

The Relationships Between the Biochemical Properties of Allergens and Their Immunogenicity

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Introduction

In the past years, proofs have been accumulated to argue for a close relationship between structure and function of proteins (1). On the other hand, progress made on allergen characterization has shown that the majority of clinically important allergens are biochemically active. Among the biochemical activities associated with allergens, most are enzymes or regulatory proteins. Thus, the relatively new idea of a link between function and allergenicity of proteins (2–5) has emerged, raising again a currently debated question about what makes an antigen an allergen.

It is no longer believed that there exist proteins that are allergenic and others that are not. An antigen is an allergen for a particular subject when it provokes, on a first contact, specific IgE synthesis and, later, anaphylactic or inflammatory reactions. In a healthy subject, the same molecule will behave as a classical antigen without any allergic disorders. As it is well-established, the hypersensitivity reactions are genetically controlled, but they are also dependent on numerous factors that include: the allergen itself, allergen dose and exposure frequency, environmental factors, and, finally, on the subject's immunological background (6). One of the main findings in the mechanisms of immunity is that T-helper lymphocytes (Th) can be subdivided into two subsets according to the cytokines they produce (7). Among cytokines pro-

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duced by Th2 cells, Interleukin-4 (IL-4) promotes IgE synthesis by B cells, whereas Th1 cells produce IFN- γ and IL-2, which prevent hypersensitivity by playing a negative regulatory role in the development of Th2 cells (8). An imbalance between Th2 and Th1 has been reported among atopic patients, but little is known about the factors that determine these restricted profiles. Nevertheless, data continue to accumulate, suggesting that most allergens possess in addition to particular epitopes a biochemical function involved in the regulation of either allergen-nonspecific IgE (i.e., total IgE) or allergen-specific IgE production. It is indeed conceivable that such activities influence both immunogenicity and/or allergenicity. As reported hereafter, a number of observations are consistent with the proposal. In this review, we would like to support the hypothesis by collecting the available information on the relationship between biochemical properties of allergens and their immunogenicity.

Biochemical Activities of Allergens

Nowadays, approx 140 allergens have been characterized with known partial or complete amino acid sequences (4,9,10). Most of them have been obtained quite recently by molecular biological approaches while the remaining allergens have been purified by conventional chemical techniques. A number of other important allergens are also mentioned in the literature, but their sequences are as yet not known.

An examination of the data regarding the 140 currently characterized allergens reveals that half of them either possess a biochemical activity or are thought to be biochemically active, according to sequence homologies. Although the second half of these characterized allergens do not show significant homology with other known proteins, the possibility of an unknown biochemical activity cannot be excluded.

The list of allergens with known or supposed biochemical functions is reported in Table 1. We have arbitrarily divided them into two broad groups: the enzymes and the binding proteins.

The Enzymes

According to the Enzymes Commission (EC), enzymes are classified on the basis of the reactions they catalyze. There are six major classes: Oxidoreductases (EC 1), Transferases (EC 2), Hydrolases (EC 3), Lyases (EC 4), Isomerases (EC 5), and Ligases (EC 6).

Allergens could be found in most of these major subdivisions (Table 1). Nevertheless, the majority of them are hydrolytic enzymes, including Esterases (EC 3.1) and Proteases (EC 3.4).

Table 1
Biochemical activities associated with allergens

Enzymatic activity	Allergens	Origins	Refs.
Oxydoreductases			
Dehydrogenases	Can a 1	Fungi	44
	Cla h 3	Fungi	45
	Alt a 10	Fungi	45
Transferases			
Glutathione-S-transferases	Der p 8	Mite	46
Hydrolases			
Esterases			
Phospholipases	Api m 1	Honey bee	47
	Bom p 1	Bumble bee	48
	Dol m 1	White face hornet	49
	Ves m 1	Yellowjacket	50
	Ves v 1	Yellowjacket	9
Ribonucleases	Asp f 1	Fungi	51
Glycosidases			
Lysozymes	Der p 2	Mite	52
	Gal d 4	Chicken	53
Amylases	Der p 4	Mite	54
	Asp o 2	Mould	55
Hyaluronidases	Api m2	Honey bee	56
	Dol m 2	White face hornet	57
	Ves m 2	Yellowjacket	58
	Ves v 2	Yellowjacket	9
Polygalacturonases	Cry j 2	Japanese cedar	59
Proteases			
Cysteine proteinases	Der p 1	Mite	60
	Der f 1	Mite	61
	Der m 1	Mite	62
	Eur m 1	Mite	63
	Gly m 1	Soybean	64
	Papain	Papaya fruit	65
	Chymopapain	Papaya fruit	66
	Bromelain	Pineapple	67
	Ananain	Pineapple	68
Serine proteinases	Der p3	Mite	52
	Der f 3	Mite	69
	Der p 6	Mite	70
	Der f 6	Mite	71
	Bom p 4	Bee	48
	Der p 9	Mite	72
Aspartic proteinases	Bla g 2	Cockroach	73
Lyases			
Dehydratases	Cla h 6	Fungi	45

(continued)

Table 1 (continued)

Enzymatic activity	Allergens	Origins	Refs.
Calycins	Mus m 1	Mouse	74
	Feld 1	Cat	75
	Equ c 1	Horse	25
	B-lactoglobulin	Cow milk	76
	Rat n 1	Rat	77
	Rat n 2	Rat	77
	Bla g 4	Cockroach	78
	Asc s 1	Worm	79
	Bos d 2	Bovine dander	80
	Bos d 1	Hen's egg	28
Proteinase inhibitors	Gal d 2	Hen's egg	81
	Hor v 1	Barley	82
Actin-binding proteins			
Profilins	Bet v 2	Birch pollen	83
	Zea m 11	Maize pollen	84
	Phl p 11	Timothy grass pollen	85
Tropomyosins	Pen a 2	Brown shrimp	86
	Met e 1	Shrimp	87
	Der f?	Mite	88
Albumins	Sin a 1	Yellow mustard	89
	Bra j 1	Oriental mustard	90
	Can f 3	Dog	91
Cation-binding proteins	Bet v 3	Birch pollen	92
	Gad c 1	Codfish	93
	BDA11	Bovine dander	94
	Gal d 3	Hen's egg	95
	Chi t 1	Diptera	96

Esterases

The phospholipase activity associated with some allergens has been described to be important in affecting the synthesis of IgE. This has been elegantly shown by using the bee venom phospholipase A2 (Api m1), the main allergen involved in bee venom anaphylaxis (3). Using that phospholipase and a mutant variant lacking enzymatic function, the authors have shown that the active form, but not its catalytically inactive variant, was able to trigger, in an IgE-independent manner, rodent mast cells *de novo* production of IL-4. Furthermore, to assess the *in vivo* relevance of these results, they have shown that repeated injections of low doses active enzyme into mice induce the synthesis of high levels of Api m1-specific IgE, whereas immunization with the inactive form yields no detectable IgE response.

Recently, murine T-cell stimulation experiments with overlapping peptides have shown that the T-helper cell-activating epitopes are

located outside the active site of the PLA2 enzyme (11). This finding confirmed that the difference in immunogenicity observed between enzymatically active and inactive PLA2 could indeed be attributed to the enzymatic activity of the allergen and not to an alteration of T-helper cell epitope in the PLA2 active site.

Proteases

Allergens from various sources have now been recognized as expressing proteolytic activity (Table 1). Among the house dust mite allergens, at least four of them are proteinases (Groups 1, 3, 6, and 9). We shall restrict our review to the Group 1 mite allergens, which are certainly the most studied. These molecules are cysteine proteases that belong to the papain (C1) family of enzymes, together with other allergens of plant origin: Gly m1 (soybean), chymopapain (papaya fruit), actinidin (kiwi fruit), bromelain, and ananain (pineapple).

This family of proteases displays closely related amino acid sequences and uses an identical catalytic group, the thiolate imidazolium pair, for their activity. A comparative modeling study of Der p 1, the major allergen of the house dust mite *Dermatophagoides pteronyssinus*, has shown that this protein shares amazing structural and mechanistic features with papain and actinidin (12).

Several direct or indirect links have already been described between cysteine proteinase activity of allergen and their immunogenicity. They are reported hereafter and summarized in Fig. 1.

DISRUPTION OF COHESION IN THE EPITHELIAL BARRIER

Herbert et al. have reported three lines of evidence that suggest that Der p 1 is capable of causing the bronchial epithelium to become increasingly permeable to macromolecules as a consequence of proteolytic injury (13). First, they demonstrated the ability of Der p 1 to detach cultured epithelial cell monolayers from both artificial and natural biopolymer substrata. Second, their experiments established that Der p 1 was capable of increasing the permeability of bronchial mucosa to macromolecules such as albumin. Third, they showed that exposure of isolated bronchial segments to Der p 1 resulted in disruption of epithelial architecture that can be visualized by histological techniques. These authors speculated that events leading to epithelial injury could have a marked influence on the recognition of allergen by the antigen-presenting cells located below the mucosal layer, thus indirectly affecting immunogenicity.

INACTIVATION OF NATURAL PROTECTIVE MOLECULES

Der p 1 has also been shown to catalytically inactivate α_1 -antitrypsin, a serine-proteinase inhibitor that protects the human lower respiratory tract against damage by proteinases released during infla-

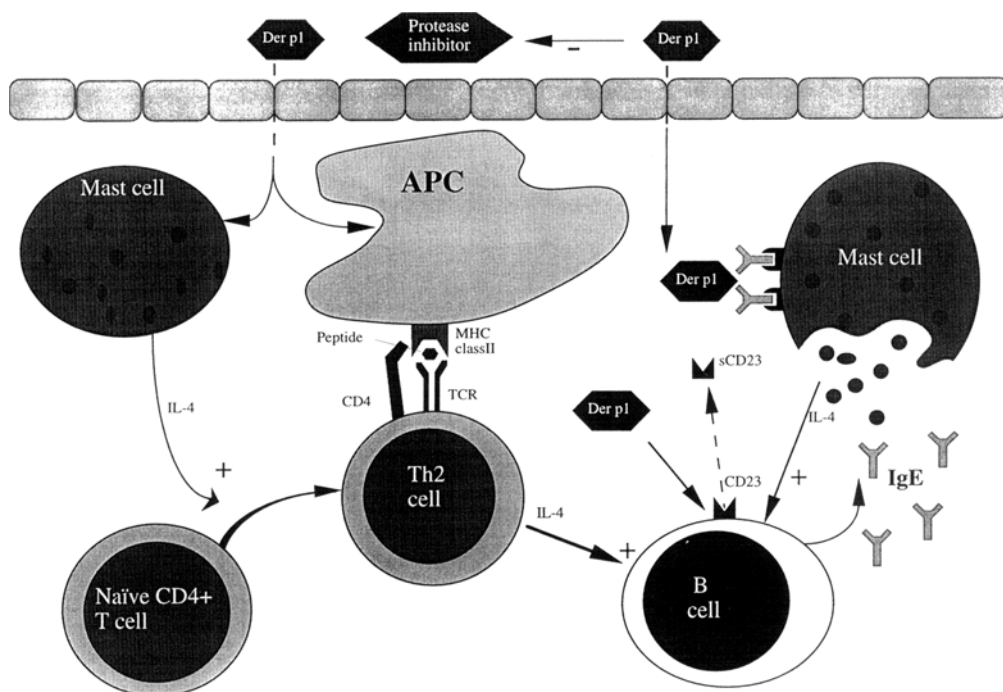


Fig. 1. Model depicting that the enzymatic activity of Der p 1 can take place in various steps and have different targets during the sensitization process. Der p 1 can cause: disruption of cohesion in the epithelial barrier, inactivation of natural protective molecules, mast-cell IgE-independent stimulation of IL-4 synthesis and secretion, and low-affinity IgE Fc receptor (CD23) cleavage from the surface of B cells. Such activities may influence both immunogenicity and allergenicity (*see text* for details). Also represented the mechanisms of IgE-mediated allergy. Allergens (Der p 1) enter via mucosal surfaces, and are taken up by local APCs, which process and present them to Th2 cells. Cognate recognition between the Th2 cell and B cell, and the release of IL-4, are required for B-cell proliferation and differentiation, which leads to the production of allergen-specific IgE. The IgE binds, via receptors, to mast cells, thus sensitizing them. When allergen subsequently reaches the sensitized mast cell, it crosslinks surface-bound IgE, causing the release of mediators that produce the clinical effects of allergy. Abbreviations: APC, antigen-presenting cell; IL, interleukin; MHC, major histocompatibility complex; TCR, T-cell receptor; sCD23, soluble CD 23.

mmation (14). Since α_1 -antitrypsin deficiency is associated with predisposition to developing respiratory disease (15), the inactivation of this major natural inhibitor of enzymes could exacerbate tissue damage and inflammation, thus accentuating asthma.

IG-E-INDEPENDENT STIMULATION OF MEDIATOR RELEASE FROM MAST CELLS

Machado et al. have reported that enzymatically active Der p 1 was able to induce, by a noncytotoxic mechanism, mast cell and basophil degranulation and to stimulate IL-4 synthesis and secretion in the

absence of antigen-specific IgE (16). In contrast, no histamine or IL-4 release was detected after challenge with enzymatically inactive Der p 1. These data are similar to those reported above for the bee venom phospholipase A2. They point to a linkage between the initial activation of cells of the innate immune system and subsequent adaptive immune responses.

DISRUPTION OF IgE NETWORK

Recently, two independent reports demonstrated that Der p 1 may upregulate IgE synthesis by virtue of its ability to cleave the low-affinity IgE Fc receptor (CD23) from the surface of cultured human B cells (17,18). The cleavage of this CD23 may deprive the B cell of an important mechanism to limit IgE synthesis and generate soluble fragments of CD23 that directly enhance IgE synthesis. Furthermore, the proteolytic effect of Der p 1 was specific for CD23, since no other B-cell markers tested were affected.

These data are consistent with the observations of Finkelman and Urban (19). They reported that repeated injections of mice with active papain stimulates an increase in serum IgE levels, compared to the response induced by the same dose of papain that has been 99% inactivated.

Similar results were obtained in our laboratory (paper in preparation), using a highly purified active papain (20) and its inactive counterpart. The papain lacking enzymatic function was generated from the active pure protease, using a specific chemical reactant without altering the protein three-dimensional structure. Thus, we concluded that the differences observed in immunogenicity between the two forms were effectively due to the enzymatic function of papain.

Finkelman and Urban further hypothesized that some proteolytic enzymes that are produced in large quantities by many helminth parasites are triggers of cytokines that induce a Th2 response. They reported that injection of papain into the footpad of mice induced within 1 h increases in the expression of messenger RNA for the cytokines, IL-4, IL-5, IL-9, but not those of IFN- γ or IL-2 (19).

In addition, studies in rats have shown that the cysteine proteinase of the nematode *Nippostrongylus brasiliensis* preferentially evokes an IgE/IgG₁ response (21), and active serine proteinases of *Schistosoma mansoni* increase the synthesis of IgE (22).

These data and those described above are all consistent with the possibility that protease activity of some antigens is involved in the induction of IgE antibody response.

The Binding Proteins

According to sequence homology, we have included in this group a large panel of allergens thought to display binding activity.

Calycins

The lipocalins and the fatty acid-binding proteins are two protein families that both function by binding small hydrophobic molecules. Their similarities of structure and sequence suggest that they form part of a larger structural superfamily that has been called the *calycins* to reflect the cup-shaped structures of its members (23). Several allergens have been shown to belong to this kind of binding proteins (Table 1). Among them, Equ c1, the major allergen of horse (*Equus caballus*), has been identified as a lipocalin (24); and recently, a model based on the complete amino acid sequence (25) confirmed that the allergen could adopt the same tertiary structure as that described for other lipocalins. This folding architecture is appropriate for binding and transport of small hydrophobic molecules such as retinoids and/or steroid hormones.

Interestingly, it has been reported that retinoids may have a regulatory effect on IgE synthesis (26). Retinoids are a group of natural and synthetic compounds that are structurally related to retinol (vitamin A) and retinoic acid. They function as important regulatory signaling molecules for cell growth (27). For example, it has been shown that retinoids are immunoregulators for lymphocyte proliferation and differentiation in cooperation with cytokines (26). More precisely, it has been reported that the presence of switch modulators such as IL-4 and IL-5, their concentration ratios, and concentrations of retinoids, are crucial factors in initiating and directing surface IgM-positive B cells switching to IgA and IgE/IgG1.

Thus it is feasible that some allergenic proteins like Equ c1 (horse), Bla g 4 (cockroach), Bos d 2 (bovine dander), and Asc s 1 (*Ascaris* worm), by virtue of their retinoid-binding properties, could influence IgE synthesis.

In this context, it is interesting to note that *Ascaris suum* extracts were used 20 yr ago without any clear explanation as an adjuvant for IgE response in mice.

Proteinase Inhibitors

The major occupational barley allergen (Hor v 1) and the hen's egg white allergens Gal d 1 and Gal d 2 show sequence homology with known proteinase inhibitors (*see* Table 1). Moreover, the last two also display an actual reactive site for inhibition of serine proteinases (28). The proteinase inhibitors are most likely involved in the regulation of all normal or pathological processes in which proteinases participate (29). Nevertheless, the link between proteolytic inhibition and immunogenicity remains to be investigated.

However, of particular interest are the observations that show that the efficiency of antigen uptake by macrophages is enhanced when antigens are combined with α_2 macroglobulin, an abundant cysteine and serine proteinase inhibitor in extracellular fluids (30,31). Mention should

also be made of α_1 -antiprotease, an inhibitor present in lung to prevent proteolytic damage by elastase; it has been reported to bind to Der p1 (18).

Finally, in an attempt to examine the role that helminth antigens themselves play in determining the so-called Th2 response in parasitic infestations, Garraud et al. identified two recombinant filarial proteins that induced IgG4 and IgE production in vitro, using PBMCs from parasite-infected patient in the absence of exogenous cytokines (32). These two proteins were shown to be analogous to cysteine proteinase and aspartyl proteinase inhibitors, respectively.

Discussion

The reasons why allergenic proteins and some parasitic antigens cause abundant IgE antibody synthesis are poorly understood. In the case of IgE-mediated allergy, even if the crucial role of genetic predisposition has been demonstrated, the possibility that certain antigens share common structural motifs that could influence class switching to IgE has been investigated extensively. Despite the considerable amount of information regarding the molecular structure of many allergens, no unifying feature has yet been discovered.

However, several factors influencing the polarization of T helper cell responses have now been well-identified.

The Nature of the Peptide Associated with MHC Molecules

The finding that signal transduction resulting from T-cell recognition of short peptides bound to MHC molecules is dependent on the nature of the peptides led Allen and coworkers to the concept of the "altered peptide ligand" (APL) (33). Using these analogs of the immunogenic peptides in which the TCR contact sites have been manipulated, they showed that TCR interaction with such APLs can result in dramatically different phenotypes of the T cells. For example, APLs can induce cytokine production without proliferation (34), anergy (35), TCR antagonism (36), or changes in the profile of cytokines produced (37).

Presence of Cytokines

The current concept is that Th2 and Th1 subsets differentiate from a common precursor (Th0) that has the capacity to secrete Th1 and Th2 type cytokines.

Numerous studies have emphasized the pivotal importance of the cytokine environment present during the priming of Th0 cells in determining their differentiation into Th1 or Th2 phenotypes. In particular, it has been shown that the presence of IL-4 is essential for Th0 cells to differentiate into Th2 cells (8).

Presence of Costimulatory Molecules

Activation of T cells by antigen-presenting cells (APC) requires not only engagement of the TCR/MHC/peptide complex, but also that of costimulatory molecules (38). Kuchroo et al. have shown that interaction of the costimulatory molecules CD80 (B7-1) or CD86 (B7-2) with their counterreceptors CD28 and CTLA4 on Th cells during antigen presentation leads to polarization of Th responses (39).

Interestingly, in the search for factors that determine Th2 profiles in allergic diseases, it has been pointed out that biochemical activity could be associated with half of the currently characterized allergens. Furthermore, we should not exclude the possibility that some still unknown functions will be ascribed to the remaining. Thus, the idea emerged of a close relationship between function and allergenicity of proteins.

One could argue that these observations are purely fortuitous and that descriptions of nonproteinic allergens like synthetic chemical reagents without evident biological action have been reported (40). Nevertheless, the above arguments for such a link are numerous, and it is likely that the biochemical actions of allergen can take place in various steps of the immune response, as reviewed herein for Der p 1.

However, once an antigen penetrates the initial host defenses, it is taken up and degraded by appropriate APCs, so that time and target for its biochemical activity, if any, are certainly limited. Consequently, possible targets are APCs themselves first.

In this context, it is noteworthy that mast cells have been reported to possess all the characteristics required to function as efficient antigen-presenting cells and promote clonal expansion of CD4⁺ T cells (41). Furthermore, the demonstration of IgE-independent mast cells and basophils activation by virtue of allergen enzymatic activity is of importance, since it possibly answers an intriguing question about the initial cellular source of IL-4, the cytokine involved in the priming of naive T cells for subsequent Th2 differentiation. Confirmations of these results could assign to mast cells and basophils a new important role at the onset of specific immunity against allergens.

Second, it is not inconceivable that other targets for allergen biochemical action are situated within the cytosol of APCs, since two recent studies (42,43) demonstrated that macrophages can take up bead-bound or soluble ovalbumin by phagocytosis or macropinocytosis and transfer the intact protein to the cytosol.

Up until now, it was not clear whether allergen-associated biochemical potencies like profilin and tropomyosin actin-binding, cation-binding, or even ribonuclease activities may influence immunogenicity and allergenicity. Although the link between biochemical properties of some molecules and their aptitude to trigger IgE responses is well-documented, there is now no available rule that allows to draw a general concept.

However, taking into account the other previously stated observations, it is obvious that non-antibody-mediated interactions between allergen and immunocompetent cells may be more important than has been previously recognized.

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