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Mast cell involvement in the adenosine mediated airway hyper-reactivity in a murine model of ovalbumin-induced lung inflammation

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1 Airway hyper-reactivity to inhaled adenosine, mediated *via* mast cell activation, is a cardinal feature of asthma. Animal models have been developed in several species to mimic this phenomenon, but only in the rat has a mast cell involvement been clearly defined. In this study, a model of ovalbumin-induced adenosine hyper-reactivity was developed in BALB/c mice to determine whether mast cells are involved in this phenomenon.

2 Sensitised mice were challenged one, two or three times, on a daily basis, and airway responses to the stable adenosine analogue NECA (5′-*N*-ethylcarboxamido adenosine) determined 4 and 24 h after each challenge. Airway hyper-reactivity was observed in ovalbumin-challenged mice 4 h after a single challenge and to a minor extent 24 h after a single challenge and 4 h after two challenges.

3 Cromolyn (20 mg ml^{-1}), given by aerosol an hour before the NECA provocation, fully inhibited the airway hyper-reactivity observed 4 h after a single allergen challenge, suggesting a role for mast cells in this response. The airway space cellular inflammation was not affected by cromolyn.

4 As observed in human asthma, an acute treatment with steroid (budesonide 3 mg kg^{-1}, given an hour before the allergen challenge) inhibited the NECA airway hyper-reactivity and significantly inhibited the airway space cellular inflammation.

5 These data suggest that the ovalbumin-challenged BALB/c mice can be considered as a suitable model to study the adenosine-induced airway hyper-reactivity phenomenon observed in human asthma.

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Abbreviations: NECA, 5′-*N*-ethylcarboxamido adenosine; PBS, Phosphate buffered saline; PC_{200}, concentration of NECA that produced a two-fold increase above baseline Penh

Introduction

Airway hyper-reactivity to bronchoconstrictor stimuli is a key feature of asthma. Histamine and methacholine are most commonly used to study this phenomenon in the clinic. These agonists cause bronchoconstriction mainly by acting directly on airway smooth muscle. On the other hand, indirect triggers can induce airflow limitation by activating cell types other than smooth muscle that in turn release bronchoconstrictor mediators. Owing to the involvement of intermediate pathways, it is believed that indirect challenges are more representative of lung inflammation and can be used as a noninvasive tool for monitoring disease progression and for assessing the response to therapies. There are a wide range of stimuli that are classified as indirect stimuli (Joos *et al*., 2003). Among them, adenosine 5′-monophosphate (AMP), and by implication adenosine because AMP is rapidly metabolised to adenosine, is of particular interest. When inhaled by asthmatic subjects, but not healthy volunteers, AMP causes a marked and rapid bronchoconstriction that last for about an hour (Cushley *et al*., 1983). Studies in human have suggested that this bronchoconstriction is occurring through mediators released from resident lung mast cells. As such, AMP-induced bronchoconstriction is accompanied by increased levels of histamine, a mediator mainly released upon mast cell activation, in the airway space and in plasma (Phillips *et al*., 1990; Polosa *et al*., 1995). More importantly, the bronchoconstrictor response to AMP is inhibited by pretreatment with nedocromil sodium and cromolyn, two compounds known to inhibit mast cell degranulation (Phillips *et al*., 1989; Richards *et al*., 1989). To further study this phenomenon, animal models have been developed in rabbit, guinea-pig and rat. In all three species, airway hyperactivity to adenosine, following allergen challenge, was demonstrated, but only in the rat has a mast cell involvement been clearly defined (Fozard & Hannon, 2000; Hannon *et al*., 2001). Murine models of allergen-driven lung inflammation are increasingly being used to study the asthma pathology. Largely because this specie has an array of immunological tools available, including neutralising antibodies and genetically modified animals. Recently, airway hyper-reactivity to adenosine has been reported in a ragweed-driven murine model of lung inflammation (Fan & Mustafa, 2002). Although the authors suggested that the A_{2b} and A_3 adenosine receptors were involved (Fan *et al*., 2003), the

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question whether or not mast cells are involved in this phenomenon was not addressed. In view of the species differences reported above, we thought it was important to determine whether or not the adenosine-induced hyper-reactivity in mice was mast cell dependent. To do so, we have developed a model of ovalbumin-induced lung inflammation that allows the measurement of adenosine hyper-reactivity and studied the effect of cromolyn, a mast cell stabiliser, on this response.

Methods

Animals and experimental protocol

Female BALB/c mice (8 weeks old) were obtained from Charles River (U.K.). All animals were housed in plastic cages in air-conditioned room at 24°C in a 12 h light–dark cycle. Food and water were available *ad libitum*. All animals were acclimated for a period of at least 7 days upon arrival before any experimental work begun. The studies reported here conformed to the U.K. Animals (scientific procedures) Act 1986.

The ovalbumin sensitisation and challenge protocol was as previously described (Trifilieff *et al*., 2003). Mice were intraperitoneally immunised on days 1 and 14 with ovalbumin in alum or alum alone (sham) and challenged from day 21 with an aerosolised solution of 5% ovalbumin in phosphate buffered saline (PBS) or PBS as a control.

Determination of airway responsiveness

Since adenosine is known to be rapidly metabolised *in vivo* by various enzymes (Deussen, 2000), we have used 5′-*N*-ethylcarboxamido adenosine (NECA) a stable analogue of adenosine that has been shown to activate all the adenosine receptors (Ralevic & Burnstock, 1998). Airway responsiveness was measured using barometric plesythmography and whole-body plesythmography (Trifilieff *et al*., 2000; Fujitani & Trifilieff, 2003). Either 4 or 24 h after the last challenge, conscious mice were placed in the plesythmography chamber (Buxco Electronics, Sharon, CT, U.S.A.) and allowed to acclimate for 5 min. Animals were then sequentially exposed to aerosolised PBS and increasing doses of NECA (100–500 $\mu g\,ml^{-1}$) by nebulisation over a 2-min period. Airway response, Penh, was then measured for 10 min. Airway responsiveness was expressed as area under the curve. For comparison between groups, a sigmoidal curve was fitted to the dose–response data and used to calculate the concentration of NECA that produced a two-fold increase above baseline Penh (PC_{200}).

Assessment of airway inflammation

At the indicated time point, terminal anaesthesia was induced with pentobarbitone sodium (60 $mg\,kg^{-1}$, intraperitoneally). The trachea was cannulated and bronchoalveolar lavage was performed by injecting four times 0.3 ml of PBS into the lung *via* the trachea. Total cell counts were measured and cytospin preparation prepared. Cells were stained with Diff-Quik and a differential count of 200 cells performed using standard morphological criteria.

Drugs treatment

Budesonide (3 $mg\,kg^{-1}$ in 50 μl of PBS containing 2% dimethyl sulphoxide) was given *via* the intranasal route, under Halothane/oxygen/nitrous oxide anaesthesia, 1 h before the allergen challenge (Fujitani & Trifilieff, 2003). Cromolyn sodium (20 and 50 $mg\,ml^{-1}$ in PBS for 10 min) was given by aerosol 1 h before the NECA provocation. The doses and route of delivery for both compounds were chosen from previous studies from our laboratory (Corteling & Trifilieff, 2004) and by others (Cieslewicz *et al*., 1999) to be effective in murine models of allergen-driven lung inflammation.

Materials

Cytospins were purchased from Shandon Scientific Ltd (Cheshire, U.K.). PBS was from Invitrogen Ltd (Paisley, U.K.). Dif-quik was from Baxter Dade AG (Dudingen, Switzerland). Alum was from Serva (Heidelberg, Germany). All other reagents were obtained from Sigma-Aldrich (Gillingham, U.K.).

Statistical analysis

Data are expressed as means ± s.e.m. and statistical significance ($P<0.05$) was determined using a Mann–Whitney test.

Results

Ovalbumin-induced airway hyper-reactivity to NECA

Different groups of actively sensitised animals were challenged one, two or three times, on a daily basis, with PBS or ovalbumin and airway response to increasing concentrations of aerosolised NECA or its vehicle (PBS) measured 4 (Figure 1a) and 24 h (Figure 1b) after the last challenge. As a control, groups of animals were sensitised with alum only (sham-sensitised), challenged one, two or three times with PBS and exposed to NECA as described above.

When compared with actively sensitised and PBS-challenged mice, in actively sensitised and ovalbumin-challenged animals, airway hyper-reactivity at all concentrations of NECA was observed 4 h after a single ovalbumin challenge but only at the 100 $\mu g\,ml^{-1}$ concentration at the 24 h time point. At 4 h after the second challenge, a small increase in the airway response to NECA, in the ovalbumin-challenged mice, was apparent for the 100 and 250 $\mu g\,ml^{-1}$ concentrations and the airway hyper-reactivity was lost by 24 h. After three challenges, no airway hyper-reactivity was observed either at 4 or 24 h postchallenge (Figure 1a, b). The airway hyper-reactivity observed on the dose–response curve to NECA, 4 h after a single challenge, was confirmed by the calculated PC_{200} values where a significant decrease in ovalbumin-challenged animals, when compared with PBS-challenged animals, was observed (Table 1). At all the time points, sham-sensitised and PBS-challenged animals also develop a concentration-dependent bronchoconstriction following NECA exposure, but the magnitude of the response was significantly lower than for actively sensitised and PBS-challenged animals (Figure 1a, b, Table 1). All together, these results show that the optimal condition to study airway hyper-reactivity to aerosolised NECA in actively sensitised and

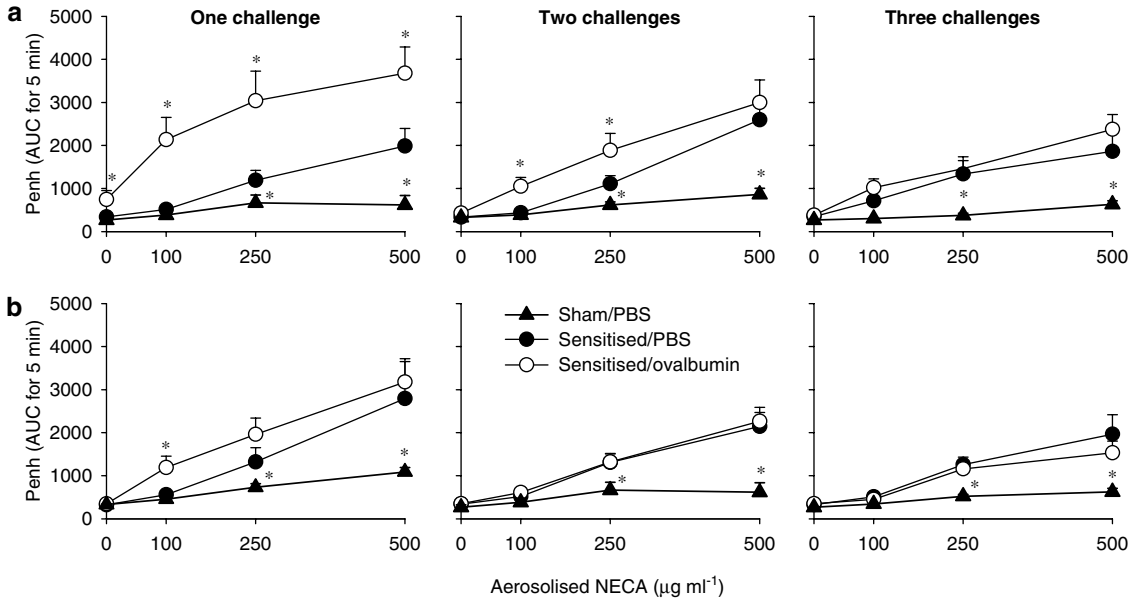

Figure 1 Ovalbumin challenge-dependency on the airway reactivity to NECA. Sham or actively sensitised mice were challenged with an aerosolised solution of PBS or ovalbumin (5%) once, two times or three times, on a daily basis. At 4 (a) and 24 h (b) after each allergen challenge, animals were sequentially exposed to NECA vehicle (PBS for 2 min) and aerosolised NECA (100–500 μg ml^{-1} for 2 min). Airway reactivity was measured for 10 min after each nebulisation. Data are expressed as mean $\pm$ s.e.m. of 10 animals per group. Significance, indicated by *, is *versus* the sensitised and PBS-challenged animals.

Table 1 Challenge-dependency on the airway response to NECA

	PC_{200} (μg ml^{-1})								
	One challenge			*Two challenges*			*Three challenges*		
	Sham/PBS	*Sensitised/PBS*	*Sensitised/ovalbumin*	*Sham/PBS*	*Sensitised/PBS*	*Sensitised/ovalbumin*	*Sham/PBS*	*Sensitised/PBS*	*Sensitised/ovalbumin*
4 h	213 $\pm$ 23*	148 $\pm$ 20	75 $\pm$ 12*	275 $\pm$ 45*	179 $\pm$ 20	122 $\pm$ 21	320 $\pm$ 51*	110 $\pm$ 21	110 $\pm$ 38
24 h	210 $\pm$ 19*	162 $\pm$ 31	92 $\pm$ 27	214 $\pm$ 9*	202 $\pm$ 37	168 $\pm$ 32	269 $\pm$ 28*	177 $\pm$ 32	163 $\pm$ 31

Data are expressed as mean $\pm$ s.e.m. ($n = 10$). Significance, indicated by *, is *versus* sensitized and PBS-challenged animals. PC_{200} was defined as the concentration of NECA that produced a two-fold increase above baseline Penh and calculated by fitting a sigmoidal curve to the dose–response data.

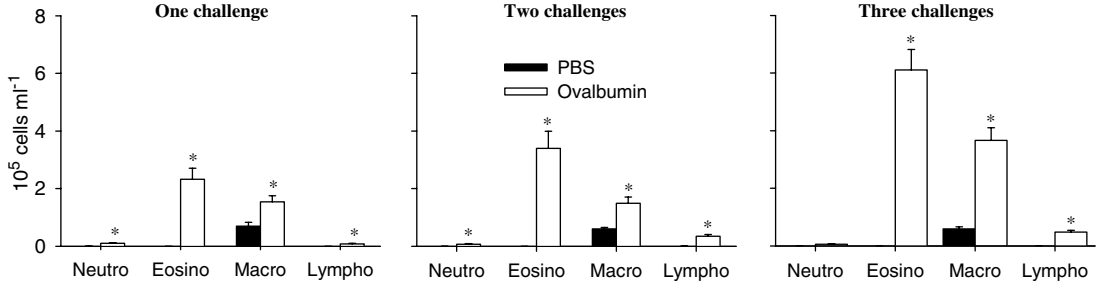

Figure 2 Ovalbumin challenge-dependency on the bronchoalveolar lavage fluid cellular inflammation. Actively sensitised mice were challenged with an aerosolised solution of PBS or ovalbumin (5%) once, two times or three times, on a daily basis. At 4 and 24 h after each allergen challenge, animals were exposed to NECA as described in the legend of Figure 1. At 48 h after the challenge, bronchoalveolar lavage was performed. Data are expressed as mean $\pm$ s.e.m. of 10 animals per group. Significance, indicated by *, is *versus* PBS-challenged animals. Neutro, neutrophil; Eosino, eosinophil; Macro, macrophages; Lympho, lymphocyte.

ovalbumin-challenged mice is 4 h after a single challenge. This protocol was therefore used in the following experiments.

When compared with actively sensitised mice challenged with PBS, the BAL cellular inflammation (eosinophils, macrophages and lymphocytes), measured 48 h after each challenge, increased with the number of ovalbumin challenges. Neutrophil numbers were significantly increased only after the first and second challenges (Figure 2). In PBS-challenged mice, the bronchoalveolar cellularity in actively sensitised and sham sensitised was comparable (data not shown).

Effect of cromolyn

It is accepted that in asthmatics (Polosa *et al*., 2002) and in ovalbumin-sensitised and challenged Brown Norway rats (Hannon *et al*., 2001), the hyper-reactivity to adenosine is a consequence of activation of resident airway mast cells. We therefore tested the effect of cromolyn, a compound know to inhibit mast cell degranulation and mediator release (Shin *et al*., 2004). When given *via* aerosol, an hour before the NECA provocation, cromolyn (20 mg ml^{-1}) fully inhibited the ovalbumin-induced airway hyper-reactivity to aerosolised NECA (Figure 3a, Table 2) but had no effect on the ovalbumin-induced bronchoalveolar fluid cellular inflammation (Figure 3b). In PBS-challenged mice, cromolyn had no effect on the airway response to NECA (Figure 3c, Table 2) or the bronchoalveolar lavage fluid cells (Figure 3d).

Effect of budesonide

Inhaled steroids are the gold standard treatment for human asthma and therefore as part of the validation of our models, we tested the effect of budesonide, a steroid widely used in the treatment of human asthma (Angus, 2002). When given intranasally, an hour before the ovalbumin challenge, budesonide (3 mg kg^{-1}) fully inhibited the airway hyper-reactivity to aerosolised NECA (Figure 4a, Table 2). The bronchoalveolar lavage fluid cellular infiltration induced by ovalbumin challenge was also significantly inhibited (Figure 4b). In PBS-challenged mice, budesonide had no effect on the airway

Table 2 Effect of drug treatment on the airway response to NECA

Group	*Treatment*	
	Budesonide (3 mg kg^{-1}) PC_{200} (μg ml^{-1})	*Cromolyn* (20 mg ml^{-1}) PC_{200} (μg ml^{-1})
Vehicle/PBS	151±15	162±11
Treated/PBS	145±21	137±18
Vehicle/ovalbumin	72±9*	55±14*
Treated/ovalbumin	145±11	172±13

Data are expressed as mean±s.e.m. ($n=8$). Significance, indicated by *, is *versus* PBS-challenged animals. PC_{200} was defined as the concentration of NECA that produced a two-fold increase above baseline Penh and calculated by fitting a sigmoidal curve to the dose–response data.

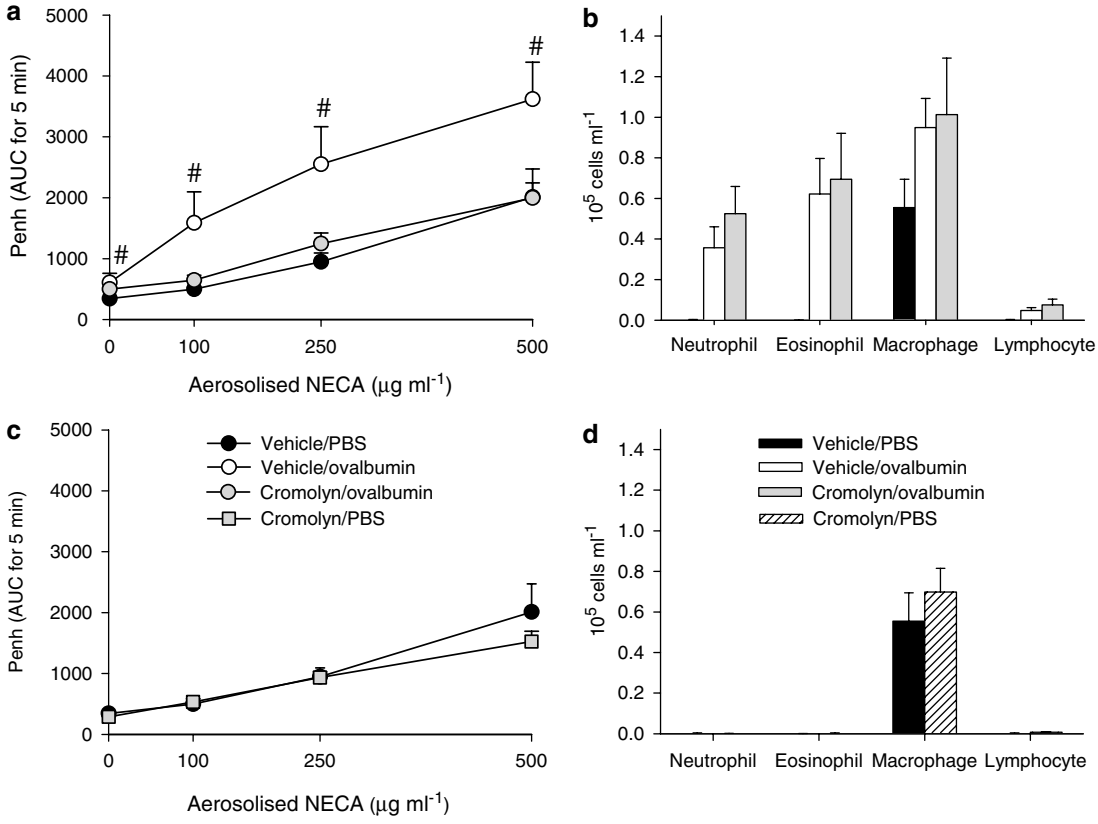

Figure 3 Effect of cromolyn on airway reactivity to NECA (a, c) and bronchoalveolar lavage fluid cellular inflammation (b, d). Sensitized mice were either challenged with PBS or ovalbumin (5%) and 4 h after, animals were placed in the plesythmography box and exposed to aerosolized NECA (0–500 μg ml^{-1}) for 2 min. Penh was measured for the following 10 min. Aerosolized cromolyn (20 mg ml^{-1} for 10 min) or its vehicle was given an hour before the start of the NECA exposure. At 24 h after the challenge, bronchoalveolar lavage was performed and differential inflammatory cell counts determined. Results are expressed as mean±s.e.m. from eight mice per group. Significance, indicated by #, is *versus* vehicle-treated/PBS-challenged animals. (a) Effect of cromolyn on ovalbumin-induced airway hyper-reactivity to NECA. (b) Lack of effect of cromolyn on ovalbumin-induced airway inflammation. (c) Lack of effect of cromolyn on airway response to NECA in PBS-challenged mice. (d) Lack of effect of cromolyn on resident airway inflammatory cells in PBS-challenged mice.

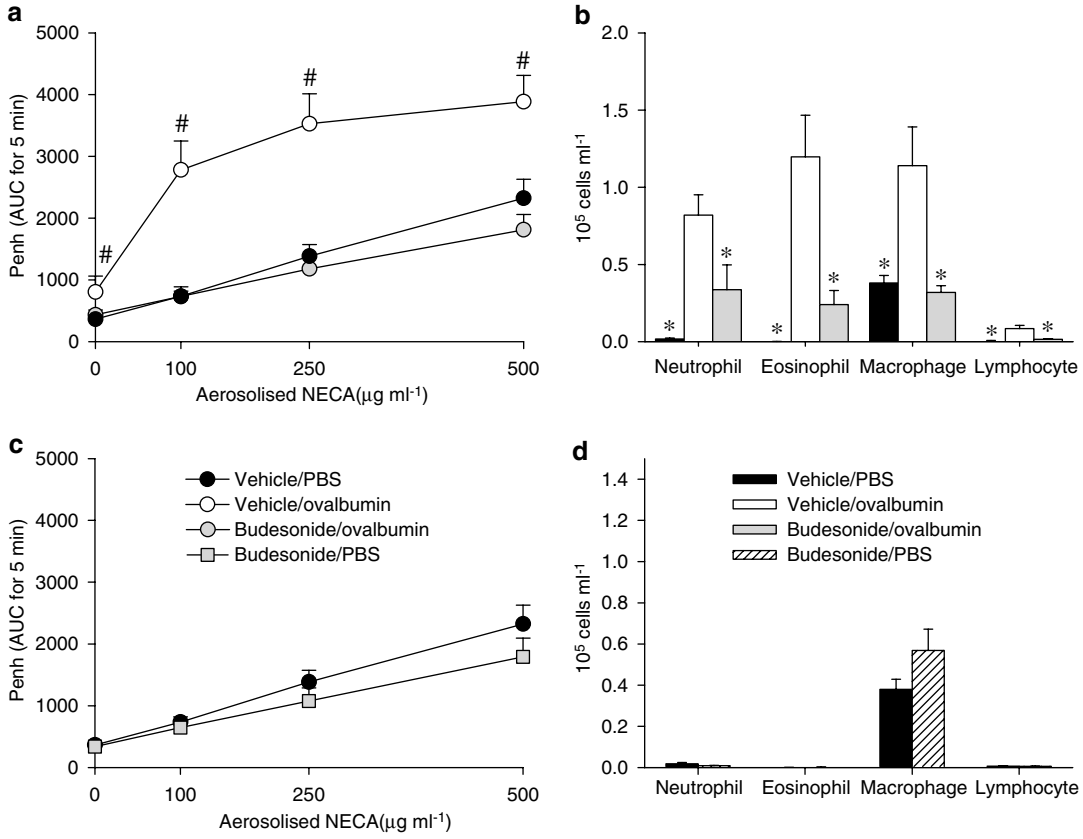

Figure 4 Effect of budesonide on airway reactivity to NECA (a, c) and bronchoalveolar lavage fluid cellular inflammation (b, d). Sensitized mice were intranasally treated with 3 mg kg^{-1} of budesonide or its vehicle 1 h before being either challenged with PBS or ovalbumin (5%). At 4 h after the challenge, animals were placed in the plesythmography box and exposed to aerosolized NECA (0–500 μg ml^{-1}) for 2 min. Penh was measured for the following 10 min. At 24 h after the challenge, bronchoalveolar lavage was performed and differential inflammatory cell counts determined. Results are expressed as mean + s.e.m. from eight mice per group. Significance, indicated by # or *, is *versus* vehicle-treated/PBS-challenged or vehicle-treated/ovalbumin-challenged animals, respectively. (a) Effect of budesonide on ovalbumin-induced airway hyper-reactivity to NECA. (b) Effect of budesonide on ovalbumin-induced airway inflammation. (c) Lack of effect of budesonide on airway response to NECA in PBS-challenged mice. (d) Lack of effect of budesonide on resident airway inflammatory cells in PBS-challenged mice.

response to aerosolised NECA (Figure 4c, Table 2) or the bronchoalveolar lavage fluid cells (Figure 4d).

When compared to the data in Figure 3, an enhanced inflammation was observed in the allergen-challenged animals in the experiment described in Figure 4. Although, in both experiments, the mice have been sensitised and exposed to the allergen in the same way, the vehicle treatment was different. In Figure 3, conscious animals were treated with aerosolised PBS for 10 min 3 h after the allergen challenge. In Figure 4, halothane-anaesthetised animals were intranasally treated with 50 μl of PBS containing 2% DMSO an hour before the allergen challenge. We believed that the difference in the route and timing of the vehicle treatment could account for the differences observed.

Discussion

Airway hyper-reactivity to direct or indirect stimuli is a cardinal feature of asthma. Among the indirect stimuli, adenosine is of interest since it has the potential to be used as a noninvasive tool to monitor disease progression and to assess the response to anti-inflammatory therapies (Spicuzza *et al*., 2003). To study this phenomenon in further detail, animal models would certainly be useful. Mouse models of allergic lung inflammation are increasingly being used to study the asthma pathology. Mainly because this specie has an array of immunological tools available, including neutralising antibodies and genetically modified animals. Therefore, the primary goal of this work was to establish a murine model of allergen-driven lung inflammation that, like in human asthma, was associated with airway hyper-reactivity to adenosine, mediated *via* mast cell activation. The description of a mast cell-dependent model of adenosine-induced airway hyper-reactivity in ovalbumin-sensitised Brown Norway rats (Hannon *et al*., 2001) led us to study whether the same was true in mice.

As observed in human, naïve mice responded weakly to adenosine receptor stimulation. The low sensitivity of the airway to adenosine *in vivo* is a feature of a number of laboratory species including rats (Pauwels & Van der Straeten, 1987; Hannon *et al*., 2001), rabbits (Ali *et al*., 1994b), guinea-pigs (Thorne & Broadley, 1994; el Hashim *et al*., 1996) and mice (Fan *et al*., 2003; Tilley *et al*., 2003). The lack of

adenosine sensitivity in naïve mice was also reported using isolated murine lung (Held *et al*., 1999). Sensitisation *per se*, despite the absence of lung inflammation, did increase the airway sensitivity to aerosolised NECA. This is in contrast with the finding in the Brown Norway rat (Hannon *et al*., 2001), but a similar phenomenon has been reported in other species such as rabbit (el Hashim *et al*., 1996) and guinea-pig (Thorne & Broadley, 1994). The mechanism responsible for this is not clear, but a possible role for altered calcium signalling in the smooth muscle cells has been suggested (Schmidt & Rabe, 2000).

Mimicking the human asthmatic response, actively sensitised mice challenged with ovalbumin developed an intense bronchoconstriction to aerosolised NECA that, for several reasons, we believe to be mediated *via* mast cell activation. First, the allergen-driven enhanced response to NECA was fully prevented by cromolyn, a compound known to inhibit mast cell degranulation and mediator release (Shin *et al*., 2004). Second, this enhanced response was only present 4 h after a single challenge and disappeared with time and the number of challenges. The disappearance of the NECA response with time after a single challenge imitates the human situation where although present at an early time point after a single allergen challenge (3 h), the hyper-reactivity to adenosine is no longer present at a later time point (24 h) (Aalbers *et al*., 1991). Moreover, the same time-dependency phenomenon is described in Brown Norway rat, where a clear mast cell involvement was demonstrated (Hannon *et al*., 2001). Third, the lack of response following repeated challenges also points towards a mast cell involvement. Indeed, mast cells are known to be desensitised following repeated allergen stimulation (Ishizaka *et al*., 1985; Shalit & Levi-Schaffer, 1995).

It is interesting to note that, 4 h after a single challenge, even in the absence of the NECA provocation, a small but significant bronchoconstriction was observed in the sensitised and ovalbumin-challenged mice when compared with the sensitised and PBS-challenged animals. Our data suggest that this allergen-induced bronchoconstriction is driven *via* mast cell activation since it was fully inhibited by cromolyn pretreatment.

In addition to its inhibition of mast cell activation, cromolyn is also known to modulate sensory nerve activation (Collier & Fuller, 1983; Verleden *et al*., 1991). Therefore, one can argue that, in our experimental system, cromolyn inhibits the adenosine airway hyper-reactivity by a mast cell-independent mechanism. Although we cannot firmly refute this possibility, experimental data generated in the rat have shown that it is the A_1 receptor that mediates activation of the bronchopulmonary sensory afferent nerves (Hong *et al*., 1998), but no evidence for the A_1 receptor involvement in the airway response to adenosine could be demonstrated in mice (Fan *et al*., 2003; Tilley *et al*., 2003). All together, these data render unlikely a role for the sensory nerves in our model.

While our results strongly support a role for mast cells in the airway responsiveness to NECA in sensitised and allergen-challenged mice, they also indicate that other cell types contribute to this response in sensitised but not challenged animals. As such, the lack of effect of cromolyn on NECA-induced bronchoconstriction in sensitised mice challenged with PBS and the lack of time and/or number of challenges dependency on this response, suggest that mast cell are not involved. The cell type(s) responsible for the observed bronchoconstriction in sensitised but nonallergen-challenged mice remain to be determined but studies in other species suggest the smooth muscle cells and nerves as possible candidates. For example, bronchoconstriction in sensitised rabbits is a consequence of activation of the A_1 receptor, which is induced in smooth muscle upon sensitisation (Ali *et al*., 1994a, b; el Hashim *et al*., 1996; Nyce & Metzger, 1997). Adenosine has also been suggested to induce bronchoconstriction partly *via* activation of the postsynaptic vagal nerve in both BDE rats (Pauwels & Van der Straeten, 1987) and asthmatics (Polosa *et al*., 1991; Crimi *et al*., 1992). Although, as discussed above, pulmonary nerves are unlikely to be involved in the response to adenosine in mice.

Local administration of budesonide before the challenge suppressed the airway hyper-reactivity to NECA in allergen-challenged mice but did not affect the airway response in PBS-challenged animals. In addition, the allergen-induced bronchoconstriction observed 4 h after a single challenge was also fully inhibited by budesonide pretreatment. The mechanism behind the budesonide inhibition of these mast cells mediated events is not clear but could be due to the general anti-inflammatory activity of this compound as measured by the inhibition of allergen-induced cellular inflammation, to the suppression of the allergen-induced increased sensitivity of mast cells to NECA, or to the direct inhibition of the interaction of NECA with the mast cells. Since budesonide did not affect the response to NECA in sensitised and PBS-challenged mice, the latter hypothesis can be reasonably excluded. Similarly, an effect of budesonide *via* its general anti-inflammatory properties seems unlikely since our data indicate that NECA hyper-reactivity is independent of the allergen-induced airway inflammation. As such, hyper-reactivity to NECA was maximal at 4 h after a single allergen challenge, the time at which we have previously shown that the lung inflammation is minimal (Trifilieff *et al*., 2000). Moreover, the hyper-reactivity to NECA was no more present following repeated challenges that induced a pronounced airway lung inflammation. The more likely explanation is therefore that budesonide is inhibiting the allergen-driven increased sensitivity of mast cells to NECA. In line with this hypothesis, we have shown that budesonide is also inhibiting the allergen-induced bronchoconstriction observed 4 h after a single challenge that is sensitive to cromolyn and therefore likely to be mast cell driven. Furthermore, this hypothesis is supported by several previously published works demonstrating blockade of allergen-mediated activation of masts cells by steroids (Wershil *et al*., 1995; Andrade *et al*., 2004). The inhibition of adenosine airway hyper-reactivity by an acute steroid treatment has also been reported in human asthma (Ketchell *et al*., 2002) and further testifies to the similarity of our model to the response observed in asthmatics.

In the present study, we did not attempt to define which of the adenosine receptors is involved in the bronchoconstrictor response. Apart from the A_{2a} receptor, which is mainly involved in the anti-inflammatory properties of adenosine (Hasko & Cronstein, 2004), all the other adenosine receptors have been implicated in the bronchoconstrictor response to adenosine. As such, it is accepted that the A_1 receptor mediates the bronchoconstrictor activity of adenosine in the rabbit (Ali *et al*., 1994a, b; el Hashim *et al*., 1996; Nyce & Metzger, 1997). In the rat, the A_1, A_{2b} and A_3 receptors (Pauwels & Joos, 1995) or an atypical adenosine receptor (Hannon *et al*., 2002) have

been proposed. In the guinea-pig, it is the A_3 receptor that drives the bronchoconstrictor response to adenosine (Thorne *et al*., 1996). In mice, all four adenosine receptors are present in the lung (Chunn *et al*., 2001; Fan & Mustafa, 2002) and can potentially be involved. However, in a recently published study, it was suggested that, in a ragweed driven model of lung inflammation, the bronchoconstriction to NECA was mediated *via* activation of the A_{2b} and A_3 receptors (Fan *et al*., 2003). Finally, in human asthmatics, indirect evidence supports a role for the A_{2b} receptor (Fozard & Hannon, 2000). The confusion around the adenosine receptor subtype(s) responsible for the bronchoconstrictor response to adenosine is likely due to the fact that there is marked species differences for adenosine agonists as well as antagonists (Fredholm *et al*., 2001) resulting in conflicting statements about the role of the different adenosine receptor across species.

In conclusion, we have developed a model of NECA-induced airway hyper-reactivity in BALB/c mice sensitised and challenged with ovalbumin. This model has similarity with the human asthmatic situation (i.e. upregulated by the disease state, present only earlier on after the allergen challenge, mediated *via* mast cell activation and sensitive to acute steroid treatment). Therefore, the ovalbumin-sensitised and challenged BALB/c mice can be considered as a suitable model to mimic the adenosine-induced airway hyper-reactivity phenomenon in human asthma.

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