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Pharmacologic modulation of airway inflammation

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Asthma is currently defined by the presence of characteristic symptoms, by reversible airway narrowing, and by airway hyperresponsiveness to inhaled bronchoconstrictor stimuli. Airway hyperresponsiveness is present in virtually all asthmatics with current symptoms (1); however, airway hyperresponsiveness is not specific for asthma, as it can be present in patients with other airway diseases (2). These physiologic abnormalities of asthma, which were the main research focus for two decades in the 1960s and 1970s, are now known to be caused by persistent airway inflammation in asthmatic airways (3, 4) and the structural abnormalities caused by this inflammation (5, 6).

A common manifestation of inflammation at any tissue site is the presence, at some time in the process, of activated inflammatory cells at the site. The type of inflammatory cell varies with the type of inflammation. The most important inflammatory cell at most sites of acute inflammation is the neutrophil. However, in other, more chronic inflammatory conditions, eosinophils, lymphocytes, or mast cells appear to be more prominent. For the purposes of this paper, airway inflammation will be defined as the presence of activated inflammatory cells in the airways. While this definition is restrictive and excludes other important components of inflammatory events, such as edema and vasodilation, quantifying numbers, as well as, occasionally, the state of activation, of inflammatory cells has been the most commonly used index of airway inflammation in studies of asthma to date. It must also be recognized, however, that the structural consequences of persistent airway inflammation in asthma may be as, or more, important than the presence of the activated inflammatory cells.

Allergen-induced airway inflammation

The identification of stimuli which can cause asthma has proven to be important in studies of airway inflammation in asthma. This is because the evidence that the presence of inflammatory cells is causally related to the development of airway hyperresponsiveness and asthma in human subjects initially depended on studies which have examined numbers of cells and cellular differentials in bronchoalveolar lavage (BAL) fluid before and after inhalation of ozone (7), the occupational sensitizing agent toluene diisocyanate (TDI) (8), and environmental allergens (9), all of which are known to cause airway hyperresponsiveness and transient asthma, or exacerbate persistent asthma. These studies have all demonstrated an acute inflammatory response in the airways associated with the development of variable airflow obstruction, airway hyperresponsiveness, and asthma.

Allergen inhalation is the most important stimulus known to cause asthma. Methods for studying inhaled allergen in the laboratory have been well standardized and extensively used for the investigation of the pathophysiology and treatment of aller-

gen-induced airway responses. By these methods, it has been shown that inhalation of allergen by a sensitized subject in the laboratory results in an early asthmatic response, during which bronchoconstriction develops within 10 min, reaches a maximum within 30 min, and generally resolves within 1–3 h (Fig. 1) (10). In 50–60% of adults and 70–80% of children who develop an early asthmatic response, a late asthmatic response occurs, during which bronchoconstriction persists or recurs 3–4 h later, reaches a maximum within 6–10 h (Fig. 1), and can last for more than 24 h if untreated (10). A third consequence of inhaling allergen is the development of airway hyperresponsiveness, the magnitude and duration of which appears to be related to the development of late asthmatic responses (11). Subjects who develop isolated early responses after inhaled allergen do not generally develop prolonged airway hyperresponsiveness (11). In contrast, subjects who develop a late asthmatic response after inhaled allergen develop airway hyperresponsiveness which can last for days or even weeks.

The factors which predict the development of a late asthmatic response (12–14) are mainly indices of high circulating IgE antibody to the inhaled allergen, such as a high IgE RAST with the allergen (13, 14), and a low antigen concentration in the skin eliciting a late cutaneous response. Boulet et al. (12) have also demonstrated that if a late cutaneous response followed a small early cutaneous response (wheal <5 mm), then a late asthmatic response was more likely to occur after an early response. Thus, an important determinant of whether a patient will develop a late asthmatic response is the level of circulating IgE antibody, which may be considered the degree of sensitization to the allergen. The other major determinant of the late asthmatic response is the size of the early response (15). Thus, the greater the degree of airway narrowing during the early response, the more likely a late response will develop. In turn, the size of the early response is dependent not only on the quantity of mediators released, but also on the degree of histamine or methacholine airway responsiveness of the subject (11).

The late response after inhaled allergen is associated with airway inflammation, with numbers of activated airway eosinophils and metachromatic cells, as assessed in BAL fluid obtained during fiberoptic bronchoscopy, being increased at 7–8 h (9) and eosinophils further increased 24 h after allergen exposure (16). More recently, a less invasive method than bronchoscopy has been developed, using sputum induced by the inhalation of hypertonic saline, to quantify and characterize inflammatory cells in asthmatic airways. Studies using this method have demonstrated that eosinophils increase markedly in sputum samples of

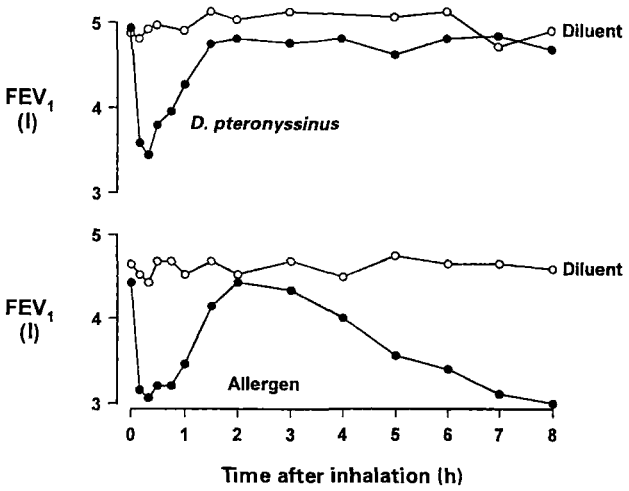


Fig. 1. Isolated early asthmatic response after inhalation of house-dust-mite extract (*D. pteronyssinus*) (upper panel). Early followed by late asthmatic response after inhalation of house-dust-mite extract (lower panel). Control measurements (open circles) were made after inhalation of diluent alone. Measurements after allergen inhalation (closed circles) were made at same time points as control measurements (reproduced with permission from O'Byrne et al. [10].

asthmatics undergoing naturally occurring exacerbation of their asthma (17), as well as 24 h after allergen inhalation, an effect which persists for 3 days after allergen exposure (Fig. 2) (18). These increases in airway eosinophils and metachromatic cells are associated with increases in circulating eosinophil progenitors measured as colony-forming units (Eo/B-CFU), 24 h after inhaled allergen (19), suggesting that inhaled allergen stimulates bone-marrow production of these progenitors. This has recently been confirmed in a study in which bone-marrow progenitors were directly measured in bone marrow aspirated 24 h after allergen inhalation in mildly asthmatic subjects (20). This study demonstrated an increased responsiveness of the bone marrow to interleukin-5 (IL-5) after allergen exposure, an effect which was associated with an increased expression of the IL-5 receptor on the progenitors (21). Taken together, these studies suggest that after allergen inhalation, signals are sent from the airways to the bone marrow (possibly IL-5), an action which increases production of Eo/B progenitors, by acting on progenitors more responsive to IL-5, thereby making more cells available to be recruited into the airways.

The mediators which cause allergen-induced bronchoconstrictor responses have also been clarified. The cysteinyl leukotrienes (22), thromboxane (23), and histamine (24) are important in causing early responses, while the leukotrienes and histamine are mainly responsible for bronchoconstriction during the late response (24).

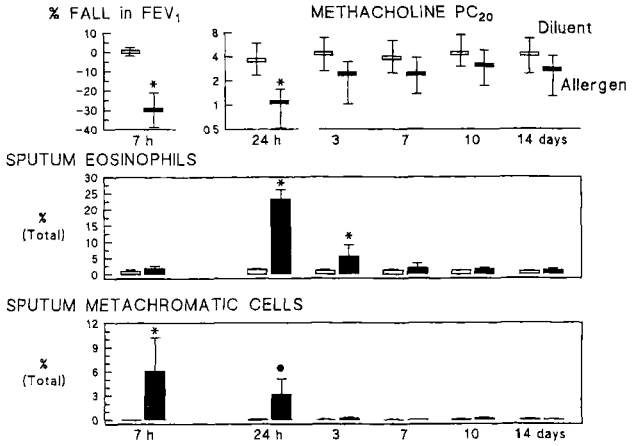


Fig. 2. Increases in sputum eosinophils and metachromatic cells followed for 14 days after inhaled allergen in subjects who developed allergen-induced dual asthmatic responses and methacholine airway hyperresponsiveness. Increases in metachromatic cells were maximal 7 h after inhaled allergen, while increases in eosinophils were maximal 24 h after allergen.

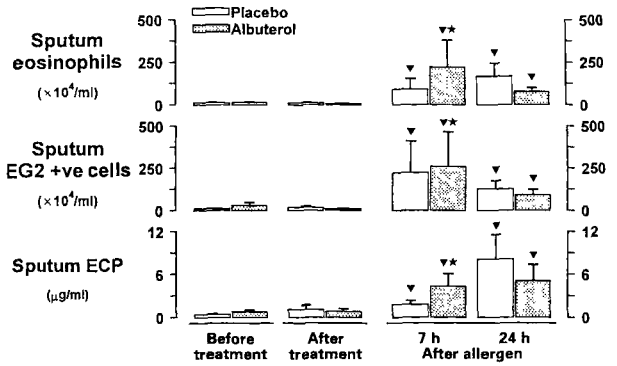


Fig. 3. Allergen-induced increase of eosinophils, activated eosinophils, and eosinophil cationic protein (ECP) in induced sputum. Number of sputum eosinophils (top panel), EG2-positive eosinophils (middle panel), and sputum fluid-phase ECP (bottom panel) before treatment, after 7 days of treatment with albuterol 800 μg/day (solid bars) or placebo (open bars), and at 7 and 24 h after allergen inhalation. All measurements increased after allergen inhalation, at both 7 and 24 h (▼), with further significant increase in sputum eosinophils and ECP 7 h after allergen after albuterol treatment (*) (reproduced with permission from Gauvreau et al. [28]).

Pharmacologic modulation of allergen-induced responses

Inhaled β_2-agonists

Pretreatment with short-acting inhaled β_2-agonists immediately before allergen inhalation abolishes allergen-induced early responses, but has no effect on the magnitude of the late response or allergen-induced airway hyperresponsiveness (25). For this reason, the effect of short-acting inhaled β_2-agonists is believed to be due to functional antagonism, rather than to the prevention of allergen-induced mast-cell-mediator release. In contrast to the effect of pretreatment before inhaled allergen, regular treatment for 1 week with short-acting inhaled β_2-agonists enhances the magnitude of both the early asthmatic response (26) and the late response (27). This enhancement of the late response is associated with increases in the number of airway eosinophils in induced sputum during the late response and increases in levels of eosinophil cationic protein (ECP), suggesting increased eosinophil activation (Fig. 3) (28).

Pretreatment with the long-acting inhaled β_2-agonists immediately before inhaled allergen abolishes both early and late asthmatic responses (29), but it does not influence the magnitude of allergen-induced airway inflammation (30).

Cromones

Pretreatment with either inhaled cromoglycate or nedocromil attenuates the magnitude of allergen-induced early and late asthmatic responses (25, 31) and allergen-induced airway hyperresponsiveness. Neither drug is a bronchodilator or functional antagonist, and this effect is thought to be due to prevention of mediators, such as histamine, being released after allergen inhalation (32). Nedocromil has also been shown to attenuate the eosinophil influx after instillation of allergen into the airway (32).

Antileukotrienes

Pretreatment with specific cysteinyl leukotriene (cys LT_1) receptor antagonists, such as Zafirlukast (33), MK-571 (34), and Pranlukast (35), as well as LT-biosynthesis inhibitors, such as MK-0591 (36) and Bay x1005 (37), have partially attenuated both the early and late asthmatic responses. None of these studies with antileukotrienes have demonstrated complete protection against allergen responses. For example, BAYx1005 and MK-0591 inhibited the maximum percentage of fall in FEV_1 during the early asthmatic response by 56.3% and 57.0%, respectively (36, 37). Treatment with BAYx1005 attenuated the maximum percentage of fall in FEV_1 during the late asthmatic response by 47.4% (37), with MK-0591 being comparably effective with mean 50% and 54% reductions in the mean percentage of fall in FEV_1 (36).

The residual allergen-induced bronchoconstriction not attenuated by antileukotrienes is probably mediated by histamine release. Increases in urinary histamine metabolites have been measured during both the early and late asthmatic responses (38). In addition, pretreatment with the combination of

an antileukotriene and an antihistamine attenuates the early and late asthmatic responses more than the antileukotriene alone (24).

Allergen-induced airway hyperresponsiveness and airway inflammation may also be mediated, in part, by cysLTs. Pretreatment with the $cysLT_1$ antagonist Pranlukast significantly reduced allergen-induced methacholine airway hyperresponsiveness (35), while the $cysLT_1$ antagonist Zifurlukast reduced allergen-induced increases in airway inflammatory cells (39).

Prostaglandin (PG)E_2

PGE_2 is known to have inhibitory effects on many inflammatory cells *in vitro*, including antigen-induced mediator release from mast cells (40), and eosinophil degranulation (41), chemotaxis, and cytokine-stimulated survival (42). PGE_2 is also known to be released in asthmatic airways after bronchoconstrictor challenges, such as exercise (43). Inhalation of PGE_2 has been reported to prevent allergen-induced early and late asthmatic responses (44) and exercise-induced bronchoconstriction (45). Pretreatment with inhaled PGE_2 immediately before allergen challenge also suppresses allergen-induced airway inflammation, as indicated by a significant attenuation of allergen-induced increases in sputum eosinophils, EG2-positive cells, and metachromatic cells up to 24 h after challenge (46).

Bronchodilation following inhaled PGE_2 may account, in part, for the attenuation of the allergen-induced early responses. However, this could not account for the attenuation of the late response. Furthermore, attenuation of the early and late responses to allergen was accompanied by reduced allergen-induced airway inflammation, and protection against allergen-induced airway hyperresponsiveness. Taken together, the results of the present study indicate that inhaled PGE_2 has anti-inflammatory effects in asthmatic airways, and that its effects on allergen-induced asthmatic responses are not those of a functional antagonist. These results also suggest that, as PGE_2 can attenuate allergen-induced airway responses long after its direct pharmacologic activity has ceased (7 and 24 h after inhaled allergen), it acts through inhibition of very early allergen-induced airway events, such as mast-cell degranulation and mediator release.

Inhaled corticosteroids

Inhaled corticosteroids are the best studied anti-inflammatory treatment in asthma. They have been shown to improve all of the clinical manifestations of asthma (47), as well as airway hyperresponsiveness (48). This is because of their ability to improve the persistent airway inflammation of asthma (49) and some of the airway structural abnormalities associated with the disease. Moreover, pretreatment with inhaled corticosteroids immediately before allergen inhalation prevents the late-response and allergen-induced airway hyperresponsiveness (25), while treatment for 1 week also significantly attenuates the early response (18).

The effect of inhaled corticosteroids on allergen-induced airway inflammation has also been evaluated. This study (18) demonstrated that treatment with the inhaled corticosteroid budesonide attenuated, but did not abolish, allergen-induced increases in eosinophils and metachromatic cells in the airways, as assessed by increases in cells in induced sputum (Fig. 4). This suggests that the effects of inhaled corticosteroids on allergen-induced airway responses are mediated through their effects on inflammatory cell migration and activation.

More recently, the effect of inhaled budesonide on the allergen-induced increases in bone-marrow eosinophil/basophil hemopoietic colony-forming cells (Eo/B-CFU), and on selective increases in $CD34^+$ hemopoietic progenitors expressing the α-subunit of the IL-5 receptor (IL-5Rα) was evaluated. Budesonide pretreatment attenuated the magnitude of the allergen-induced early and late asthmatic responses, and the degree of increase in sputum and blood eosinophils, as well as the baseline numbers of total bone-marrow $CD34^+$ cells, $CD34^+$ IL-$34\alpha^+$ cells, and Eo/B-CFU grown in the presence of IL-5 (50). As we have previously shown, allergen inhalation increased Eo/B-CFU grown in the presence of IL-3, GM-CSF, or IL-5, as well as the number of $CD34^+$ IL-$5R\alpha^+$ cells. However, these allergen-induced increases in bone-marrow Eo/B-CFU and $CD34^+$ IL-$5R\alpha^+$ cells were not affected by budesonide treatment. These data demonstrate that short-term inhaled budesonide treatment has a systemic effect in inhibiting the turnover of a subpopulation of bone-marrow-derived progenitors, but that inhalation of allergen overcomes this inhibitory effect. This lack of effect of inhaled budesonide on the allergen-induced increases in IL-5-responsive Eo/B-CFU and on $CD34^+$ IL-$5R\alpha^+$ progenitors was an unexpected result. This is because of our previous observations of the ability of inhaled budesonide to abolish inhaled allergen-induced increases in GM-CFU in allergic dogs (51), and to attenuate the number of circulating Eo/B-CFU in asthmatic subjects with an acute exacerbation (52). This suggests that inhaled budesonide may pre-

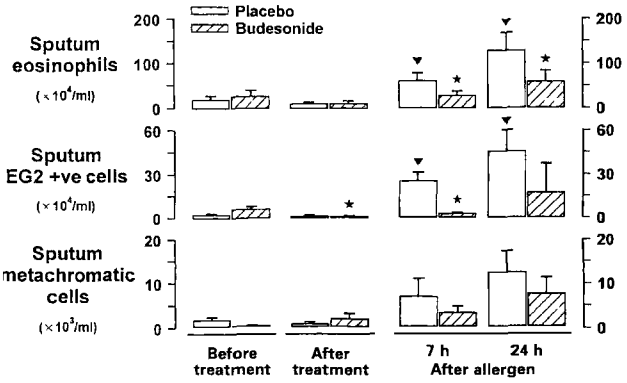


Fig. 4. Allergen-induced increase of eosinophils, activated eosinophils, and metachromatic cells in induced sputum. Number of sputum eosinophils (top panel), EG2-positive eosinophils (middle panel), and metachromatic cells (bottom panel) before treatment, after 7 days of treatment with budesonide 400 μg/day (solid bars) or placebo (open bars), and at 7 and 24 h after allergen inhalation. Sputum eosinophils and EG2-positive cells increased after allergen inhalation, at both 7 and 24 h after placebo (▼), with significant attenuation of these increases after budesonide treatment (*) (reproduced with permission from Gauvreau et al. [18]).

vent maturation of the eosinophil progenitors in the bone marrow, or may prevent allergen-stimulated release of the mature cells from the bone marrow.

Conclusions

Allergen inhalation is an important cause of asthma, and methods to study inhaled allergen in the laboratory have been very useful in evaluating the pro- or anti-inflammatory properties of drugs used for asthma therapy. Studies have shown that the regular use of short-acting inhaled β_2-agonists can enhance allergen-induced airway inflammation, while long-acting inhaled β_2-agonists are not known to have this effect. Antileukotrienes can attenuate all allergen-induced responses, suggesting an important role for the cysteinyl leukotrienes in causing these effects. A similar degree of attenuation has been demonstrated with inhaled PGE_2, but the mechanisms of this effect are not yet known. Finally, inhaled corticosteroids also attenuate all allergen-induced physiologic responses and airway inflammation, an effect possibly mediated, in part, through inhibition of eosinophil and basophil maturation or release from the bone marrow.

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