effects of formoterol on airway responsiveness to adenosine 5'-monophosphate and histamine. *Eur Respir J* 2002;19:611-6.
94. Taylor DA, Jensen MW, Aikman SL, Harris JG, Barnes PJ, O'Connor BJ. Comparison of salmeterol and albuterol-induced bronchoprotection against adenosine monophosphate and histamine in mild asthma. *Am J Respir Crit Care Med* 1997;156:1731-7.
95. Soler M, Joos L, Bolliger CT, Elsasser S, Perruchoud AP. Bronchoprotection by salmeterol: cell stabilization or functional antagonism? Comparative effects on histamine- and AMP-induced bronchoconstriction. *Eur Respir J* 1994;7:1973-7.
96. Barnes PJ. Scientific rationale for inhaled combination therapy with long-acting beta2-agonists and corticosteroids. *Eur Respir J* 2002;19:182-91.
97. Pauwels RA, Lofdahl CG, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ, et al. Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. *N Engl J Med* 1997;337:1405-11.
98. Greening AP, Ind PW, Northfield M, Shaw G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. Allen & Hanburys Limited UK Study Group. *Lancet* 1994;344:219-24.
99. Woolcock A, Lundback B, Ringdal N, Jacques LA. Comparison of addition of salmeterol to inhaled steroids with doubling of the dose of inhaled steroids. *Am J Respir Crit Care Med* 1996;153:1481-8.
100. Shrewsbury S, Pyke S, Britton M. Meta-analysis of increased dose of inhaled steroid or addition of salmeterol in symptomatic asthma (MIASMA). *BMJ* 2000;320:1368-73.
101. Aziz I, Tan KS, Hall IP, Devlin MM, Lipworth BJ. Subsensitivity to bronchoprotection against adenosine monophosphate challenge following regular once-daily formoterol. *Eur Respir J* 1998;12:580-4.
102. O'Connor BJ, Aikman SL, Barnes PJ. Tolerance to the nonbronchodilator effects of inhaled beta 2-agonists in asthma. *N Engl J Med* 1992;327:1204-8.
103. Chong LK, Morice AH, Yeo WW, Schleimer RP, Peachell PT. Functional desensitization of beta agonist responses in human lung mast cells. *Am J Respir Cell Mol Biol* 1995;13:540-6.

Correction

With regard to the August 2004 letter entitled "Body burden of mercury is associated with acute atopic eczema and total IgE in children from southern Germany" (2004;114:457-9): In **Table II** (page 458), the adjusted change in geometric mean for urinary mercury concentration was presented incorrectly. A correct version of the Table is presented here:

TABLE II Linear regression analyses for total serum IgE levels (log-transformed) as dependent variable and metal concentrations in urine

	n	Adjusted change* in geometric means (95% CI)
Urinary cadmium concentration	264	1.35 (0.93-1.95)
Urinary arsenic concentration	262	1.18 (0.68-2.04)
Urinary mercury concentration	260	1.54 (1.12-2.11)

*Change in geometric mean value (95% CI) when changing exposure from the 5th to the 95th percentile (0.45 µg/L mercury, 0.44 µg/L cadmium, and 11.8 µg/L arsenic). Adjusted for sex, social status, persons in home, parental history of AE, exposure to environmental tobacco smoke, urinary creatinine, and case/control status.