



Effect of adenosine A2A receptor activation in murine models of respiratory disorders

Olivier Bonneau, Daniel Wyss, Stephane Ferretti, Clare Blaydon, Christopher S. Stevenson and Alexandre Trifilieff

AJP - Lung 290:1036-1043, 2006. First published Dec 9, 2005; doi:10.1152/ajplung.00422.2005

You might find this additional information useful...

This article cites 43 articles, 22 of which you can access free at:

<http://ajplung.physiology.org/cgi/content/full/290/5/L1036#BIBL>

Updated information and services including high-resolution figures, can be found at:

<http://ajplung.physiology.org/cgi/content/full/290/5/L1036>

Additional material and information about *AJP - Lung Cellular and Molecular Physiology* can be found at:

<http://www.the-aps.org/publications/ajplung>

This information is current as of April 8, 2006 .



Effect of adenosine A_{2A} receptor activation in murine models of respiratory disorders

Olivier Bonneau,¹ Daniel Wyss,¹ Stephane Ferretti,¹ Clare Blaydon,¹
Christopher S. Stevenson,¹ and Alexandre Trifilieff²

Novartis Institutes for BioMedical Research, Respiratory Diseases Area, ¹Horsham, England and ²Basel, Switzerland

Submitted 4 October 2005; accepted in final form 2 December 2005

Bonneau, Olivier, Daniel Wyss, Stephane Ferretti, Clare Blaydon, Christopher S. Stevenson, and Alexandre Trifilieff. Effect of adenosine A_{2A} receptor activation in murine models of respiratory disorders. *Am J Physiol Lung Cell Mol Physiol* 290: L1036–L1043, 2006. First published December 9, 2005; doi:10.1152/ajplung.00422.2005.—Activation of the adenosine A_{2A} receptor has been postulated as a possible treatment for lung inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD). In this report, we have studied the anti-inflammatory properties of the reference A_{2A} agonist CGS-21680, given intranasally at doses of 10 and 100 µg/kg, in a variety of murine models of asthma and COPD. After an acute ovalbumin challenge of sensitized mice, prophylactic administration of CGS-21680 inhibited the bronchoalveolar lavage fluid inflammatory cell influx but not the airway hyperreactivity to aerosolized methacholine. After repeated ovalbumin challenges, CGS-21680 given therapeutically inhibited the bronchoalveolar lavage fluid inflammatory cell influx but had no effect on the allergen-induced bronchoconstriction, the airway hyperreactivity, or the bronchoalveolar lavage fluid mucin levels. As a comparator, budesonide given intranasally at doses of 0.1–1 mg/kg fully inhibited all the parameters measured in the latter model. In a lipopolysaccharide-driven model, CGS-21680 had no effect on the bronchoalveolar lavage fluid inflammatory cell influx or TNF-α, keratinocyte chemoattractant, and macrophage inflammatory protein-2 levels, but potently inhibited neutrophil activation, as measured by bronchoalveolar lavage fluid elastase levels. With the use of a cigarette smoke model of lung inflammation, CGS-21680 did not significantly inhibit bronchoalveolar lavage fluid neutrophil infiltration but reversed the cigarette smoke-induced decrease in macrophage number. Together, these results suggest that activation of the A_{2A} receptor would have a beneficial effect by inhibiting inflammatory cell influx and downregulating inflammatory cell activation in asthma and COPD, respectively.

chronic obstructive pulmonary disorder therapies; anti-asthmatic agents; animal models

ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) are chronic inflammatory disorders of the airways. Asthma is characterized by infiltration of the lung with inflammatory cells such as eosinophils and lymphocytes, mucus hypersecretion, and by the presence of bronchial hyperresponsiveness to a variety of stimuli (26). COPD is characterized by slowly progressive and irreversible airway obstruction, mucus hypersecretion, and infiltration of activated neutrophils and macrophages into the lungs (24). Adenosine is a purine nucleoside that is released during tissue hypoxia, and inflammation and has both pro- and anti-inflammatory roles mediated by four different G protein-coupled receptors, A₁ (G_i coupled), A_{2A} (G_s coupled), A_{2B} (G_s and G_{q11} coupled), and A₃ (G_s and G_{q11}

coupled). Each receptor can be implicated either beneficially and/or detrimentally in the development of inflammation. However, a lot of evidence points toward a predominant role for the A_{2A} receptor subtype in the anti-inflammatory activities of adenosine. As such, specific activation of the A_{2A} receptor with agents such as CGS-21680 or ATL-146e has demonstrated anti-inflammatory activities in animal models of septic arthritis (6), reperfusion injury (3, 11, 31), and bacterial meningitis (37). In addition, the use of A_{2A} receptor-deficient mice has clearly demonstrated a nonredundant role for this receptor in downregulating ongoing inflammation (30). Despite the fact that A_{2A} receptor activation has been postulated as a way to downregulate inflammation in airway diseases such as asthma and COPD (16, 28, 36), only one animal study has addressed this question and demonstrated that activation of the A_{2A} receptor with CGS-21680 reduces inflammatory cell infiltration in a Brown Norway rat model of asthma (15). However, in this work the authors did not study the effect of the compound on airway hyperreactivity and mucin production, two cardinal features of asthma. In addition, no data are available from animal models of COPD that would support the concept of a beneficial effect of an A_{2A} receptor agonist in this disease.

In this study, we have used CGS-21680 (10) to delineate the potential anti-inflammatory role of the adenosine A_{2A} receptor in two different allergen-driven murine models of asthma characterized by inflammatory cell infiltration, airway hyperreactivity and mucin secretion. The effect of CGS-21680 was also studied in murine COPD-related models, i.e., lipopolysaccharide-driven models of airway neutrophilic infiltration and activation and a model of cigarette smoke-induced lung inflammation.

MATERIALS AND METHODS

Materials. All materials were obtained from Sigma-Aldrich (Gillingham, UK) unless specified otherwise.

Animals. Eight-week-old female BALB/c mice (Charles River, Margate, UK) were acclimatized for a period of at least 7 days upon arrival before any experimental work began. The studies conformed to the United Kingdom Animals (scientific procedures) Act of 1986 and were approved by the institutional review board.

Ovalbumin models of allergic lung inflammation. CGS-21680 was given prophylactically in an acute challenge model (41) and given therapeutically in a repeated challenge model (5). Briefly, mice were immunized intraperitoneally with 10 (acute model) or 20 (repeated model) µg of ovalbumin in Alum (0.2 ml) on days 0 and 14. For the acute model, animals were exposed for 20 min to an aerosol of 5%

Address for reprint requests and other correspondence: A. Trifilieff, WSJ-386.5.10, Novartis Pharma, Postfach CH-4002 Basel, Switzerland (e-mail: alexandre.trifilieff@novartis.com).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.



ovalbumin on day 21. For the repeated model, animals were exposed for 20 min to an aerosol of 1% ovalbumin on days 21, 22, and 23 and to an aerosol of 5% ovalbumin on day 26. Control animals were immunized with ovalbumin and challenged with the ovalbumin vehicle, PBS.

Lipopolysaccharide models of neutrophilic lung infiltration and activation. To study airway neutrophil infiltration, mice were challenged intranasally, under halothane/oxygen/nitrous oxide anesthesia, with 0.3 mg/kg of lipopolysaccharide or its vehicle (PBS, 50 μ l). To study neutrophil activation, airway neutrophilia was established as described above. Twenty-four hours later, *N*-formyl-Met-Leu-Phe (fMLP, 5 mg/kg) or its vehicle (50 μ l of 1% DMSO in PBS) was applied intranasally, and the mice were killed 2 h thereafter (8).

Cigarette smoke model of lung inflammation. Mice were exposed to tobacco smoke generated from five cigarettes (2R4F research cigarette; Univ. of Kentucky, Louisville, KY) or air on three consecutive days as described previously for rats (35) and killed 24 h after the last exposure.

Lung function measurement. Lung function changes [enhanced pause (Penh)] were measured using barometric plesythmography and whole body plesythmography (Buxco Electronics, Sharon, CT). The allergen-induced bronchoconstriction in the chronic model was measured for 5 min, 4 h after the end of the last challenge, and baseline respiratory parameters were measured for 5 min, just before the last allergen challenge. Airway reactivity to aerosolized methacholine was measured 24 h after the last allergen challenge (41).

Determination of bronchoalveolar lavage parameters. At the indicated time point, terminal anesthesia was induced, and bronchoalveolar lavage and differential cell counts were performed as described previously (41). Bronchoalveolar lavage fluid supernatants were assayed for neutrophil elastase activity and mucin level as previously described by our group. Neutrophil elastase activity was measured using the fluorogenic substrate (Me-Suc-Ala-Ala-Pro-Val-MCA; Bachem, Weil am Rhein, Germany). Samples and standard human neutrophil elastase were incubated with the substrate at 37°C, and the cleavage of the substrate was measured using a fluorometer set at 370 nm excitation and 460 nm emission (8). Mucin levels were determined by a mucin enzyme-linked lectin assay. Samples and purified human mucin standard (Novartis, Horsham, UK) were added to Ulex europaeus agglutinin-1 (UEA-1)-precoated wells. After a 1-h incubation at 37°C, horseradish peroxidase-conjugated UEA-1 was added for 1.5 h, followed by addition of the substrate (orthophenyldiamine dihydrochloride). The reaction was stopped by addition of 4 M H₂SO₄ (2).

Drug treatment. Mice were treated via the intranasal route, under halothane/oxygen/nitrous oxide anesthesia, with CGS-21680 or budesonide in PBS containing 2% DMSO. Control mice received 50 μ l of vehicle (41). The dose range selected was based on previous *in vivo* studies (9, 15). The treatment schedule is detailed in RESULTS.

Data analysis. Whenever possible, the dose that induced 50% inhibition of the signal (ED₅₀) was calculated by fitting a sigmoidal curve to the dose-response data (i.e., 2 doses and the positive and negative control groups). Data are expressed as means $\pm$ SE, and statistical significance ($P < 0.05$) was determined using the Mann-Whitney test with Bonferroni correction for multiple comparison.

RESULTS

Effect of CGS-21680, given prophylactically, in the acute ovalbumin model of allergic lung inflammation. We first studied the prophylactic effect of CGS-21680 given half an hour before and 3 h after a single ovalbumin challenge. When compared with sensitized and PBS-challenged mice, mice challenged with ovalbumin had an increased bronchoconstrictor response to aerosolized methacholine measured 24 h post-

challenge. CGS-21680, given prophylactically at doses of 10 and 100 μ g/kg, had no effect on the ovalbumin-induced airway hyperreactivity to aerosolized methacholine (Fig. 1A). We have previously shown that budesonide is fully effective in inhibiting the airway hyperreactivity to aerosolized methacholine in this model (9).

Immediately after completion of the airway reactivity measurement, mice were killed and bronchoalveolar lavage was performed. When compared with the PBS-challenged animals, there were significant increases in the numbers of neutrophils, eosinophils, macrophages, and lymphocytes recovered in the bronchoalveolar lavage fluid. CGS-21680 dose dependently inhibited all inflammatory cell influx except the macrophage (Fig. 1B). Its effect was ~ 10 times more potent than budesonide (Table 1). No significant increase in the bronchoalveolar lavage fluid mucin levels could be detected (0 and 0.5 ± 0.3 U/ml for the PBS- and ovalbumin-challenged animals, respectively; $P = 0.18$), and therefore the effect of CGS-21680 or budesonide on this parameter could not be studied.

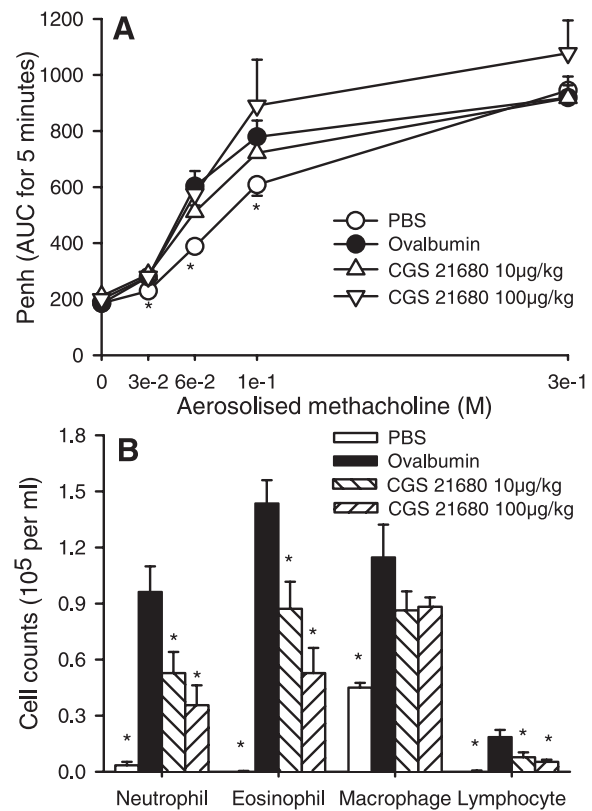


Fig. 1. Effect of CGS-21680, given prophylactically, on airway hyperreactivity to methacholine (A) and bronchoalveolar lavage (BAL) fluid cellular inflammation (B) after a single ovalbumin challenge. Sensitized mice were either challenged with an aerosol of PBS or 5% ovalbumin. Animals were intranasally treated with 10 or 100 μ g/kg of CGS-21680 or its vehicle half an hour before and 3 h after the challenge. Twenty-four hours after the last challenge, animals were placed in the plesythmography box and sequentially exposed to increasing concentrations of aerosolized methacholine (0–0.3 M) for 2 min. Enhanced pause (Penh) was measured for 5 min after each nebulization. Immediately after the measurement of the airway response, BAL was performed, and differential inflammatory cell counts were determined. AUC; area under the curve. Significance (* $P < 0.05$) is vs. vehicle-treated/ovalbumin-challenged animals (ovalbumin). Results are expressed as means $\pm$ SE from 8 mice/group.



Table 1. *ED₅₀ values for CGS-21680 and budesonide against ovalbumin-induced lung inflammation*

	ED ₅₀ (μg/kg)	
	CGS-21680	Budesonide
Acute model		
Neutrophil	16 ± 3	286 ± 35
Eosinophil	21 ± 5	231 ± 15
Macrophage	Not active	Not active
Lymphocyte	19 ± 2	188 ± 16
Repeated model		
Allergen-induced bronchoconstriction	Not active	115 ± 19
Neutrophil	82 ± 2	232 ± 81
Eosinophil	100 ± 22	271 ± 33
Macrophage	31 ± 6	117 ± 15
Lymphocyte	17 ± 5	289 ± 89
Mucus	Not active	174 ± 26

Results are expressed as means ± SE from 8 mice/group. Budesonide's ED₅₀ values in the acute model were calculated from our previously published work (9).

Effect of CGS-21680 and budesonide, given therapeutically, in the repeated ovalbumin challenge model of allergic lung inflammation. We next studied the effect of therapeutic dosing with CGS-21680 against ovalbumin-induced lung inflammation. To do so, animals were challenged with ovalbumin for three consecutive days to establish a baseline inflammation. Two days after the third challenge, animals were either exposed to PBS (OA/PBS) or ovalbumin (OA/OA). Control animals were exposed four times to PBS (PBS/PBS). CGS-21680 (10 or 100 μg/kg) was given twice daily starting 30 min before the last ovalbumin challenge. Four hours after the last challenge, no bronchoconstriction was observed in the PBS/PBS or the OA/PBS groups. In contrast, animals challenged four times with ovalbumin developed a bronchoconstriction, and CGS-21680 had no effect on this phenomenon (Fig. 2A, left). Although markedly attenuated, the bronchoconstriction observed in the OA/OA group was still present 24 h after the challenge as shown by the significant increase in baseline Penh when compared with the PBS/PBS and OA/PBS groups (Fig. 2B, left). No difference in the airway reactivity to aerosolized methacholine, measured 24 h after the last challenge, was observed between the PBS/PBS and OA/PBS groups. When compared with the OA/OA group, animals treated with 100 μg/kg of CGS-21680 had a twofold increase in the baseline Penh value and an increased sensitivity to the two lower doses of aerosolized methacholine (Fig. 2B, left).

Forty-eight hours after the last challenge, macrophages were the predominant cell type found in the bronchoalveolar lavage fluids of the PBS/PBS group. As expected, when compared with the PBS/PBS group, the airway inflammatory cell numbers (i.e., neutrophil, eosinophil, macrophages, and lymphocytes) were increased in the OA/PBS group and further increased in the OA/OA group. Although CGS-21680 dose dependently inhibited all inflammatory cell influx (Fig. 2C, left), it was less potent when compared with the acute challenge model. However, it was still more potent than budesonide (Table 1).

Forty-eight hours after the last challenge, mucin could be detected in the bronchoalveolar lavage fluid of the OA/PBS group, and the last ovalbumin challenge induced a further

fivefold increase in mucin levels (OA/OA group). A small inhibition of the mucin production was observed in animals treated with both doses of CGS-21680. However, this was not significant and no dose relationship was observed. No mucin were detected in the bronchoalveolar lavage fluids from the PBS/PBS animals (Fig. 2D, left).

When given therapeutically once a day starting 1 h before the last challenge, budesonide dose dependently inhibited the allergen-induced bronchoconstriction (Fig. 2A, right), the airway hyperreactivity to aerosolized methacholine (Fig. 2B, right), the bronchoalveolar lavage fluid cell influx (Fig. 2C, right), and the increased mucin levels (Fig. 2D, right). For all these parameters, budesonide had an ED₅₀ of 100–300 μg/kg (Table 1).

Effect of CGS-21680 in the lipopolysaccharide model of neutrophilic lung inflammation. Three hours after the challenge, lipopolysaccharide increased the number of bronchoalveolar lavage fluid neutrophils and to a lesser extent the number of lymphocytes. At that time point, the macrophage numbers were decreased by lipopolysaccharide, probably reflecting their activation status (Fig. 3A, left). Twenty-four hours postchallenge, lipopolysaccharide increased the number of bronchoalveolar lavage fluid neutrophils, macrophages, and lymphocytes (Fig. 3B, left). At the 3-h time point, in animals treated with CGS-21680, there was a trend for reversing the lipopolysaccharide-induced decrease in macrophage numbers, but this was not significant (Fig. 3A, left). Similarly, at both the 3- and 24-h time points, a trend toward inhibiting the lipopolysaccharide-induced increases in lung inflammatory cells was observed for the 100 μg/kg dose of CGS-21680 (given half an hour before and 6 h after the challenge), but this did not reach statistical significance (Fig. 3, A and B, left).

At the 3-h time point, a trend for inhibiting lipopolysaccharide-induced TNF-α, keratinocyte chemoattractant (KC; murine equivalent of human IL-8), or macrophage inflammatory protein-2 (MIP-2; the murine equivalent of human GRO-α) release was observed for CGS-21680, although this did not reach statistical significance (Fig. 3A, right). In contrast, CGS-21680 potentially inhibited the lipopolysaccharide-induced elastase release, measured at the 24-h time point, with full inhibition observed at the 100 μg/kg dose (Fig. 3B, right).

At both time points, no mucin could be detected in the bronchoalveolar lavage fluids of the lipopolysaccharide- or PBS-challenged animals.

Effect of CGS-21680 in the lipopolysaccharide/fMLP model of lung neutrophil activation. To study further the inhibitory effect of CGS-21680 on neutrophil activation, animals were sequentially challenged with lipopolysaccharide and fMLP. Animals challenged with lipopolysaccharide only had an increased level of bronchoalveolar lavage fluid elastase when compared with the PBS-challenged group. The bronchoalveolar lavage fluid elastase level was further enhanced in the animals challenged with lipopolysaccharide and fMLP. CGS-21680, given intranasally half an hour before the fMLP challenge, fully inhibited the fMLP-induced increase in elastase levels with a calculated ED₅₀ of 6 ± 2 μg/kg (Fig. 4).

Effect of CGS-21680 in the cigarette smoke model of lung inflammation. One day after the last of three daily exposures, cigarette smoke increased the number of bronchoalveolar lavage fluid neutrophils and decreased the macrophage numbers.

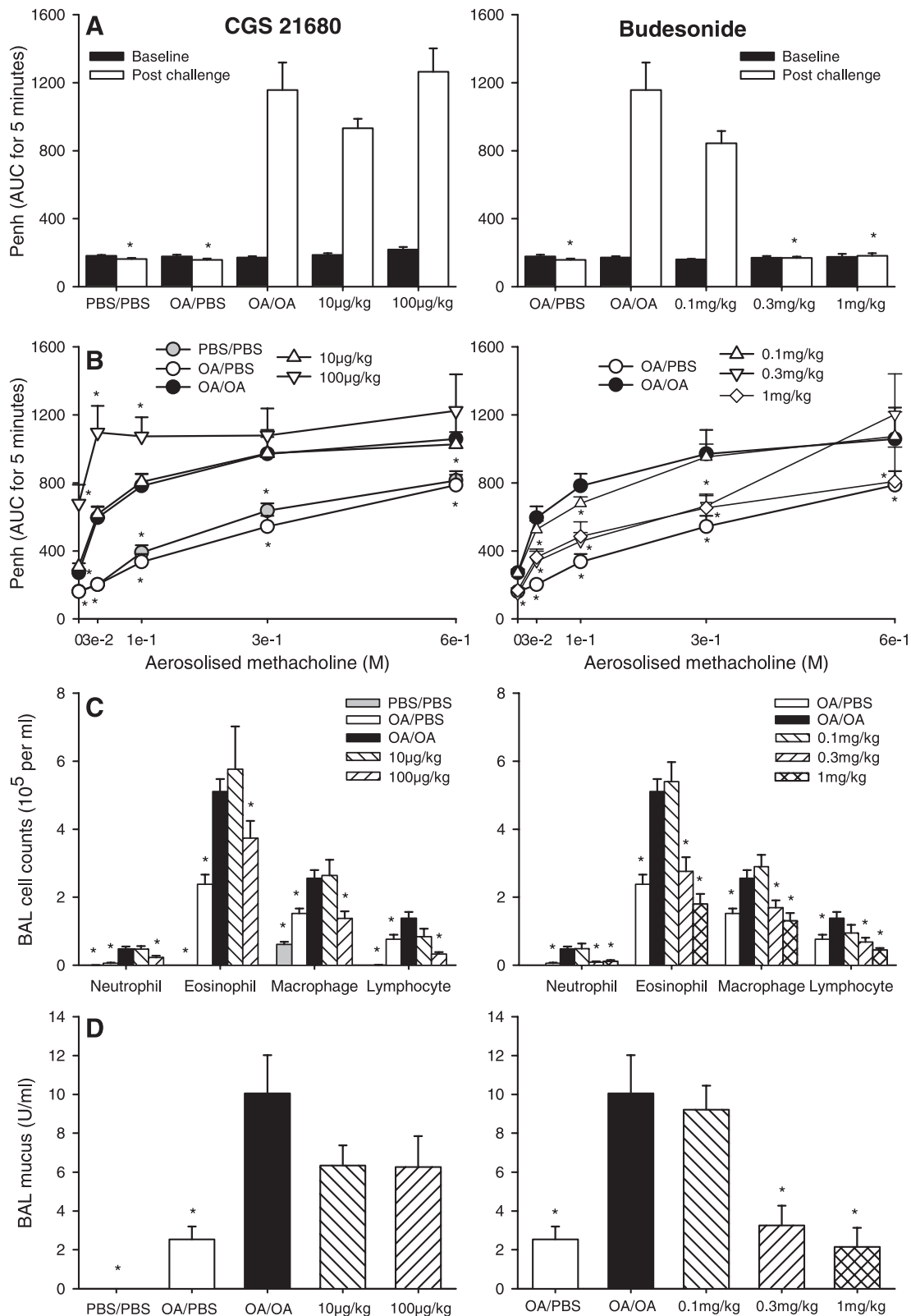


Fig. 2. Effect of CGS-21680 (left) and budesonide (right) given therapeutically on allergen-induced bronchoconstriction (A), airway hyperreactivity to methacholine (B), BAL fluid cellular infiltration (C), and BAL fluid mucin levels after repeated ovalbumin challenge (D). Sensitized mice were challenged with a 1% ovalbumin solution on days 21–23. On day 26, mice were either challenged with a 5% ovalbumin solution (OA/OA) or its vehicle PBS (OA/PBS). A control group of sensitized mice was challenged with PBS on days 21–23 and 36 (PBS/PBS). Animals were intranasally treated with CGS-21680 (twice daily), budesonide (once daily), or their vehicle (twice daily), starting half an hour or 1 h before the last challenge for CGS-21680 and budesonide, respectively. Allergen-induced bronchoconstriction and airway reactivity to aerosolized methacholine were measured 4 and 24 h after the last allergen challenge, respectively. Two days after the last challenge, BAL was performed and differential inflammatory cell counts and mucin levels were determined. Significance (**P* < 0.05) is vs. vehicle-treated/ovalbumin-challenged animals (OA/OA). Results are expressed as means ± SE from 8 mice/group.

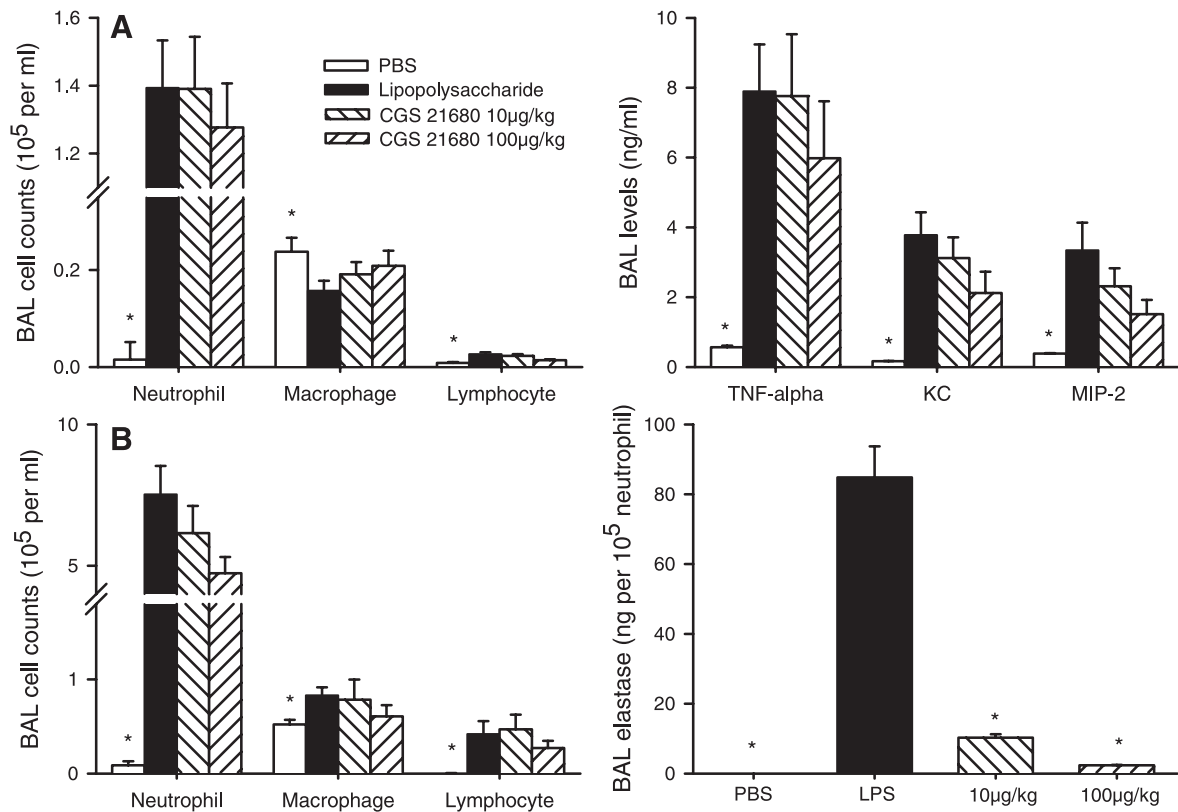


Fig. 3. Effect of CGS-21680 on lipopolysaccharide-induced airway inflammation 3 (A) or 24 h (B) after the challenge. Mice were either challenged with an intranasal application of PBS (50 µl) or lipopolysaccharide (0.3 mg/kg). Animals were intranasally treated with 10 or 100 µg/kg of CGS-21680 or its vehicle half an hour before and 6 h after the challenge. BAL was performed, and inflammatory parameters were monitored. Significance (**P* < 0.05) is vs. vehicle-treated/lipopolysaccharide-challenged animals (lipopolysaccharide). Results are expressed as means ± SE from 2 different experiments each including 8 mice/group.

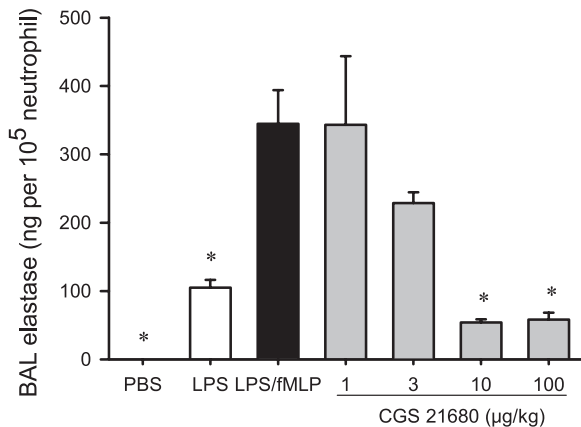


Fig. 4. Effect of CGS-21680 on BAL fluid neutrophil elastase activity after lipopolysaccharide/N-formyl-Met-Leu-Phe (LPS/fMLP) challenge. Mice were either challenged with an intranasal application of PBS (50 µl) or LPS (0.3 mg/kg). Twenty-four hours after, fMLP (5 mg/kg) or its vehicle (50 µl of 1% DMSO in PBS) was applied intranasally, and animals were killed 2 h thereafter. Animals were intranasally treated with 10 or 100 µg/kg of CGS-21680 or its vehicle half an hour before the fMLP challenge. BAL was performed, and differential inflammatory cell counts and neutrophil elastase activity in the BAL fluid supernatant was determined. Significance (**P* < 0.05) is vs. LPS- and fMLP-challenged animals treated with compound vehicle (LPS/fMLP). Results are expressed as means ± SE from 8 mice/group.

CGS-21680 (10 and 100 µg/kg) given intranasally half an hour before and 6 h after the two last smoke exposures inhibited the increase in neutrophil numbers, although this was not significant. The decrease in macrophage numbers was dose dependently reversed by CGS-21680 with a significant reversal observed for the dose of 100 µg/kg (Fig. 5). No neutrophil elastase activity could be detected in the bronchoalveolar lavage fluids of the cigarette smoke or the sham-exposed animals.

DISCUSSION

In this report, we have shown that activation of the A_{2A} receptor inhibited the inflammatory cell influx in allergen-driven models of lung inflammation but did not affect other asthma-like parameters such as airway hyperreactivity, allergen-induced bronchoconstriction, or mucus secretion. On the other hand, in COPD murine models, A_{2A} receptor activation does not inhibit airway neutrophil influx but reverses the decrease in macrophage numbers and potentially inhibits neutrophil activation.

The adenosine A_{2A} receptor belongs to the seven-transmembrane family of receptors and is associated with the stimulatory G protein, G_s. Thus the A_{2A} receptor is positively coupled to adenylate cyclase, and its stimulation increases cAMP, activating intracellular pathways known to have anti-inflammatory properties (29). In addition, the use of A_{2A} receptor-deficient mice has provided direct and conclusive evidence for a non-

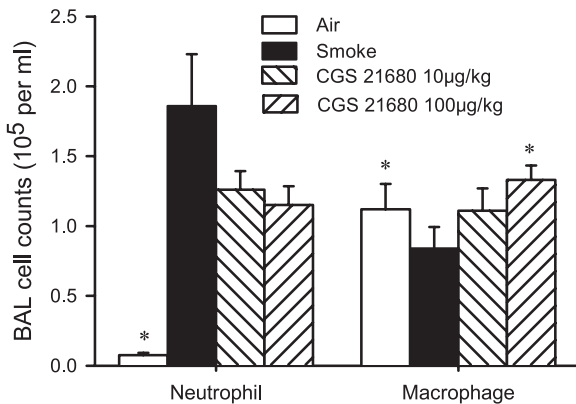


Fig. 5. Effect of CGS-21680 on BAL fluid cellular inflammation after cigarette smoke exposure. Mice were exposed to either air or cigarette smoke (smoke) for 3 consecutive days. Twenty-four hours after the last exposure, animals were killed and BAL was performed. From the 2nd day of exposure, animals were intranasally treated with 10 or 100 µg/kg of CGS-21680 or its vehicle ½ h before and 4 h after the smoke exposure. Significance (* $P < 0.05$) is vs. smoke-exposed animals treated with compound vehicle. Results are expressed as means $\pm$ SE from 8 mice/group.

redundant role of this receptor in the downregulation of inflammation in vivo (30). As such, in models of acute liver inflammation and sepsis, A_{2A} receptor-deficient mice had dramatically increased tissue damage and a prolonged inflammatory reaction (30).

Our data in the acute ovalbumin challenge model in the mouse confirmed previously published work that demonstrates that activation of the A_{2A} receptor leads to a potent inhibition of inflammatory cells infiltrating the rat airways after allergen challenge (15). The inhibitory effect on inflammatory cell influx was confirmed in a more stringent model of murine asthma (i.e., compound given therapeutically), although a loss of potency for the compound was observed. The mechanism of action for this anti-inflammatory effect still remains to be elucidated, but it has been demonstrated that activation of T cells, which play a crucial role in the recruitment of inflammatory cells into the airways in both human asthma (7) and murine models of allergic inflammation (12), is suppressed by activation of the A_{2A} receptor (25). In addition, activation of mature dendritic cells that play an important role in the initiation and regulation of immune response (23) is also inhibited by activation of the A_{2A} receptor (34). Therefore, activation of the A_{2A} receptor in the pathogenesis of asthma has the potential to not only interfere with an established airway inflammation by inhibiting T cell activation but also to inhibit early inflammatory events, such as antigen presentation by down-regulating dendritic cell activation.

Although CGS-21680 is widely used as an A_{2A} selective agonist, one cannot exclude a possible activation of the A₃ receptor when this compound is given at a high dose. In cell-based assays using transfected human adenosine receptors, we have determined a functional potency for CGS-21680 at the A_{2A} receptor of 60 nM, a selectivity ratio of ~15 against the A₃ receptor, and a selectivity ratio of >100 for the A_{2B} and A₁ receptors. However, when extrapolating the human selectivity data to laboratory animals, one has to take into account differences in the pharmacology of the A₃ adenosine receptors from different species (17). In addition, it is always difficult to

extrapolate the receptor selectivity profile of a compound based only on its pharmacological profile in vitro; an in vivo evaluation is always more relevant. In this respect, the anti-inflammatory effect of CGS-21680 in a rat allergen-driven model of lung inflammation has been shown to be fully inhibited by pretreatment with ZM-241385, a specific A_{2A} antagonist (15). We did attempt to inhibit the anti-inflammatory effect of CGS-21680 with ZM-241385. Unfortunately, in our hands, when used at a dose of 3 mg/kg in the murine ovalbumin model, adverse events were observed and a meaningful conclusion could not be drawn from this experiment. However, assuming that CGS-21680 is reaching high enough local concentrations within the lung to activate the A₃ receptor, it is very unlikely that it could account for the anti-inflammatory effect observed. Indeed, activation of the A₃ receptor, in a murine model of lung inflammation, has been shown to have a proinflammatory effect (42, 43).

In the repeated allergen challenge model, animals chronically treated with a high dose of CGS-21680 (100 µg/kg) developed a bronchoconstriction. The A_{2B} and A₃ adenosine receptors have been suggested to be responsible for the adenosine-induced bronchoconstriction in a murine model of allergen-induced lung inflammation (14). Therefore, a putative activity of CGS-21680 at the murine A₃ receptor, as discussed above, could explain this effect. However, if this was the case, one would have expected to see the same phenomenon in the acute challenge model. Interestingly, a recent study has reported that, when compared with wild-type mice, mice deficient for the A_{2A} receptor had a higher baseline lung function and a significantly higher dose response to aerosolized methacholine (13). This suggests that the observed bronchoconstriction in our study could be due to desensitization of the A_{2A} receptor after chronic treatment with a high dose of CGS-21680.

In the two asthma models presented in this study, CGS-21680 was more than or at least as potent as budesonide in inhibiting airway inflammation. On the other hand, CGS-21680 was ineffective against other asthma-like parameters that were fully inhibited by budesonide (i.e., airway hyperreactivity, allergen-induced bronchoconstriction, and mucus production). Altogether, the results obtained in our murine asthma models would predict that an A_{2A} agonist therapy in asthma would not be as efficacious as steroids but would still inhibit airway cellular inflammation.

In contrast to the effect seen in the allergen-driven models, CGS-21680 was ineffective in decreasing inflammatory cell influx (e.g., neutrophil) in the lipopolysaccharide and the cigarette smoke models. This result was rather unexpected since in an in vitro system, A_{2A} activation has been shown to inhibit neutrophil very late antigen-4 expression and thereby diminished neutrophil binding to VCAM-1, an essential primary step for neutrophil migration into tissues (38). However, after adherence to the endothelium, neutrophils need a chemotactic signal to migrate into the airways. Our data, using the lipopolysaccharide model, show that CGS-21680 treatment does not significantly inhibit the production of such chemotactic signals (TNF-α, KC, and MIP-2). These cytokines/chemokines have been shown to play a crucial role in the neutrophil recruitment following either lipopolysaccharide (19, 21, 33) or cigarette smoke challenge (4, 35, 39) in mice and rats. It is

therefore not surprising that CGS-21680 was unable to inhibit the neutrophil influx in our murine models of COPD.

Regardless of the lack of effect on inflammatory cell influx, CGS-21680 potentially inhibited neutrophil activation, as measured by inhibition of the neutrophil elastase in two different models. This observation is in line with the published literature demonstrating a potent effect of adenosine A_{2A} agonists on neutrophil activation in vitro (1, 40). In addition, although not significant, a clear trend for inhibition of the lipopolysaccharide-induced KC and MIP-2 release was observed. This could reflect the inhibitory action of CGS-21680 on neutrophil activation. Indeed, upon activation with lipopolysaccharide, the neutrophil has been shown to be able to release IL-8 (22, 32). Although indirect, our data also indicate an inhibitory effect of CGS-21680 on macrophage activation. In the cigarette smoke exposure model, the decrease in bronchoalveolar lavage fluid macrophage numbers was reversed by the compound. This decrease in macrophages has been reported previously following both cigarette smoke (35) and lipopolysaccharide (20) exposure in rats and has been attributed to macrophage activation resulting in an increased adherence to the lung matrix protein (27, 35).

Together, our results suggest that therapies aimed at activating the A_{2A} receptor could be beneficial in airway diseases such as asthma and COPD. Despite the fact that the anti-inflammatory activity of CGS-21680 was restricted to the inhibition of inflammatory cell infiltration in our asthma models, one can still envisage using such a therapy in patients poorly controlled by inhaled steroids. Indeed, the fundamental differences in the mechanism of action for an A_{2A} agonist and an inhaled steroid might lead to a beneficial effect in this patient category. The potent inhibition of neutrophil and macrophage activation seen in our COPD models also suggests that A_{2A} receptor agonists would have a beneficial effect on patients with COPD. However, important issues would need to be overcome before such a therapy would be available. The A_{2A} receptor has a wide tissular distribution and is responsible for a variety of systemic and central effects (18). Therefore, it is likely that a selective action within the lung will be necessary to avoid unacceptable side effects.

REFERENCES

1. Al Ayadhi LY and Al Tuwajri AS. The synergistic effect of adenosine A_{2A} receptors agonist, type IV phosphodiesterase inhibitor and ATP-sensitive K channels activation on free radicals production and aggregation of human polymorphonuclear leukocytes. *Pharmacol Res* 50: 157–163, 2004.
2. Beckmann N, Tigani B, Sugar R, Jackson AD, Jones G, Mazzoni L, and Fozard JR. Noninvasive detection of endotoxin-induced mucus hypersecretion in rat lung by MRI. *Am J Physiol Lung Cell Mol Physiol* 283: L22–L30, 2002.
3. Cassada DC, Tribble CG, Long SM, Laubach VE, Kaza AK, Linden J, Nguyen BN, Rieger JM, Fiser SM, Kron IL, and Kern JA. Adenosine A_{2A} analogue ATL-146e reduces systemic tumor necrosis factor- α and spinal cord capillary platelet-endothelial cell adhesion molecule-1 expression after spinal cord ischemia. *J Vasc Surg* 35: 994–998, 2002.
4. Churg A, Wang RD, Tai H, Wang X, Xie C, and Wright JL. Tumor necrosis factor- α drives 70% of cigarette smoke-induced emphysema in the mouse. *Am J Respir Crit Care Med* 170: 492–498, 2004.
5. Cieslewicz G, Tomkinson A, Adler A, Duez C, Schwarze J, Takeda K, Larson KA, Lee JJ, Irvin CG, and Gelfand EW. The late, but not early, asthmatic response is dependent on IL-5 and correlates with eosinophil infiltration. *J Clin Invest* 104: 301–308, 1999.
6. Cohen SB, Gill SS, Baer GS, Leo BM, Scheld WM, and Diduch DR. Reducing joint destruction due to septic arthritis using an adenosine A_{2A} receptor agonist. *J Orthop Res* 22: 427–435, 2004.
7. Corrigan CJ. The role of the T cell in the immunopathogenesis of asthma. *Chem Immunol* 78: 39–49, 2000.
8. Corteling R, Wyss D, and Trifilieff A. In vivo models of lung neutrophil activation. Comparison of mice and hamsters. *BMC Pharmacol* 2: 1, 2002.
9. Corteling R and Trifilieff A. Gender comparison in a murine model of allergen-driven airway inflammation and the response to budesonide treatment. *BMC Pharmacol* 4: 4, 2004.
10. Cristalli G, Lambertucci C, Taffi S, Vittori S, and Volpini R. Medicinal chemistry of adenosine A_{2A} receptor agonists. *Curr Top Med Chem* 3: 387–401, 2003.
11. Day YJ, Marshall MA, Huang L, McDuffie MJ, Okusa MD, and Linden J. Protection from ischemic liver injury by activation of A_{2A} adenosine receptors during reperfusion: inhibition of chemokine induction. *Am J Physiol Gastrointest Liver Physiol* 286: G285–G293, 2004.
12. Deurloo DT and Van Oosterhout AJ. Role of T cell co-stimulation in murine models of allergic asthma. *Clin Exp Allergy* 34: 17–25, 2004.
13. Fan M, Ledent C, and Mustafa SJ. Augmentation of airway inflammation in A_{2A} adenosine receptor deficient allergic mice. *Proc Am Thoracic Soc* 2: A97, 2005.
14. Fan M, Qin W, and Mustafa SJ. Characterization of adenosine receptor(s) involved in adenosine-induced bronchoconstriction in an allergic mouse model. *Am J Physiol Lung Cell Mol Physiol* 284: L1012–L1019, 2003.
15. Fozard JR, Ellis KM, Villela Dantas MF, Tigani B, and Mazzoni L. Effects of CGS-21680, a selective adenosine A_{2A} receptor agonist, on allergic airways inflammation in the rat. *Eur J Pharmacol* 438: 183–188, 2002.
16. Fozard JR and Hannon JP. Adenosine receptor ligands: potential as therapeutic agents in asthma and COPD. *Pulm Pharmacol* 12: 111–114, 1999.
17. Fozard JR and McCarthy C. Adenosine receptor ligands as potential therapeutics in asthma. *Curr Opin Investig Drugs* 3: 69–77, 2002.
18. Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, and Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 53: 527–552, 2001.
19. Frevert CW, Huang S, Danaee H, Paulauskis JD, and Kobzik L. Functional characterization of the rat chemokine KC and its importance in neutrophil recruitment in a rat model of pulmonary inflammation. *J Immunol* 154: 335–344, 1995.
20. Frevert CW, Warner AE, Weller E, and Brain JD. The effect of endotoxin on in vivo rat alveolar macrophage phagocytosis. *Exp Lung Res* 24: 745–758, 1998.
21. Goncalves de Moraes V, Vargaftig B, Lefort J, Meager A, and Chignard M. Effect of cyclo-oxygenase inhibitors and modulators of cyclic AMP formation on lipopolysaccharide-induced neutrophil infiltration in mouse lung. *Br J Pharmacol* 117: 1792–1796, 1996.
22. Hayashi F, Means TK, and Luster AD. Toll-like receptors stimulate human neutrophil function. *Blood* 102: 2660–2669, 2003.
23. Holt PG and Upham JW. The role of dendritic cells in asthma. *Curr Opin Allergy Clin Immunol* 4: 39–44, 2004.
24. Jeffery PK. Comparison of the structural and inflammatory features of COPD and asthma. Giles F. Filley Lecture. *Chest* 117: 251–260, 2000.
25. Koshiba M, Kojima H, Huang S, Apasov S, and Sitkovsky MV. Memory of extracellular adenosine A_{2A} purinergic receptor-mediated signaling in murine T cells. *J Biol Chem* 272: 25881–25889, 1997.
26. Maddox L and Schwartz DA. The pathophysiology of asthma. *Annu Rev Med* 53: 477–498, 2002.
27. Marwick JA, Kirkham PA, Stevenson CS, Danahay H, Giddings J, Butler K, Donaldson K, MacNee W, and Rahman I. Cigarette smoke alters chromatin remodeling and induces proinflammatory genes in rat lungs. *Am J Respir Cell Mol Biol* 31: 633–642, 2004.
28. Marx D, Ezeamuzie CI, Nieber K, and Szelenyi I. Therapy of bronchial asthma with adenosine receptor agonists or antagonists. *Drug News Perspect* 14: 89–100, 2001.
29. Moore AR and Willoughby DA. The role of cAMP regulation in controlling inflammation. *Clin Exp Immunol* 101: 387–389, 1995.
30. Ohta A and Sitkovsky M. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* 414: 916–920, 2001.

31. Okusa MD, Linden J, MacDonald T, and Huang L. Selective A_{2A} adenosine receptor activation reduces ischemia-reperfusion injury in rat kidney. *Am J Physiol Renal Physiol* 277: F404–F412, 1999.
32. Sabroe I, Prince LR, Jones EC, Horsburgh MJ, Foster SJ, Vogel SN, Dower SK, and Whyte MK. Selective roles for Toll-like receptor (TLR)2 and TLR4 in the regulation of neutrophil activation and life span. *J Immunol* 170: 5268–5275, 2003.
33. Schmal H, Shanley TP, Jones ML, Friedl HP, and Ward PA. Role for macrophage inflammatory protein-2 in lipopolysaccharide-induced lung injury in rats. *J Immunol* 156: 1963–1972, 1996.
34. Schnurr M, Toy T, Shin A, Hartmann G, Rothenfusser S, Soellner J, Davis ID, Cebon J, and Maraskovsky E. Role of adenosine receptors in regulating chemotaxis and cytokine production of plasmacytoid dendritic cells. *Blood* 103: 1391–1397, 2004.
35. Stevenson CS, Coote K, Webster R, Johnston H, Atherton HC, Nicholls A, Giddings J, Sugar R, Jackson A, Press NJ, Brown Z, Butler K, and Danahay H. Characterization of cigarette smoke-induced inflammatory and mucus hypersecretory changes in rat lung and the role of CXCR2 ligands in mediating this effect. *Am J Physiol Lung Cell Mol Physiol* 288: L514–L522, 2005.
36. Sullivan GW. Adenosine A_{2A} receptor agonists as anti-inflammatory agents. *Curr Opin Investig Drugs* 4: 1313–1319, 2003.
37. Sullivan GW, Linden J, Buster BL, and Scheld WM. Neutrophil A_{2A} adenosine receptor inhibits inflammation in a rat model of meningitis: synergy with the type IV phosphodiesterase inhibitor, rolipram. *J Infect Dis* 180: 1550–1560, 1999.
38. Sullivan GW, Lee DD, Ross WG, DiVietro JA, Lappas C, Lawrence MB, and Linden J. Activation of A_{2A} adenosine receptors inhibits expression of $\alpha 4/\beta$ integrin (very late antigen-4) on stimulated human neutrophils. *J Leukoc Biol* 75: 127–134, 2004.
39. Thatcher TH, McHugh NA, Egan RW, Chapman RW, Hey JA, Turner CK, Redonnet MR, Seweryniak KE, Sime PJ, and Phipps RP. The role of CXCR2 in cigarette smoke-induced lung inflammation. *Am J Physiol Lung Cell Mol Physiol* 289: L322–L328, 2005.
40. Thibault N, Burelout C, Harbour D, Borgeat P, Naccache PH, and Bourgoin SG. Occupancy of adenosine A_{2A} receptors promotes fMLP-induced cyclic AMP accumulation in human neutrophils: impact on phospholipase D activity and recruitment of small GTPases to membranes. *J Leukoc Biol* 71: 367–377, 2002.
41. Trifilieff A, El-Hashim A, and Bertrand C. Time course of inflammatory and remodeling events in a murine model of asthma: effect of steroid treatment. *Am J Physiol Lung Cell Mol Physiol* 279: L1120–L1128, 2000.
42. Young HW, Molina JG, Dimina D, Zhong H, Jacobson M, Chan LN, Chan TS, Lee JJ, and Blackburn MR. A₃ adenosine receptor signaling contributes to airway inflammation and mucus production in adenosine deaminase-deficient mice. *J Immunol* 173: 1380–1389, 2004.
43. Zhong H, Shlykov SG, Molina JG, Sanborn BM, Jacobson MA, Tilley SL, and Blackburn MR. Activation of murine lung mast cells by the adenosine A₃ receptor. *J Immunol* 171: 338–345, 2003.

