

CORRESPONDENCE

Proteinases and sensitization: house mites and enzyme detergents

The closely reasoned editorial by Robinson, Wan and Winton concerning the potential role of proteinases in house mite faeces in initiating sensitization to these and possibly other allergens [1], was of particular interest to me as the issue had already arisen in another context.

In the investigation of chest illness in the manufacture of the early 'biological' detergents which used proteinases [2], it was found that although direct proteolytic effects could occur, the predominant clinical problem was asthma caused by sensitization to the enzyme [3,4]. However, this had not precluded a possible proteolytic role in this sensitization. By facilitating access to the tissues of the respiratory tract directly, or indirectly such as through inactivation of defence mechanisms [5], proteolytic activity might have played a part in initiating sensitization, or even in triggering allergic responses in those sensitized. The fact that the other early reports of sensitization from enzymes were confined to proteases, such as trypsin [6] and papain [7], gave support to the possibility that proteolytic capacity was not necessarily coincidental.

However, the discovery that α -amylase could also cause sensitization and asthma, and at the clinical level appeared even more potent in this respect than papain, made it seem likely that enzymes were sensitizers due to being high molecular weight proteins, whether they were proteolytic or not [8]. Other nonproteolytic enzymes have since been shown to be sensitizers [9] and, in the case of amylase, it has been confirmed that minimal exposure can cause sensitization and symptoms [10]. Thus, it seems that although proteolytic capacity might play a facilitating role in causing sensitization to enzymes, it is not essential. In respect of those already sensitized, it had been found that positive skin prick tests and inhalation challenge tests could be obtained with enzyme heat treated to destroy proteolytic activity [11].

The editorial referred to the investigation by Piacentini *et al.* into the effects of house dust mite allergen avoidance on bronchial epithelial shedding in allergic asthmatic children [12]. Whatever the interpretation of the results, this was interesting and useful work. I noted that they had taken care to exclude variables such as sensitization to furred pets, and avoidance of the pollen season. However, although there was no indication that their results had been affected by an unconsidered factor, I submit that in any investigation of domestic allergens, let alone of the role of proteinases, account should be taken of the possible presence of proteinases and other enzymes in presoak and other washing products used in many homes. Although products are

often clearly marked as to whether or not they are 'biological' or contain enzymes, sometimes the enzyme content is only recorded in small print at the end of the ingredient list.

It is still possible that in spite of the introduction of granulated enzymes, consumer sensitization to these allergens can occur [2,13]. Apart from the clinical importance, failure to recognize this could cause anomalous research results. For example, there would not have been clear cut findings in the investigation of Piacentini *et al.* if one or more of the children was also sensitized to a proteolytic or nonproteolytic enzyme component of detergents, and the bedding in the residential house at altitude was washed in an enzyme-containing product.

Proteolytic activity was not the only potential adjuvant factor to be considered in the original detergent enzyme investigation. Nonenzyme detergent dust was present, although it had not previously been found to cause more than slight irritant effects. Case analyses, and the later findings with α -amylase and papain in factories which did not handle nonenzyme detergents, had reduced the likelihood of a significant sensitization role in a workplace context, but there will be residues from them, as from proteolytic and nonproteolytic enzymes, where these have been used in fabrics laundered with them. Originally, I had thought of the theoretical role of nonenzyme detergents as physically rendering respiratory tissues more accessible to noxious or allergenic substances, but it seems that detergents can act as adjuvants in potentiating immunological processes [14].

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Response by Robinson, Wan and Winton

Proteinases/peptidases and sensitization: house mites and enzyme detergents

We were delighted to hear that the interesting paper by Dr Piacentini and colleagues [1] and our accompanying editorial [2] had generated further interest in the allergenic nature of enzymes.

Dr Flindt raises an important point about the design of investigational studies and issues a timely reminder that exposure to allergenic materials from unconsidered sources may compromise the interpretation of data. We cannot comment on whether Dr Piacentini's study considered factors such as exposure to enzymes from 'biological' detergents or the adjuvant action of detergents, but inspection of the published data shows the results, whatever their explanation, to be persuasive and certainly meriting further investigation [1].

There is now a growing understanding of the efferent arm of allergic responses. However, the afferent components of these responses — that is the ways in which inhaled allergens are able to gain access to the pulmonary immune system — have been less extensively investigated and knowledge concerning them is rudimentary [3]. This paucity of knowledge reflects the difficulties in obtaining allergens in suitable quantity and unambiguous purity for use in such studies. The fundamental question is how foreign macromolecules cross the physical and biochemical barriers which exist to prevent the ingress of antigens. Intuitively, the interactions between potential allergens

and the airway lining must be of great significance in the process of allergic sensitization yet, despite this, little is known about the disposition and effects of allergens when they impact upon airway epithelium. The careful work which implicated industrial enzymes as the cause of occupational asthma in the manufacture of 'biological' detergents [4,5] was one factor which fuelled our interest in the notion that the enzymatic activity of house dust mite (HDM) allergens may contribute to the process of allergic sensitization by facilitating delivery of foreign proteins to antigen-presenting cells (APCs) [3].

Any attempt to explain the mechanisms which facilitate allergen presentation must take account of the fact that not all inhaled allergens are enzymes, and not all of the allergens that are enzymes have peptidase activity. This situation suggests that the interactions between allergens and the airway lining that are necessary to facilitate allergen presentation may be more diverse than the paradigm in which epithelial permeability is increased by an exogenous peptidase interacting with an (as yet) unidentified protein substrate within the airway epithelium [3]. The existence of multiple pathways of interaction between allergens and lung epithelium is exemplified by our recent discovery that focally restricted apoptosis represents a further component of the cellular response to HDM allergens [6] and also by the fact Der p 3 and Der p 9 initiate the release of cytokines from airway epithelial cells [7]. The enigma of how allergens interact with APCs is thus fertile ground for research directed towards understanding the basis of allergic disease.

However, it may not be the case that every allergen has to have some biological effect upon the airway lining for sensitization to occur. The hypothesis that breakdown of the epithelial barrier by peptidase allergens is a key event in allergic sensitization creates two corollaries which are capable of explaining the occurrence of allergy to multiple allergens in a single individual. Firstly, allergens with peptidase activity might facilitate delivery of nonproteolytic allergens which are encountered at the same time. Secondly, any environmental factor which perturbs the structure of the airway epithelium might facilitate the transepithelial delivery of allergenic proteins. Such environmental factors might include, for example, the presence of concurrent or recent infection since microbial products are known to possess potent activities on mucosal surfaces [8,9]. Many allergens exist within complex matrices that are comprised of components which have unknown or poorly characterized biological effects. In the case of many allergens encountered in the natural environment, these other components may be other allergens; in the case of materials encountered occupationally, they may be product contaminants or other materials handled within the factory. Under these circumstances sensitization should be considered as the net effect of all of these interactions and where the outcome may be

allergy to single or multiple components of the allergen load in the environment. We thus fully endorse the possibility suggested by Dr Flindt that adjuvant factors may be important in allergic sensitization.

Dr Flindt reminds us that sensitization to α -amylase, and other nonproteolytic enzymes, can occur readily in an occupational setting [10,11]. It is noteworthy that group 4 allergens of HDM also have amylase activity [12]. To the best of our knowledge the effects of amylase have not been investigated specifically in the context of the biophysical events which underlie sensitization. However, for the reasons described above, it should be remembered that the presence of allergic sensitivity to amylase does not necessarily mean that exposure to amylase alone was responsible for facilitating sensitization to this enzyme. Evidence exists to suggest that amylase activity is (like some HDM allergens) able to induce cytokine release (IL-1, IL-6, TNF α) [13], creating the possibility that the immunogenicity of amylases may be linked to their having a cascade of effects on dendritic cell dynamics and the formation of a TH2 phenotype from naïve CD4T cells [14]. Given the potency of amylases as allergens, it would therefore be of considerable interest to explore the epithelial disposition and effects of these enzymes as this information may yield some valuable clues to the events which facilitate delivery of allergens to APC.

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Response by Piacentini and Boner

Airway epithelial damage in asthma: is it all due to allergy?

In his letter Flindt [1] suggests that proteinases contained in biological detergents can act as adjuvants in potentiating immunological processes.

The issue of a potential role of proteinases in the mechanisms of allergic sensitization and expression of disease appears to be particularly relevant not only in consideration of the risks connected to biological detergents but also because it has been demonstrated that house dust mite allergen retains proteinase activity.

In a recent issue of *Clinical and Experimental Allergy* we have proposed that proteolytic-mediated damage to adhesive structures could concur, at least in part, to cylindrical cell detachment from airway mucosa and therefore cause epithelial disruption in asthma [2]. This hypothesis has been discussed in an interesting editorial by Robinson, Wan and Winton in the same issue [3] in which the idea that proteinase activity of Der p 1 is of greater significance to the process of allergic sensitization than the frank injury in established disease was stressed. We agree with Robinson *et al.* [3] that our original article did not provide direct evidence of a causally linked reduction in epithelial shedding after mite proteinase avoidance. However, it seems difficult to also exclude this possibility. In fact, most of the criticisms from Robinson *et al.* [3] were not based on convincing evidence contrasting with our results. In particular, a wide section of the editorial has been dedicated to

comment on the vision of the role of eosinophils in our original study. The small number of eosinophils recovered in the induced sputum has been described as a weak criterion to refute the importance of eosinophil involvement in our study, in consideration of a supposed, yet unlikely, relationship between the number of cells resident in the airway submucosa and those recoverable in the sputum. However, a good agreement between the number of eosinophils in sputum, bronchial lavage and biopsies has been previously demonstrated [4]. Furthermore, Robinson *et al.* [3] propose that the activation state may be more relevant than the absolute number of the infiltrating cells. In our study the mean percentage of eosinophils in the sputum was about 2% when the children were exposed to mite antigen and 1.5% when they were not exposed. It is difficult to accept that such small changes in the numbers of eosinophils, even in the case of hyperactivated cells, could be responsible for a significant change in the architecture of the epithelium. Similarly, it is difficult to accept that proteolytic enzymes contained in such a small number of eosinophils could be of practical relevance in accounting for noncytotoxic loss of cells from the airway. In fact, despite our agreement with the authors of the editorial that very little detail is known about the role of proteolytic enzymes from eosinophils in asthma, it is also in this case difficult to accept that the enzymatic content of a very low number of eosinophils in the airway could be responsible for an evident cell loss. If this were the case, a complete disruption of airway architecture would be expected when, as very frequently happens in asthmatic patients, the eosinophil percentage is significantly higher.

Obviously, the 'conventional' view that a diminution in the frequency and intensity of IgE-dependent reactions has played a pivotal role in the outcome of our study is fully accepted by us. However, in agreement with Robinson *et al.* [3,5], we are still tempted to consider that prolonged exposure to high concentrations of Der p 1 may result in frank injury of airway lining and to perpetuate IgE-mediated inflammatory events. In our opinion, from the data presently available in the literature it is not possible to form conclusions about the relevance of a direct effect of mite proteinases on airway epithelial shedding. This issue will require further studies evaluating the eventual relationship between epithelial shedding and level of exposure to mite allergens in nonallergic subjects.

We fully agree with the authors of the editorial [3] about the interest of the enzymatic activity of mite antigen in the process of allergic sensitization. The proteolytic activity could be one of the factors influencing the capability of mite

antigen to generate an allergic response and the efficacy of this enzymatic activity could account for the particularly relevant percentage of patients who present sensitization to mite in early childhood.

The results of our study do not allow us to draw any firm conclusions with regard to Dr Flindt's hypothesis. We do not know what kind of detergent was used in the homes of the children. We know that the laundry in the residential house is in the basement, far away from the room where the children used to sleep. We also know from the literature [6] that enzyme-induced allergy is generally limited to factory workers exposed to dust for long periods but we cannot exclude some effect of the residues in the laundered fabrics [6].

If this effect could be proven, the increased use in detergent should be explored as a possible additional cause of the increase in atopic disease, which has been observed in recent decades.

Taken together, the comments by Robinson *et al.* [3] and Flindt [1] suggest the opportunity to further evaluate the possible significance of protease activity in allergen presentation and sensitization.

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