

EDITORIAL

Chronic allergen challenge as an experimental model: necessary, significant or useful?

Is there a necessity for the development of improved models for asthma? Models are used to determine pathophysiological mechanisms and to assess efficacy of therapeutic agents in the treatment of asthma. Standard methodologies include acute allergen challenge, chronic administration of low doses of allergen and natural allergen exposure. However, perhaps the most appropriate model in asthma research methodology should be the 'wild-type' subject with chronic clinical disease.

Asthma is characterized by Th2-type inflammation with a predominance of the cytokines, interleukin (IL)-4, 5 and 13 [1]. Pathologically, there is infiltration of CD4+ lymphocytes and eosinophils, epithelial erosions, basement membrane thickening and mucus gland and smooth muscle hyperplasia [2]. One of the pathophysiological consequences of these changes is airway hyperreactivity (AHR). Several animal models have shown that repeated allergen exposure can cause AHR, bronchoalveolar lavage (BAL) eosinophilia and goblet cell, submucosal gland and smooth muscle hyperplasia: pathological changes identical to those in chronic asthma in humans [3,4].

Acute allergen challenge in humans involves inhalation of an allergen protein via a nebulizer at increasing doses until there is a drop of at least 15% in FEV₁ [5,6]. A positive skin prick test, and a RAST test determine the type of allergen used for inhalation [7]. Approximately 50% of patients with atopic asthma will develop both an early asthmatic reaction (EAR) between 30 min and 2 h, and a late asthmatic reaction (LAR) 4–10 h following inhalation of allergen [8]. The EAR is thought to be a reflection of mast cell degranulation and histamine release, while the LAR reflects multicellular events more analogous to the inflammatory response seen in clinical asthma. However, there are problems with specificity and sensitivity using this model. In 1991, Sedgwick and colleagues showed that it was possible to induce a LAR in atopic subjects who did not have asthma [9]. In addition, there are several examples of pharmaceutical trials employing the acute allergen challenge model in which there were no effects on AHR or LAR, despite clear reductions in inflammatory cells, suggesting a dissociation between cellular infiltrate and physiological consequences [10]. Muller *et al.* suggested that acute allergen challenge bears no relation to natural allergen exposure as there is no chronicity [11]. From a safety perspective, acute allergen challenge causes significant bronchoconstriction with a propensity to anaphylactic reaction [12]. In terms

of repeatability, Gauvreau and colleagues have shown that both sputum eosinophils and the LAR take at least 3 weeks to return to normal following acute challenge [13]. A further consideration in methodology of allergen challenge is circadian rhythm. Mohiuddin and Martin found that in the evening more subjects had a more severe late phase than those receiving challenge in the morning [14].

More recently a chronic allergen challenge model has been utilized in order to emulate the natural history of allergic asthma. Proponents of this method suggest that chronic administration of allergen at low doses creates a cellular milieu similar to that seen in clinical disease, and that this can then be used as an experimental method of determining pathological basis of disease and the response to new therapies.

In 1988, Ihre and colleagues showed that isolated LARs could be provoked by inhalation of low doses of allergen [15]. In addition, the following year it was shown that in two subjects in whom single administration of high-dose allergen caused no increased AHR or LAR, repeated inhalation of high-dose allergen caused AHR [16]. This suggests that repeated exposure at short time intervals led to priming of AHR. These two studies initiated further clinical trials using chronic repeated low-dose allergen challenges.

In one study, subjects with mild allergic asthma had inhalations of either one or 10 biological units of allergen daily for 5–7 days and histamine reactivity was measured before and after challenges. There was an increase in AHR following this regimen without clinical deterioration of asthma [17]. Several other studies have been performed since then using a variety of protocols ranging from daily inhalations for 5 consecutive days [18] to inhalations three times weekly for 4 weeks [19]. Doses also range from 1/100th dose used for the acute allergen challenge [20], to a dose causing a 10% reduction in FEV₁ [21]. Investigators have also variously examined effects on asthma symptoms and β_2 -agonist usage [18,19] lung function and AHR [16,17,20,22], cytokine expression in peripheral blood [20], sputum eosinophils and IL-5 levels [18] and BAL eosinophils [22] and macrophages [21]. Three studies have tried to determine the amount of time required for these surrogate markers to return to normal. Sulakvelidze and colleagues suggest that sputum eosinophils and IL-5 levels along with AHR and β_2 -agonist usage return to normal within 3 days following daily administration of low-dose allergen for 5 days [18]. In contrast to this Arshad *et al.*

found that it took at least 2 weeks for AHR to return to baseline following three times weekly inhalations of allergen for 4 weeks [19]. This difference may be explained by the different methods of allergen administration. However, despite these variations in methodology, investigators have repeatedly shown that chronic administration of low doses of allergen cause an inflammatory cell infiltrate and Th2 cytokine pattern [20], along with physiological changes in AHR similar to that seen in chronic asthma.

As an extension of these findings, Gauvreau and colleagues [23] report a study in the current issue of this journal which examines the value of repeated low-dose allergen challenge, and the effect of inhaled steroids on AHR and sputum eosinophils following such challenges. The authors have used the model of repeated low-dose allergen inhalation over 4 days to provoke AHR and sputum eosinophilia in eight atopic asthmatic subjects. They analysed the effects of two doses of once daily inhaled budesonide, or placebo, on AHR and sputum eosinophils. Although the higher of the two doses of budesonide caused a reduction in both AHR and sputum eosinophil numbers, when the lower dose was administered, there was a dissociation between sputum eosinophils and AHR. This finding has relevance to the discussion regarding the role of eosinophils in AHR and in clinical asthma. The study also addresses the question of the role of allergen challenge as a model, and suggests that administration of chronic low-dose allergen by inhalation is more reflective of clinical allergic asthma.

A third model used in the assessment of inflammation and AHR in asthma is the natural allergen exposure model. This has the advantage of being more analogous to clinical asthma, but with the disadvantage of difficulty in standardization. Djukanovic and colleagues investigated the cellular changes which occur in the lungs of patients with grass pollen-sensitive asthma, and found increased numbers of CD4+ T cells and increased expression of the IL-2 receptor which is a marker of T-cell activation in BAL fluid during the grass pollen season [24]. In 1997, Hilterman *et al.* examined the effects of photochemical air pollution and allergen exposure on inflammatory markers in patients with asthma. They showed a significant correlation between ozone exposure and pollen, and eosinophils and ECP during a 3-month period [25]. In contrast Wanger and Dockhorn in 1999, used the more controlled model of 'live-cat-room' challenge to induce symptoms in patients known to be allergic to cat hair [26]. However, these studies are dogged by polysensitization, and the multifactorial nature of asthma deterioration make the results difficult to interpret.

From a pathophysiological perspective the chronic administration of low doses of inhaled allergen causes changes similar to that seen in clinical asthma. When comparing this method to acute allergen exposure, it may

be that chronic allergen challenge induces a more 'natural' type of reaction such as that seen in a grass-pollen season for example [24]. In addition, as the chronic model does not generally induce bronchoconstriction and the effects on AHR seem to be transient, this method could be considered to be safer than that using high doses of allergen. A further development of the chronic administration of allergen may be in its utility in determination of the effect of immunotherapy. For example, Weller *et al.* showed that the late skin response to repeated intradermal challenge was diminished following a course of immunotherapy [27].

There are clear issues with methodology, such as consideration of circadian rhythms, allergen dosing regimens and equipment used which require urgent attention and a standardized protocol for chronic allergen challenge should be developed. In spite of these methodological problems, models are required for the determination of pathophysiological processes of chronic lung inflammation. The chronic low-dose inhaled allergen challenge model affords a repeatable controlled method of achieving this and as such is necessary, significant and useful.

References

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