

Clinical & Experimental Allergy

What makes an allergen *more* than an allergen?

This editorial discusses the findings of the paper in this issue by M. Milovanovic et al. [16] pp. 671–8.

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The speculations of what makes an allergen an allergen are almost as old as the field of allergology. Current knowledge would suggest that there is more than one single factor leading to the 'successful' induction of an IgE response. Thus, the usual suspects of inhalation allergy: pollen, fungal spores, house dust mite (HDM) droppings and pet dander, all seem to have some common features but there are also differences to be found. It goes without saying that many host-specific factors including genetic disposition, age and maturity, concomitant or previous diseases [1], or even the overall microbiota in the intestines, on the skin and at mucosal surfaces play an important role, but these will not be discussed further here.

A key feature of allergy development is the initial exposure of mucous membranes to certain biological sources, followed by an extraction and uptake by proteinaceous antigens. There is likely to exist a minimal combination of dose and administration regimen under which sensitization does not occur, but in general the immune system responds with IgE production to extremely small dosages of inhaled antigen sources, with estimates of pollen major allergen dosages going down to less than a microgram per season [2].

It is likely that we are exposed to many aerobiological sources but some are clearly more efficient in inducing IgE responses than others. Physical characteristics such as a small particle size, i.e. the ability to remain airborne for a long time, and to avoid sequestering and thus penetrating deep into the airways, seem to be important features for an allergen particle. The aerobiology and dynamics of pollen have been studied in most detail, and while the consensus suggests a minor role for pollen fragments,

i.e. the *paucimicronic* (2–10 µm size) and *submicronic* (< 1 µm) particles, these are not completely free from suspicion [3]. Both traditional indoor allergen particles, such as pet dander and HDM, and potential new sources created by nanotechnology need much more scientific attention to elucidate the interplay between the physical form and size, aerodynamics and the immune system. In this respect, it may be worth noticing that in a parenteral immunization model, the same antigen preparation – but with a different particle size – differentially stimulated a T-helper (Th)1 or a Th2 response [4].

Recently, natural adjuvants, substances that will increase the immunogenicity (allergenicity) of the proteins with which they are co-administered, have been identified. Pollen seem to contain lipids, the so-called phytoprostanes with some resemblance to prostaglandins, that increase the allergic response by inhibiting IL-12 production of dendritic cells and thus skewing the T cells towards a Th2 profile [5]. Allergic sensitization is one the Janus heads of the Th2 and IgE immune response, with helminthic infections being the other; interesting lipids have been isolated from parasites, such as an ascaris-derived phosphatidylserine fraction, which may also induce Th2 responses, and lyso-phosphatidylserine from *Schistosoma mansoni*, which tends to induce regulatory T cells [6], reviewed in [7]. Anthropogenic adjuvants such as diesel exhaust particles that may adsorb to pollen have been demonstrated to have a strong IgE-inducing effect [8] in human experimental studies and regarding occupational sensitization tobacco smoke has also been incriminated as an adjuvant [9].

When the allergenic particle settles on a mucosa, high extractability of the protein, i.e. fast solubility at neutral pH, seems to be a prominent feature of the most common inhalation allergens [10]. From the mucosal surface, small particles or dissolved allergens may penetrate across the epithelial barrier, either by active uptake by dendrites of dendritic cells or by passive diffusion, perhaps aided by a reduced integrity of the mucus layer and the underlying epithelium. This is the time when the allergenic molecule actually encounters the immune system, and the

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microenvironmental context determines how it is recognized and responded to. But some proteins are likely not to be recognized at all. When cats and dog live with human beings under various housing conditions, the animal dander, some of which is derived from epithelium (e.g. the allergens Can f 1 and Fel d 3), is released into the indoor environment. It is conceivable that human beings also release dander to the surroundings, but this rarely causes any problems. Unless you are a dog that recognizes his master's proteins. The human keratinocyte-derived protein cystatin A has actually been identified as a putative allergen for atopic dogs [11], and provides a good example of the decisiveness of the immune system in selecting what to react to. It is also likely that non-self proteins in the environment are left unnoticed or recognized with anergy by the immune system.

A protease, a protease, a most unusual protease, etc. (with acknowledgments to W.S. Gilbert and Martin D. Chapman et al.)

That important inhalant allergens, such as the HDM allergens Der p 1, p 3, p 6 and p 9 have strong enzymatic activity, *in casu* cysteine or serine proteases has raised considerable interest. It was found that Der p 1 selectively cleaves CD23 from activated B cells and it was speculated that this would regulate the IgE synthesis [12]. Later, other activities were discovered such as cleavage of CD25, a part of the IL-2 receptor, cleavage of protease-activated receptor 2 and the disruption of the lung epithelium due to cleavage of proteins involved in tight junctions, occludin and claudin-1 (reviewed in [13]). This falls well in line with findings that a cysteine protease from the parasite *Leishmania mexicana* induces a strong Th2 response in mice, an effect that like those of Der p 1, could be abolished by specific protease inhibitors [14]. On the other hand, as pointed out by Chapman et al. [13], there are several allergen systems such as cockroach and cat where proteases have not been found among the important allergens, and so the protease activity may not be linked to the allergenicity. It may also be mentioned that industrially produced proteases (*subtilisins*) have long been known to cause occupational allergies, but this is likely to reflect the fact that proteases were developed first and in large quantities, and also industrially produced amylases and lipases may induce occupational allergies [15].

The innate immune system

In this volume of Clinical and Experimental Allergy, Milovanovic et al. [16] investigated commercial allergen extracts of dog, grass pollen and HDM for their ability to stimulate B-lymphocytes and surprisingly it was found that all extracts induced IL-10 production from the cells. Interestingly, the IL-10-producing B cells were preferen-

tially CD27⁺, i.e. memory B cells, but the high number of cells would suggest that this cannot be an allergen-specific effect and this was confirmed by an additional IL-10 production after allergen stimulation, even when B-cell receptors were polyclonally stimulated. The IL-10 produced was sufficient to inhibit IgE production, but other activities of IL-10 could also be envisaged. For instance, the B cells may interact with the T cells in an antigen-specific manner, where the B cell acts as the antigen-presenting cell. In such a situation, the result of IL-10 production may be not only an autocrine effect on the B cell but also a dampening of the T cell response.

IL-10-producing B cells have been described as regulatory B cells in animal models of autoimmune diseases, such as experimental autoimmune encephalomyelitis, inflammatory bowel disease, type 1 diabetes and collagen-induced arthritis (reviewed in [17]). When the total B cell population was eliminated in the animal models, the disease severity surprisingly increased. A small population of CD1d^{hi}CD5⁺ B cells seems to be responsible for the inhibitory actions mainly mediated via IL-10 production, and thus they have been named B10 regulatory B cells. Much less is known about a possible human counterpart of such cells, but there have been reports on development of psoriasis after treatment of patients with Rituximab, which will eliminate all human B cells. The mechanisms by which such cells may operate have not been elucidated but it may be via a non-antigen-specific action as was found by Milovanovic and colleagues, and this would suggest that B cells may also be operative in the innate part of the immune system. Further studies that dissect the active components in the allergen extracts and relate the IL-10 production to immune status [allergic, healthy and naïve (e.g. cord blood)] will be necessary before any conclusions can be drawn.

The paper by Milovanovic and colleagues falls in line with recent findings of allergens and allergen extracts stimulating the (innate) immune system by hitherto unknown mechanisms.

Using an epithelial cell model, Nathan and et al. [18] demonstrated that HDM extract stimulated the cells to release the chemokine CCL20 (formerly known as MIP3 α), which is a strong chemoattractant for immature dendritic cells. The results were confirmed in a primary cell system with human bronchial epithelial cells. In spite of the above-mentioned enzymes in HDM extracts, protease inhibitors did not abolish the response, and likewise lipopolysaccharide (LPS) contamination of the extract was ruled out. Instead, β -glucans in the extract were incriminated, and indeed the response was abolished by glucanases and partly inhibited by dectin-1 ligands [18]. Interestingly, the investigators found some of the same biological activity, albeit smaller, in a ragweed extract.

Finally, Trompette and et al. [19] have recently demonstrated that the recombinant form of HDM allergen

Der p 2 – one of the mite allergens without protease activity – has the ability to replace MD-2, which is a cofactor for LPS stimulation via Toll-like receptor 4 complex. In an asthma mouse model that is dependent on low-dose LPS, the recombinant Der p 2 actually induces a Th2 response.

It is not easy to reconcile the above findings into a unified theory. It is likely that the coming years will bring a vast amount of biological activities that can be ascribed to both the allergens themselves (although different from just being an IgE-crosslinking allergen) and to adjuvant factors in the biological sources (and likely also the pharmaceutically produced allergen extracts). The challenge for allergists and immunologists will be to fit these pieces well together with epidemiological findings into the big puzzle of why susceptible individuals become allergic to certain biological source materials containing allergens.

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