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Allergen-induced airway responses

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ABSTRACT Environmental allergens are an important cause of asthma and can contribute to loss of asthma control and exacerbations. Allergen inhalation challenge has been a useful clinical model to examine the mechanisms of allergen-induced airway responses and inflammation. Allergen bronchoconstrictor responses are the early response, which reaches a maximum within 30 min and resolves by 1–3 h, and late responses, when bronchoconstriction recurs after 3–4 h and reaches a maximum over 6–12 h. Late responses are followed by an increase in airway hyperresponsiveness. These responses occur when IgE on mast cells is cross-linked by an allergen, causing degranulation and the release of histamine, neutral proteases and chemotactic factors, and the production of newly formed mediators, such as cysteinyl leukotrienes and prostaglandin D₂. Allergen-induced airway inflammation consists of an increase in airway eosinophils, basophils and, less consistently, neutrophils. These responses are mediated by the trafficking and activation of myeloid dendritic cells into the airways, probably as a result of the release of epithelial cell-derived thymic stromal lymphopoietin, and the release of pro-inflammatory cytokines from type 2 helper T-cells. Allergen inhalation challenge has also been a widely used model to study potential new therapies for asthma and has an excellent negative predictive value for this purpose.

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Environmental allergens are an important cause of asthma

<http://ow.ly/NxkGi>

Received: April 3 2015 | Accepted after revision: May 8 2015 | First published online: July 23 2015

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Introduction

In his publication *A Treatise of the Asthma*, published in 1698, Floyer identified the periodicity of “asthma fits” and recognised, from his own experience as an asthmatic, that there was a seasonal variation to his asthma symptoms [1]. In 1819, BOSTOCK [2] provided the first clinical description of what is now recognised as seasonal allergic rhinitis and asthma. About 10 years later, the terminology of hay fever and hay asthma appeared in the English language [3] because of the relationship of these allergic symptoms to the haying season. In 1873, BLACKLEY [4] published his classic monograph documenting that pollen, grass pollen in particular, was the cause of these seasonal symptoms. Subsequently, many other allergens have been identified and allergic diseases, particularly asthma and allergic rhinitis, have become strikingly more prevalent worldwide [5].

Asthma has been defined by the most recent iteration of the Global Initiative for Asthma (GINA) strategy as “a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms, such as wheeze, shortness of breath, chest tightness and cough that vary over time and intensity, together with variable expiratory airflow limitation” [6]. The physiological hallmarks of asthma are variable airflow limitation and the presence of airway hyperresponsiveness (AHR) to a wide variety of bronchoconstrictor stimuli [7], which is most often documented by measuring the airway responses to inhaled methacholine or histamine [8]. Airway inflammatory responses in asthmatics vary in patients and from time to time. The most characteristic inflammatory response is manifested by the presence of airway eosinophils [9], type 2 helper T (Th2)-cells [10], mast cells [11] and basophils [12]. In some patients, however, an airway neutrophilia is prominent [13], while in a small minority, there is no evidence of an increase in airway inflammatory cells [14]. The relationship of the presence of airway inflammation, the physiological hallmarks of asthma and the development of symptoms remains an area of intense research.

Environmental allergens as a cause of asthma

There are a number of lines of evidence that environmental allergens are an important cause of persistent asthma and asthma exacerbations. First, the majority of asthmatics (particularly those with childhood-onset asthma) are atopic [15], and studies of birth cohorts have demonstrated a greatly increased risk of diagnosed asthma and AHR in atopic children [16], particularly those sensitised to house dust mite (HDM), cats and dogs [17]. Second, the presence of airway allergic inflammation predisposes children to rhinovirus-induced recurrent wheezing and asthma exacerbations [18], and this interaction between allergic sensitisation and virus exposure predisposes to the development of asthma in childhood [19], possibly through an effect of type-2 cytokines (such as interleukin (IL)-4 and IL-13) on the innate immune responses to airway viral infection [20]. Third, seasonal asthma, associated with specific environmental allergen exposure, is a well-described clinical entity [21]. Fourth, asthma morbidity and mortality have been associated with inhalation of allergens in soybean in Spain [22], high levels of *Alternaria* in the USA [23], and thunderstorms and the deposition of rye grass pollen [24]. Fifth, some (but not all) studies have shown that avoidance of allergens results in improvements in AHR, overall asthma control and reduced treatment requirements [25, 26]. Finally, and perhaps most convincingly, occupational asthma can be caused by inhalation of allergens in the workplace and removal of the patient from this environment can cure asthma [27]. Thus, the weight of evidence strongly supports that the inhalation of environmental allergens plays an important role in the initiation and persistence of asthma in some patients.

Allergen-induced airway responses

Allergen inhalation by atopic asthmatics in the laboratory results in many of the physiological and inflammatory manifestations of asthma, including reversible airflow obstruction, AHR, and eosinophilic and basophilic airway inflammation. The first description of allergen-induced bronchoconstrictor responses was made by HERXHEIMER [28] in the early 1950s, who identified two distinct constrictor components, which he called the early (EAR) and late asthmatic responses (LAR). The EAR develops within 10–15 min after inhalation, reaches a maximum within 30 min and generally resolves by 1–3 h. In approximately 60% of adults and 80% of children, bronchoconstriction recurs after 3–4 h and reaches a maximum over 6–12 h [29]. The prevalence of the LAR appears to be allergen specific, being >75% for some allergens, such as HDM or cat allergen, and much less frequent for others, such as grass pollen. Subsequently, COCKCROFT *et al.* [30] demonstrated that allergen-induced airway responses were followed by an increase in AHR to inhaled histamine or methacholine, which could last several days to weeks, and which was greater in patients who develop allergen-induced LAR [31]. These airway responses, although fairly prolonged, do not mimic the chronic, low-dose exposure to environmental allergens. This fact resulted in the development of low-dose inhaled allergen challenge, where doses causing minimal bronchoconstriction are administered daily over 5–7 days. These challenges do not cause late responses, but do cause airway eosinophilia and AHR [32]. Another method used to study the mechanisms of allergen-induced airway responses has been to instil allergen into the airway segment *via* a bronchoscope and then return to this segment to perform

bronchial washes at a predetermined time (*e.g.* at 18 h) [33]. This method does not allow for the measurements of the physiological responses to the allergen. Allergen inhalation challenge has become the mainstay for models of asthma developed in many species, including mice [34], rats [35], guinea pigs [36], dogs [37], rabbits [38] and primates [39].

Mechanisms of allergen-induced bronchoconstriction

The development of airway responses to a specific allergen begins when an inhaled allergen is presented by the professional antigen-presenting cells in the airways, dendritic cells (DCs), to naïve T-cells, which stimulates the development of Th2 cells (figure 1). The Th2 cells produce a variety of cytokines, which promote the production of IgE (IL-4), eosinophilopoiesis (IL-5), mast cell development (IL-9), and goblet cell hyperplasia and AHR (IL-13); these pro-inflammatory cytokines can also be produced by other cells, most notable type 2 innate lymphoid cells. These cytokines are currently best referred to as type-2 cytokines.

The allergen-induced responses are initiated when IgE binds to high-affinity receptors (FcεRI) located on the surface of mast cells and basophils. The IgE is subsequently cross-linked by allergen, causing mast cell degranulation and the release of preformed mediators such as histamine, neutral proteases and chemotactic factors, and the activation of eicosanoid pathways to produce newly formed mediators such as cysteinyl leukotriene (CysLT)C₄ and CysLTD₄, and their metabolite cysLTE₄, as well as prostaglandins (PG), especially PGD₂. CysLTs are the most potent bronchoconstrictor mediators yet described [40] and are an important cause of allergen-induced bronchoconstriction. This was initially suggested by the demonstration of increased levels of LTE₄ measured in bronchoalveolar lavage (BAL) fluid [41] and in urine after allergen challenge [42]. More convincing evidence was obtained by studies using leukotriene receptor antagonists (LTRAs), which demonstrated that CysLTs account for approximately 50% of the decrease in the forced expiratory volume in 1 s (FEV₁) that occurs during the EAR [43, 44]. CysLTs also increase microvascular permeability [45] and stimulate secretion of mucus [46]. Histamine also contributes to bronchoconstriction during the EAR. Increases in urinary histamine metabolites have been measured during the EAR [47], and combined treatment with antihistamines and LTRAs abolish the EAR [48, 49].

The bronchoconstriction that occurs during the LAR is also caused by cysLTs and histamine release. Once again, the most convincing evidence is that the LAR is partially attenuated by specific LTRAs [43, 50] as well as a 5-lipoxygenase activating protein antagonist [51]. Further attenuation is achieved with the combination of an LTRA and an antihistamine [52]. This effect of LTRAs and antihistamine on the LAR is probably mediated through the prevention of airway smooth muscle constriction, airway oedema and mucus production. LTRAs also cause a reduction of allergen-induced airway eosinophilia [44]. In addition, allergen-induced AHR, which is associated with the LAR [31], is also partly mediated by CysLTs. Several studies have demonstrated that treatment with LTRAs also attenuates allergen-induced increases in AHR [53, 54].

FIGURE 1 The role of airway epithelium, dendritic cells and IgE in the initiation of allergic airway inflammation. TSLP: thymic stromal lymphopoietin; pDC: plasmacytoid dendritic cell; mDC: myeloid dendritic cell; MHC: major histocompatibility complex; Treg: regulatory T-cell; Th: helper T-cell; TCR: T-cell receptor; IL: interleukin; LT: leukotriene; PG: prostaglandin.

Mast cells have been implicated as the effector cells for the release of the bronchoconstrictor mediators causing the EAR. There is less certainty about the cells of origin of these mediators during the LAR. Both airway basophils and eosinophils are increased during the LAR, and both of these cell types have the ability to synthesise CysLTs [55, 56]; however, only basophils can produce histamine, which suggests that basophils are, at least in part, involved in causing the LAR. The importance of eosinophils in causing the LAR is less certain, as the one study that examined the effects of an anti-IL-5 human monoclonal antibody (hMAb) on allergen-induced airway responses successfully attenuated the influx of airway eosinophils, but had no effect on the LAR [57].

Mechanisms of allergen-induced airway inflammation

Allergen-induced late responses are associated with an increase in airway inflammatory cells, which have been measured in airway biopsies [58], BAL [59, 60] and induced sputum [61]. The most prominent increase is in airway eosinophils [58, 61] but also airway basophils [12] and, less consistently, neutrophils [62].

The allergen-induced airway inflammatory response could be initiated by a number of mechanisms. The cross-linking of IgE on mast cells causes the release of pro-inflammatory cytokines from mast cells, which include IL-3, IL-4, IL-5 and IL-6 [63]. In addition, CysLTs that are also released from mast cells, particularly LTE₄, can cause eosinophil chemotaxis in allergic asthmatics [64] and allergen-induced airway eosinophilia is partially attenuated by LTRAs [44, 54]. However, allergic asthmatics who develop an isolated EAR have a smaller increase in airway inflammatory cells when compared to those who develop LARs [65], even though the magnitude of the bronchoconstriction was similar, which implies that mechanisms other than mast cell degranulation and activation are involved. In addition, exercise, which is a stimulus that also causes mast cell degranulation and bronchoconstriction in asthmatics and which is independent of IgE, does not cause eosinophilic airway inflammation [66].

Another important mechanism for allergen-induced airway inflammation involves DCs, which are the most potent lung antigen-presenting cells, and play a central role in initiating primary and secondary immune responses [67]. In humans, two different DC types are distinguished by their dissimilar cell surface antigens and function [68]. Myeloid dendritic cells (mDCs), derived from myeloid precursors, and plasmacytoid dendritic cells (pDCs), derived from lymphoid precursors. In studies in mice, mDCs induce Th2-dominated sensitisation to inhaled allergen, leading to eosinophilic airway inflammation and goblet cell hyperplasia [69], and mDCs are essential in the presentation of antigen to previously primed Th2-cells, leading to eosinophilic airway inflammation [70]. By contrast, pDCs in mice have been shown to suppress the generation of effector T-cells, which are induced by mDCs [71], and that the absence of pDCs led to Th2-cell sensitisation and features of asthma [72].

There is a rapid reduction in the numbers of circulating mDCs within 3 h after inhaled allergen in asthmatic subjects [73] with trafficking of immature mDCs from blood into the airway [74], while trafficking of mDCs from the airways into the regional lymph nodes probably occurs through the lymphatic system [75]. Allergen inhalation also causes an increase in airway DCs in airway biopsy samples [76], and the number of both sputum mDCs and pDCs are higher 24 h after challenge [77] in asthmatic subjects.

mDCs in peripheral blood do not represent a homogenous population but rather consist of two subsets, mDC1s and mDC2s [78]. The mDC2s express a high level of thrombomodulin. The morphology, endocytic capacity and maturation are quite similar; however, mDC2s do not express the Fc receptors CD32, CD64 and FcεRI [79]. There is a possible role of mDC2s in allergic asthma. Studies have demonstrated that, while all three DC subpopulations are present in asthmatic lungs [80], BAL fluid [81] and in induced sputum [82], mDC2s are more frequent than mDC1s and pDCs [80]. Allergen exposure upregulates thrombomodulin in mDCs and mDC2s induce a greater type-2 cytokine response by allergen-specific T-cells [78]. mDC2s increase in the sputum of subjects with asthma after allergen challenge, suggesting this subtype is involved in the regulation of allergen responses in the lung [83].

The immune and inflammatory processes of the LAR are complex and involve the increase in type-2 airway inflammation, predominantly with eosinophils, but also includes an increase in airway neutrophils [84] and basophils [12]. These increases in airway eosinophils are associated with increases in circulating eosinophil progenitors measured as colony-forming units 24 h after inhaled allergen, suggesting that inhaled allergen stimulates bone marrow production of these progenitors [85, 86]. Furthermore, allergen inhalation causes the upregulation of the IL-5 receptor on bone marrow eosinophil progenitors following allergen inhalation [86].

Patients who develop allergen-induced LAR show evidence of impaired regulation by regulatory T (Treg)-cells and an imbalance in the ratio of inhibitory to effector T-cells. It was observed that the ratio of Treg-cells to CD4⁺ cells in induced sputum after inhaled allergen is lower in patients with LAR when compared with patients with EAR [87].

Finally, epithelial cell-derived mediators have emerged as key players in cellular inflammation in asthmatic airways. In particular, the epithelium-derived cytokine thymic stromal lymphopoietin (TSLP) has been suggested as a master switch for allergic inflammation. GAUVREAU *et al.* [88] have demonstrated that an anti-TSLP hMAb attenuated both allergen-induced EAR and LAR, and reduced the number of eosinophils in induced sputum and the exhaled nitric oxide fraction ($FeNO$). Treatment with the hMAb also significantly reduced the numbers of blood and sputum eosinophils and $FeNO$ in these allergic asthmatic subjects prior to allergen challenge. This suggests that there is constitutive epithelial production of TSLP that drives the persisting airway inflammatory response in allergic asthma.

Allergen inhalation challenge to study new drugs for asthma

The allergen inhalation challenge model is commonly used as an experimental tool to better understand the pathophysiology of allergic asthma and the blocking effects of investigational therapies [29]. The ability of pharmaceutical agents to inhibit allergen-induced outcomes provides reasonable support for clinical efficacy and helps to identify critical pathways involved in allergic airway responses. Each of the currently approved asthma therapies, including inhaled corticosteroids (ICS) [89–91], ICS and long-acting inhaled β_2 -agonists [92, 93], LTRAs [50, 94], and anti-IgE [95], show inhibition of late-phase asthmatic responses to inhaled allergen.

Inhibition of the allergen-induced late response is typically selected as the primary outcome in clinical trials assessing efficacy of anti-inflammatory therapies for the treatment of asthma. The late response is most closely associated with airway inflammation. The reproducibility of the late responses has been examined and sample sizes of less than 10 subjects are estimated to provide sufficient power to detect 50% inhibition with a crossover study design [61, 96]. Furthermore, parallel-group studies designed with as few as 15 subjects per treatment arm can also detect 50% inhibition of LAR [97]. As such, clinical trials employing allergen inhalation challenge are generally quite small. Other outcomes could be used to power these studies. These include allergen-induced AHR or airway eosinophilia. These outcomes may be more appropriate for some studies. Allergen-induced AHR is more variable than the LAR and so larger studies would be required. Allergen-induced airway eosinophilia is as reproducible as the LAR and may be an appropriate outcome variable, particularly in studies that are evaluating specific therapies that target eosinophil [98]; however, not all laboratories can measure induced sputum eosinophils and it is often not reported in clinical trials of allergen-induced airway responses.

The selection of the correct subjects to include in trials evaluating new therapies using allergen-induced airway responses is of paramount importance. These need to be mild, stable allergic asthmatic subjects, not on regular anti-inflammatory treatment. Baseline lung function is usually close to normal but such subjects usually have evidence of low-grade persisting baseline airway inflammation, with increased blood and sputum eosinophils and increased $FeNO$ [88]. This careful selection is necessary because of the safety issues of delivering inhaled allergens into the airways of patients with poor asthma control and the fact that regular therapy (with, for example, ICS or LTRA) will markedly influence the allergen-induced airway responses.

The choice of a crossover *versus* a parallel-group design is usually based on knowledge of the pharmacokinetics of the drug being tested. While crossover studies are smaller in size and can often be completed more quickly, almost all biologics have a long half-life, making a parallel-group design the only practical option.

Historically, the allergen-inhalation challenge model has demonstrated an excellent negative predictive value for the development of new drugs for asthma [99]. There are several examples of potential therapies found to be ineffective in both the allergen challenge model as well as in clinical applications, and these include platelet-activating factor antagonists [100], inhaled formulations of antileukotrienes or anti-IgE [101] and esterase-sensitive steroids [91]. The allergen challenge model has a moderate positive predictive value for identification of efficacious therapies. The number of false positive therapies, to-date, are limited and include inhaled PGE_2 [102], furosemide [103] and antihistamines [104]. There have also been potential therapies that have demonstrated inhibition of the LAR but failed to be further developed for treatment of asthma for reasons including unsuitability (adverse events) [105] or lack of business opportunity. To date, there are no cases of therapies giving false-negative results in the allergen challenge model and this statistic is of value to pharmaceutical companies when debating the prospect for further clinical development of a compound.

To test the inhibitory effects of an asthma medication on the magnitude of allergen-induced early and late responses, subjects are challenged with the same dose of allergen after receiving treatment with test therapy and again after receiving treatment with placebo in a randomised crossover study design. When the half-life of the investigational therapy is long (monoclonal antibodies) or unknown, a parallel-group design is utilised with administration of the same dose of allergen determined to induce early and late responses during a screening period. If the onset/offset of activity of the investigational therapy is

unknown, multiple allergen challenges can be conducted post-dosing to cover a larger timespan. With repeated allergen challenges in either crossover or parallel-group designs, it is critical that sufficient time is allowed between challenges for FEV₁ and AHR to return to baseline.

ICS are regarded as the gold standard for comparison of investigational anti-inflammatory therapies, owing to their nearly complete attenuation of the allergen-induced LAR in addition to their suppressive effects on allergen-induced AHR and airway inflammation [90]. Recently, a nonsteroidal glucocorticoid receptor agonist was reported to share these inhibitory properties in the allergen challenge model [106] but the carryover effects observed following treatment with the nonsteroidal glucocorticoid receptor agonist could indicate a longer duration of action than other ICS; however, this needs to be confirmed in a future study. In contrast, regular treatment with salbutamol has been shown to increase the allergen-induced LAR and airway eosinophils (figure 1) [107, 108]. Clearly, the most effective controllers of allergic inflammation in the airways are medications with anti-inflammatory properties, while bronchodilators may mask underlying inflammation and thereby worsen responses to allergen [108, 109].

Many other compounds have been evaluated for their ability to inhibit LAR through targeting of specific inflammatory cells, receptors, enzymes or mediators thought to play central roles in the allergic inflammatory cascade (table 1). Eosinophils have been a key therapeutic target, leading to the development of antibodies against IL-5 (mepolizumab and reslizumab) and its specific receptor subunit IL-5R α (benralizumab). Although anti-IL-5 antibodies have been reported to effectively reduce eosinophil levels in the circulation and airways, one study of treatment with anti-IL-5 did not show any inhibition of the LAR but did markedly attenuate allergen-induced airway eosinophilia (table 2) [57]. However, blocking receptors

TABLE 1 Asthma treatments and new molecular entities studies using inhaled allergen

Inhaled glucocorticosteroids

Budesonide [44, 90, 92, 110]
Fluticasone furoate [111, 112]
Mometasone furoate [113]
Ciclesonide [89]
Beclomethasone [114]
CS receptor agonist [106]

Long-acting β_2 -agonists

Vilanterol [111]
Salmeterol [112]
Formoterol [92]

Short-acting β_2 -agonist

Salbutamol [108, 115]

Lipid mediators

Leukotriene A₄ hydrolase inhibitor [116]
5-lipoxygenase-activating protein inhibitor [51, 117]
CysLT receptor-1 antagonist [43, 44, 52, 118]
CysLT1/2 antagonist [119]
Leukotriene B₄ receptor antagonist [120]
Tachykinin NK₁/NK₂ receptor antagonist [121]
Prostaglandin E₂ [102]
COX-2 selective inhibitor [122]
Platelet-activating factor [100]

MAbs

IL-13 MAb [97, 123]
OX40L MAb [124]
IL-5 MAb [57]
IgE MAb [95, 125]
CD11a MAb [105]
TSLP MAb [88]
M1-prime MAb [126]
IL-9 MAb [127]
C5a MAb [128]

Antihistamines

Desloratadine [52]
Cetirizine [114]
Terfenadine [129]

Protein inhibitors/antagonists

PDE4 inhibitors [84, 130, 131]
Pan-selectin antagonist [132, 133]
Mast cell tryptase inhibitor [134]
CRTH2 antagonist [135]
IL-4R mutant [136]
VLA-4 antagonist [137, 138]
TNF receptor antagonist [139]
Antisense to CCR3 and Bc [140, 141]
CpG immunostimulatory oligonucleotide [142]
PPAR- γ agonist [143]
Inhaled A_{2A}-receptor agonist [144]

Others

Calcium channel blocker [145]
Cationic airway lining modulator [146]
Heparin disaccharide [147]
Cromones [148, 149]
Nonsteroidal anti-inflammatory drug [129, 150]
Lysine ASA [151]
Synbiotics [152]
 ω -3 polyunsaturated fatty acids [153]
Furosemide [103]

CS: corticosteroid; CysLT: cysteinyl leukotriene; NK: neurokinin; COX: cyclo-oxygenase; MAb: monoclonal antibody; IL: interleukin; TSLP: thymic stromal lymphopoietin; PDE: phosphodiesterase; CRTH2: chemoattractant receptor-homologous molecule expressed on type 2 helper T-cells; IL-4R: IL-4 receptor; VLA: very late antigen; TNF: tumour necrosis factor; Bc: common β -chain; PPAR: peroxisome proliferator-activated receptor; ASA: acetylsalicylate.

TABLE 2 Effects of available and experimental asthma treatments on allergen-induced airway responses

	EAR	LAR	AHR	Eosinophilia	F _{ENO}
Attenuate responses	ICS SABA LABA LTRA Cromoglycate Theophylline Anti-IgE Anti-IL-13 Anti-TSLP PGE ₂ Furosemide Lysine ASA Antihistamines	ICS LABA LTRA Cromoglycate Theophylline Anti-IgE Anti-IL-4 Anti-IL-13 Anti-TSLP PDE4 inhibitors PGE ₂ Furosemide Lysine ASA Antisense against CCR3 and Bc	ICS LABA LTRA PDE4 inhibitors Anti-TSLP	ICS LTRA PDE4 inhibitors Anti-IL-5 Anti-TSLP Antisense against CCR3 and Bc	ICS Anti-TSLP
No effect on responses	Anti-IL-5	Anti-IL-5	Anti-IL-5 Anti-IL-13 Furosemide Lysine ASA Theophylline	Anti-IL-13	
Enhance responses		SABA		SABA	

EAR: early asthmatic response; LAR: late asthmatic response; AHR: airway hyperresponsiveness; F_{ENO}: fraction of exhaled nitric oxide; ICS: inhaled corticosteroid; SABA: short-acting inhaled β_2 -agonist; LABA: long-acting inhaled β_2 -agonist; LTRA: leukotriene receptor antagonist; IL: interleukin; TSLP: thymic stromal lymphopoietin; PG: prostaglandin; ASA: acetylsalicylate; PDE: phosphodiesterase; Bc: common β -chain.

of the eosinophil growth factors IL-3, IL-5 and granulocyte-macrophage colony-stimulating factor in combination with the chemokine receptor CCR3 has been shown to inhibit airway eosinophils and the LAR [140]. This suggests that either antieosinophil therapy needs to effectively block eosinophilopoiesis or that other proallergy cells sharing this repertoire of receptors, such as mast cells and basophils, need to be targeted in combination with the eosinophil for effective control of allergic inflammation.

Therapies directed at other cytokines central to the pathogenesis of allergic asthma have been generated and tested in the allergen challenge model. Due to its pleiotropic role in asthma, IL-13 has been the focus of considerable attention. Several therapeutic approaches have been utilised to impede the actions of IL-13, including monoclonal antibodies blocking binding to IL-13R α 1 (IMA-026) or IL-4R α (IMA-638 and lebrikizumab) and therapies blocking both receptors, such as an IL-4 variant (pitakinra). Studies conducted using the allergen challenge model consistently demonstrated that blockade of the IL-4R α -binding domain of IL-13 attenuates the LAR [97, 123, 154], while blockade of the IL-13R α 1-binding domain of IL-13 binding to has no significant effect [97]. Furthermore, blockade of IL-13/IL-4R α binding (dupilumab) was

FIGURE 2 The provocative concentration of allergen causing a 15% fall in forced expiratory volume in 1 s (FEV₁) is inhibited by anti-inflammatory drugs (inhaled corticosteroids) and can be exaggerated, as observed following regular use of salbutamol. SABA: short acting β_2 -agonist; ICS: inhaled corticosteroid.

FIGURE 3 Allergen-induced early (0–2 h) (EAR) and late (3–7 h) airway responses (LAR). The LAR is inhibited by anti-inflammatory drugs (inhaled corticosteroids [ICS]). The LAR can be exaggerated, as observed following regular use of salbutamol. SABA: short acting β_2 -agonist.

associated with improved lung function when studied in patients with uncontrolled asthma [155], demonstrating consistency of efficacy between the allergen challenge model and larger asthma clinical trials. More recently, an antibody to TSLP (AMG 157) has been reported to attenuate the LAR and many other allergen responses measured; anti-TSLP antibody inhibited the allergen-induced EAR, the shift to AHR, the increase in circulating and airway eosinophils, and the elevated levels of exhaled nitric oxide [88]. Unexpectedly, the anti-TSLP antibody also reduced the low-grade inflammation (circulating and blood eosinophils, and F_eNO) in the mild asthmatic subjects measured at baseline before allergen challenge. This comprehensive reduction in inflammation has not been observed before, not even following treatment with inhaled steroids [89]. It is believed that the upstream position of TSLP in the allergic cascade is critical for the success of the anti-TSLP approach in this model.

In another model of allergen challenge, inhibition of the early-phase response can be more carefully examined using an approach whereby increasing concentrations of allergen are administered until a 20% or 15% fall in FEV₁ is reached and the provocative concentration of the allergen causing that fall (PC₂₀ or PC₁₅, respectively) is calculated. An elevation in the PC₂₀ following drug treatment *versus* placebo treatment demonstrates a protective effect on allergen-induced bronchoconstriction. Drugs that block components of the allergic cascade, for example, by preventing crosslinking of IgE receptors by allergen (anti-IgE) [125, 156] or by interfering with mediators of bronchoconstriction (CysLTs and histamine), have been shown to increase the allergen PC₂₀ [49]. Treatment with antihistamines [52], antileukotrienes [44] or anti-IgE [95] also attenuate the fall in FEV₁ during the early and late responses after allergen challenge, which suggests that events occurring during the early response are linked to the development of the late response. Pre-treatment with β -adrenergic receptor agonists also increases the allergen PC₂₀ [157] but the mechanism is by functional antagonism rather than by blocking inflammatory pathways. Surprisingly, regular use of salbutamol has been reported to increase the responsiveness to allergen, as shown by a decrease in the allergen PC₂₀ [109] (figure 2). Consistent with this finding, when allergen is inhaled after regular use of salbutamol, there is enhancement of the EAR and LAR when compared to the response after placebo [107] (figure 3). The mechanism for the increase in LAR may involve increased airway inflammation [108]. While the allergen challenge model has been used for proof of concept in drug development to identify key therapies that reduce responses to inhaled allergen, the model can also identify therapies that increase responses to inhaled allergen.

Conclusions

Environmental allergens are an important cause of asthma, and their inhalation can contribute to loss of asthma control and exacerbations. Allergen inhalation challenge has been a useful clinical model to examine the mechanisms of allergen-induced airway responses and inflammation. It has also been a widely used model to study potential new therapies for asthma and has an excellent negative predictive value for this purpose. The predictive value of this clinical model for new drug development is likely because allergen-induced airway responses include physiological and inflammatory responses, which are central to asthma symptoms and exacerbations, such as bronchoconstriction, airway oedema, mucus production, and eosinophilic and basophilic airway inflammation. The inhaled allergen challenge model will probably continue to be extensively employed for studying the pathogenesis of airways inflammation and for assessment of new therapeutics in clinical proof-of-concept studies.

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