

EDITORIAL

The allergen challenge

Challenging an allergic patient by the inhaled, nasal or cutaneous route with an allergen to which they are sensitive provides a convenient and well established clinical model for studying allergic diseases [1–3]. Indeed, these challenges have been extensively employed to assess the complex cellular and molecular mechanisms of eosinophil influx, and also to evaluate the efficacy of new drugs intended to combat asthma and allergy. This Editorial describes how a new repeated washout procedure [4], as well as studies with drugs [5], are influencing our understanding of the mechanisms of tissue recruitment of eosinophils [6–9].

Repeated segmental washout prior to allergen challenge

In this issue of *Clinical and Experimental Allergy*, Dr Loren W. Hunt and colleagues from the Mayo Clinic in Rochester, Minnesota describe a novel and elegant technique, consisting of repeated segmental washout, to study responses to segmental allergen challenge in patients with asthma [4]. This involves performing 12 successive bronchoalveolar lavages (BALs), to deplete the washed segment of the majority of recoverable airway surface cells. It was noted that 24h after allergen challenge the washed segment contained increased numbers of eosinophils, and the authors suggest that macrophages on the surface of the airways may protect against eosinophilic migration from lung tissue into the lumen of the airway.

At the outset it is important to consider safety issues in relation to the process of repeated segmental washout, since this is demanding for both the patient and the bronchoscope operator. Indeed, investigation of induced sputum is increasingly being used as a relatively non-invasive means of studying airway-derived cells, as an alternative to bronchial biopsy or lavage. In particular, it is noted that among the six subjects studied a patient withdrew due to coughing during the washout procedure, while another subject became febrile 12h after bronchoscopy. It therefore looks as though the technical complexity of the challenge and washout procedures could limit the application of this study design.

The phenomenon of increased BAL eosinophils after allergen challenge in a washed segment could be caused by a variety of mechanisms, and it remains speculative to implicate macrophage depletion. Firstly, when considering cellular mechanisms, repeated lavage depletes surface macrophages as well as lymphocytes and other cells. To pinpoint the responsible cell types, it should be possible to deplete cell populations following serial washout, and a possibility would be to add an enriched population of autologous macrophages prior to allergen challenge. However, although the repeated lavage process depletes surface cells, it would be interesting to assess whether the washings are also altering the numbers of cell populations within the respiratory mucosa. Indeed, one could speculate that tissue cells might be more influential on eosinophil extravasation than luminal cells.

Secondly, we should consider soluble factors that could be involved in eosinophil recruitment. The physical effect of repeated washing of a segment of the lung could cause topical stimulatory effects on the airways and lung parenchyma, and stimulate epithelial cells to produce chemotactic factors for eosinophils. Alternatively, another mechanism for repeated lavage causing an amplified eosinophil response to allergen could be through the removal of mucoproteins, surfactants and/or soluble receptors that might inhibit chemotactic responses. Indeed the binding of chemokines to the glycosaminoglycans heparin [10] and heparan [11,12], is well described, and binding to mucoproteins is likely to also inactivate chemokines. An important control would be to have a sequential washout of a segment without subsequent allergen challenge, followed by a wash to assess the degree of eosinophil infiltrate at 24h. Analysis of the fluid removed by repeated lavage might demonstrate the release of epithelial-derived chemotactic factors for eosinophils, and/or depleted levels of mucus and factors that might inhibit chemotaxis.

A third possible reason to explain the eosinophilia is that allergen challenge to one segment of the lung could then increase eosinophil responses to allergen in the subsequent washed and allergen-challenged next segment, since this order of challenges was preserved in the study design. To control for this it would be helpful to study responses to sequential wash in a segment without preceding allergen challenge in another or to randomise these procedures. In carrying out these additional controls and further studies on the repeated lavage model, it should be possible to clarify the mechanism of increased eosinophilic infiltration in this system.

The eosinophil hypothesis

In a distinguished series of landmark publications Gerry Gleich and his research team at the Mayo Clinic have been the major proponents of the 'eosinophil hypothesis' that the eosinophil is central to the pathophysiology of asthma [13–23]. Asthma has been described as a chronic eosinophilic bronchitis due to the conspicuous eosinophil infiltrate found within the airways [24–28]. Furthermore, the importance of the eosinophil in asthma is supported by clinical studies correlating numbers of eosinophils with the severity of asthma [29,30], while in patients that have died from asthma, postmortem histological analysis of the airways sometimes shows airway luminal mucous plugs and large numbers of eosinophils [31–33]. Indeed, the eosinophil has been associated with the late asthmatic reaction (LAR) following allergen inhalation as well as airway hyper-reactivity (AHR) [32–34]. In particular, major basic protein (MBP) can directly damage airway epithelium, impair ciliary motility, and increase vascular permeability [14,35].

Eosinophils through the release of growth factors, such as transforming growth factor- β (TGF- β) [36], could mediate

some of the structural changes comprising airway remodelling, including sub-basement membrane collagen deposition, smooth muscle hypertrophy and epithelial shedding [37]. Eosinophils have the capacity to produce a range of cytokines and chemokines and act as immunoregulatory cells [38]; being capable of secreting both Th1 and Th2 cytokines, interleukins associated with acute phase responses, TGF- β [39], tumour necrosis factor- α (TNF- α) [40], IL-8 [41], RANTES [42,43] and other chemokines [44]. Remodelling of the airway may be mediated by fibroblasts acting in conjunction with eosinophils [35]. Eosinophils adhere to fibroblasts by means of β_2 -integrin adhesion [45] and can produce TGF- β and TNF- α to cause fibroblast proliferation [46]. Fibroblasts are able to express intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) through which they are able to bind β_2 -integrins on eosinophils [47]. IL-4 causes increased endothelial and lung fibroblast expression of VCAM-1 [48] that may contribute to selective eosinophil tissue recruitment.

However, these associations with eosinophils are not proof of causation, and recent studies have questioned the role of the eosinophil as a harmful cell [49–52], and even suggested that eosinophils can act as bystanders [53] or part of complex cell interactions and repair mechanisms [24,35,54]. Recruitment of eosinophils is linked with Th2 immune responses [54–56], since eosinophil production and recruitment appear to be mediated by the Th2 cytokines IL-5, IL-4 and IL-13 [57,58]. It is interesting that eosinophils can express HLA-DR [59,60] and also express costimulatory cell surface receptors such as CD28 and CD86 [61], suggesting that they can act as antigen-presenting cells. Hence it is postulated that tissue eosinophils can process inhaled antigens, traffic to regional lymph nodes, and stimulate CD4⁺ T cell responses [54].

Effects of drugs on inhaled allergen challenge responses

Allergen challenge responses have been extensively studied in relation to drugs, and the effects of drugs of known mechanism on cellular influx, the early and late asthmatic reactions (EAR and LAR) as well as airway hyper-reactivity provide important mechanistic insights. The inhaled allergen challenge offers the opportunity to study effects on the EAR and LAR, blood and sputum eosinophils, exhaled breath nitric oxide (NO) and methacholine airway responsiveness (PC₂₀). The reproducibility of the inhaled allergen challenge is excellent, and 12 patients is adequate to reliably demonstrate 50% attenuation of the EAR or LAR with >90% power [62]. Recently the method of bolus as opposed to incremental allergen challenge has been validated [1,2]. Inhaled allergen challenge should ideally not be repeated at less than 3-week intervals, due to residual AHR [63].

Of the anti-inflammatory drugs effective in controlling asthma, all inhibit the LAR to allergen: this includes steroids, theophyllines, leukotriene antagonists, cromones, cyclosporin A, anti-IgE (see Table 1) [64,65]. However, a number of other agents that are not effective therapy also cause some inhibition of the LAR: including furosemide, heparin, PGE₂. Hence, when studying the effects of a new therapeutic on the inhaled allergen challenge LAR, it is useful for positioning a drug relative to established asthma therapy, but offers a relatively low hurdle due to the false positives. However, we cannot identify anti-inflammatory therapeutics for asthma that are 'false

negatives': in which there is no effect on the LAR but clinical efficacy.

Of especial interest in relation to inhaled allergen challenge responses has been a monoclonal antibody directed against IL-5, since IL-5 has an established role as the major terminal differentiation factor during eosinopoiesis in the bone marrow [66,67]. CCR-3 expression on myeloid eosinophils may be up-regulated by IL-5 as a terminal event in eosinopoiesis [68]. In addition, in a range of animal species monoclonal antibody (MoAb) against IL-5 causes long-term inhibition of pulmonary eosinophilia, reduced AHR and inhibits the LAR [69,70].

An initial study in humans involved a single intravenous infusion of a humanized MoAb directed against IL-5 (SB240563) being given to mild allergic asthmatics in a parallel-design double-blind clinical trial [71]. There was pronounced suppression of peripheral blood eosinophil levels for 16 weeks and considerably reduced numbers of sputum eosinophils after allergen challenge. However, despite these clear effects, anti-IL-5 did not protect against the allergen-induced LAR and did not inhibit baseline or post-allergen AHR. Hence, the eosinophil does not seem to be a prerequisite for either AHR or the LAR, and this study suggests that anti-IL-5 therapy may not be clinically useful in the short-term for asthma therapy. Interestingly, recent clinical studies in human allergic asthma have also found dissociation between AHR and eosinophil levels [72,73].

Interpretation of the anti-IL-5 effects on inhaled allergen challenge must be made with caution, because even though eosinophil numbers in blood and sputum were reduced by anti-IL-5, there were still residual eosinophils in the airways and these could be activated and surrounded by abundant released eosinophil granule proteins. In addition, the primary aim of the study was to assess tolerability, and having groups of

Table 1. Effects of selected therapeutics on inhaled allergen challenge responses

Therapeutics	References
Inhaled steroids	[76–82]
Prednisone	[83]
Theophyllines	[84,85]
Leukotriene antagonists	[86–90]
Cromones	[91–93]
Salbutamol	[93–96]
Terbutaline	[97]
Salmeterol	[98–107]
Ipratropium bromide	[96]
Formoterol	[108,109]
Cyclosporin A	[110,111]
Anti-IgE	[112,113]
IL-12, IL-11 (mice)	[114,115]
Anti-IL-5	[71]
Antihistamine	[116]
Aspirin	[117]
Indomethacin	[118]
Prostaglandin E2	[119–121]
Heparin	[122]
Furosemide	[123]
Phosphodiesterase (PDE) 4 inhibitor	[124,125]
Mast cell tryptase inhibitor	[126]
Platelet activating factor (PAF) antagonist	[127–129]

only eight subjects in a multicentre study, there was not enough power to exclude a minor effect of anti-IL-5 on the LAR [62]. A remaining concern is that an inhaled allergen challenge study is an artificial 'model' situation, and effects on clinical symptoms and lung function in patients with symptomatic asthma were not measured.

In addition to the allergen challenge study, there are preliminary reports that MoAb vs. IL-5 is not clinically effective in treating asthma of different degrees of severity. A single infusion of a humanized MoAb against IL-5 (SCH55700) caused a pronounced reduction in circulating eosinophils in severe asthma, but effects on symptoms and lung function were unconvincing [74,75]. In addition, in mild-to-moderate asthma there was pronounced reduction in blood and airway mucosal eosinophils but again initial verbal reports describe unimpressive clinical effects. It is accepted that further clinical studies are required since anti-IL-5 could prevent or inhibit airway remodelling, although long-term studies will probably be required to assess this potential.

In the immediate future, the inhaled allergen challenge model is likely to be extensively employed in studying the pathogenesis of asthma inflammation, and analysis of the relationship with bronchoconstriction and AHR. Allergen challenge is also likely to remain at the forefront for assessment of new therapeutics in initial clinical trials, and to define novel and relevant targets for new drugs. However, with our increasing understanding of the allergen challenge model is the recognition that this response involves complex interplay between leucocytes, numerous tissue cell types, cytokines, chemokines and inflammatory mediators.

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