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The Biological Function of Allergens: Relevant for the Induction of Allergic Diseases?

Key Words

Allergens
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T cell epitopes
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CD23
CD25

Abstract

During the last 10 years an increasing number of allergens were identified and biochemically as well as immunologically characterized. More and more information on the primary structure of these molecules, revealed by recombinant technologies, became available. Furthermore for several allergens the biological functions could be elucidated. Most recently it was shown that beside IgE-binding capacity functional properties such as enzyme activity may contribute to the allergenicity and the induction of allergic inflammation. These findings could stimulate novel concepts in allergy prevention and therapy. This review summarizes recent data in this area.

Introduction

Type 1 allergic diseases are the result of a complex interaction between compounds called allergens, unspecific factors like infections and pollution as well as an obvious genetic predisposition on the mucosa and the human skin leading to an immunological hyperreactivity and an allergic inflammation. There is no doubt that allergens play an important role in this process and it is now well established that the recognition of allergens by immunoglobulin E (IgE) is the key step to the specific allergic response. The allergen-IgE complex is bound to the high affinity receptor of IgE on effector cells (i.e. mast cells) and if this occurs and at least two IgE receptors are cross-linked, mediator release is induced ultimately leading to the allergic inflammation [1]. When patients produce specific IgE antibodies they are sensitized and there is a certain immunological milieu promoting a sensitization characterized by a predominance of T helper 2 cells (Th2) mainly producing various cytokines such as IL-4, IL-5 and IL-13 [2]. Among these IL-4 is the most prominent of the cytokines activating IgE production.

That allergens are involved in this process of sensitization and of allergic responses is quite clear but it is still undecided whether defined structural features or functional properties of these molecules are responsible for their capacity to modulate the immune response in a certain direction (Th2 response). Here we want to review recent findings in this field and discuss the significance of this knowledge for the understanding of pathogenesis of allergic diseases.

Structural Features of Allergens

Since the dawn of recombinant technology a great number of allergens has been cloned and their primary structures were revealed, as reviewed elsewhere [3]. Many authors tried to define the epitopes on the allergens recognized by IgE antibodies and the specific T cell receptors [4–8]. While it is still difficult to determine the exact amino acid sequences of all the different and rather variable IgE epitopes, several T cell epitopes of different allergens were identified [9]. It became evident that none of the T cell epitopes on the allergens had a specific sequence especially designed to induce an allergic immune response. Furthermore up until now there has been no indication for a certain structural character of B cell or IgE epitopes found on all aller-

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gens. It was shown that cross-reactivity between related allergenic molecules such as tree pollen allergens is structurally based [10, 11] and it was also demonstrated that there are several IgE epitopes on one allergen and that these epitopes are mainly different in structure [7, 12]. In contrast to that the recently determined structure of the major group V allergens of grass pollen revealed a repetitive structural motif on the molecule probably bearing some repetitive IgE epitopes [13]. Though there exists no special pattern of IgE epitopes it became obvious that effective IgE binding to allergens highly depends on the three-dimensional (3-D) structural integrity of these molecules. A substantial decrease of IgE reactivity was demonstrated when the allergen Bet v 1 was split into two and thus lost its 3-D structure [14]. The same was true for Der f 2, a major allergen of mites, which was mutated at only two amino acids leading to the dissolution of a disulfide bond and complete loss of IgE-binding activity [15]. Taken together 3-D structural integrity by now is obviously the only important structural property of allergens for the induction of an effective allergic response. This property of molecular stability may not only be decreased but can be enhanced by limited proteolytic fragmentation as was shown for the group V allergen of timothy grass [16]. This mechanism could play a pathogenetic role in the activation of allergens on the mucosa. When structural stability of allergens guarantees an allergic response it may as well secure other functional features such as enzymatic activity. Whether this kind of biological functions of allergens is involved in the induction of allergic inflammation is still debated.

Table 1. Majority of the clinically relevant allergens for which the biological functions are known

Function	Allergen source	Allergen group (example)	Ref. No.
Enzymes			
Cysteine protease	Mites	Group I (Der p1)	17
	Grass pollen	Group I (Phl p 1)	18
Other proteases	Bumblebee	Group IV (Bom p 4)	19
Trypsin	Mites	Group III (Der p 3)	20
Chymotrypsin	Mites	Group VI (Der p 6)	19
Amylase	Mites	Group IV (Der p 4)	19
Nucleases	Grass pollen	Group V (Phl p 5)	21
	Tree pollen	Group I (Bet v 1)	22
PA2	Insects	Group I (Api m 1)	19
Hyaluronidase	Insects	Group II (Api m 2)	19
Lysozyme	Chicken	Group IV (Gal d 4)	19
Enzyme inhibitors			
Protease inhibitor	Mustard seed	Group I (Ric c 1)	23
	Castor bean	Group I (Sin a 3)	23
Trypsin inhibitor	Grass pollen	Group XI (Lol p 11, by homology only)	24
	Barley	Group I (Hor v 1)	25
Structural Proteins			
Profilin	Tree pollen	Group II (Bet v 2)	26
	Grass pollen	Group XI (Phl p 11)	27
Tropomyosin	Shrimps	Group II (Pen a 2)	19
Melitin	Insects	Group IV (Api m 4)	19
Storage Proteins			
Vicilin	Peanuts	Group I (Ara h 1)	8
	Soybean	Group I (Gly m 1)	28
Albumin	Chicken		
	(Ovalbumin)	Group II (Gal d 2)	19
	(Conalbumin)	Group III (Gal d 3)	
	Mustard	Group I (Sin a 1)	
Transport Proteins			
Hemoglobin	Midges	Group I (Chi t 1)	19

Influence of Functional Properties of Allergens

Table 1 shows a major part of those allergens where biological functions are known. Allergens do not seem to have specific biological functions explaining their allergenicity, but it is demonstrated more and more that biological activity of allergens may promote the immunological conditions necessary for specific sensitization towards allergens. The best-analyzed example in this context is Der p 1, the major allergen of mites known to be a cysteine protease [17]. It was shown to augment its own permeability in the bronchial epithelium by proteolytic activity [29]. An *Aspergillus fumigatus* proteinase was also able to induce human epithelial cell detachment [30]; tree and grass pollen enzymes led to injury of murine airway epithelial cells [31]. These examples do not explain the fact of how and why patients are specifically sensitized towards these allergens but they describe that allergenic molecules with enzymatic activity can irritate the human mucosal surface and facilitate their own processing. They may also enhance airway inflammation by means other than their IgE-binding activity through protease-dependent mechanisms as was shown by culturing airway epithelial cells in the presence of mite and grass pollen extracts leading to cytokine production with cell detachment only when protease activity was present [32]. Additionally, if α_1-antitrypsin which normaly protects the lower respiratory tract against damage by proteinase is catalytically inactivated by Der p 1, airway inflammation is promoted [33]. Other antiproteases block the activity of Der p 1 in vitro, but whether the equilibrium between pro-

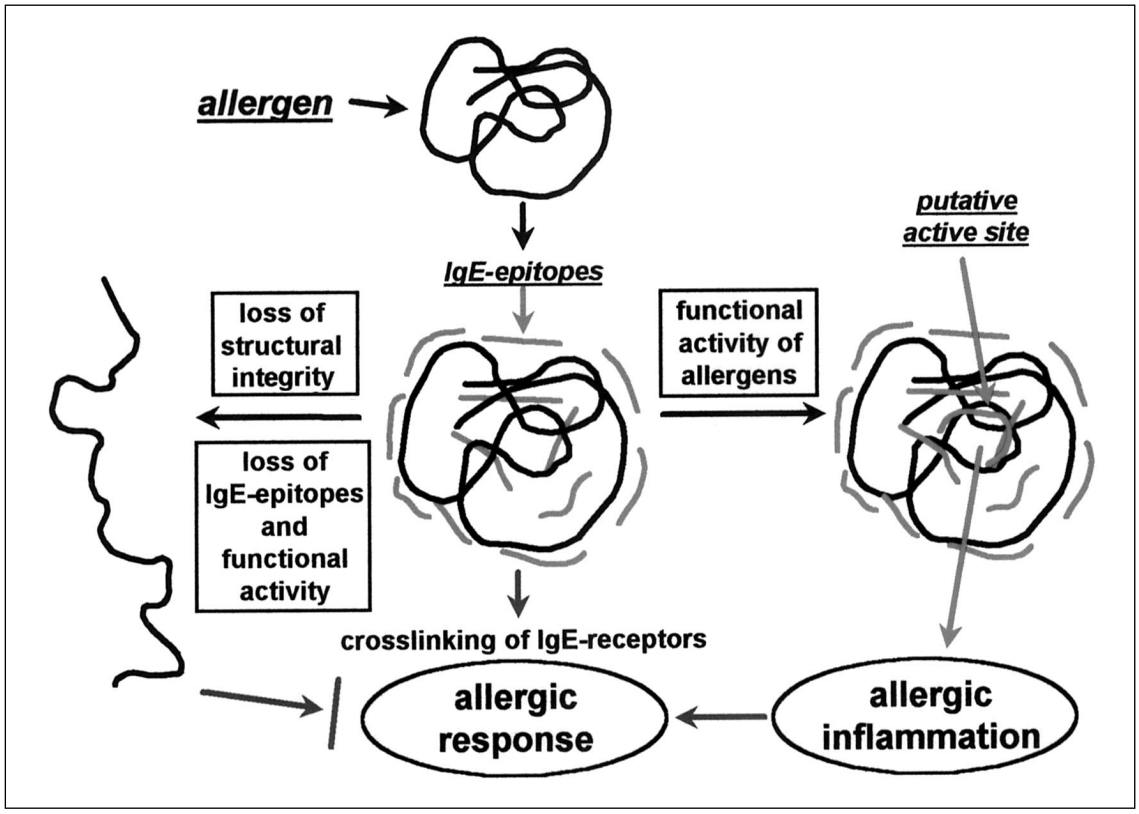

Fig. 1. Influence of structural integrity of an allergen on allergic response and allergic inflammation. Loss of structural integrity leads to loss of IgE-binding epitopes and loss of functional activity. Whereas presence of IgE-binding epitopes secures allergic response, functional activity may activate and influence allergic inflammation independent of IgE binding.

tease/protease inhibitor influences the process of sensitization of allergic patients is an open question. This balance seems to play an important but still unexplained role in the control of human IgE and IgG4 synthesis. Not only was evidence presented that α_1-antitrypsin specifically upregulates human B cell differentiation towards IgE production [34], but it was demonstrated that Der p 1 can cleave CD23, the low affinity receptor for IgE on B cells and monocytes, leading to an increase of soluble CD23 which is also known to enhance IgE production [35, 36]. The cleavage of CD23 by Der p 1 can be inhibited by α_1-antitrypsin [35]. Since tabacco smoke blocks the mucosal antiproteases, this mechanism may be the basis to explain the role of smoking as a risk factor for allergic asthma. Interestingly Der p 1 not only cleaves CD23 but also CD25, the receptor for IL-2, and if this happens the proliferation of Th1 cells is diminished and a bias of the immune response towards Th2 cells is created [37]. Taken together the balance of protease/antiprotease seems to be an important factor in the regulation of allergic disease, and an allergen disturbing this balance might promote a shift of the immune system towards an allergic response only by its enzymatic activity.

At the moment these observations on functional aspects of allergens are basically restricted to the activity of proteases. Most recently two major allergen groups were discovered to exhibit nuclease activity [21, 22]. For the timothy grass pollen allergen Phl p 5 we could show that the proteolytic degradation of the N-terminal part of the molecule led to a significant increase of the enzymatic activity of the remaining C-terminal fragment [38]. Exactly this activated portion of Phl p 5 was left as a stable degradation product when the holoallergen was incubated with human nasal secretion [16]. One might speculate that the encounter of Phl p 5 with the mucosa is associated with an activation of the functional properties of this allergen. There is no direct evidence on the effect of the active nuclease Phl p 5 on mucosal cells or other elements of the immune system. Interestingly an important molecule of allergic inflammation, the eosinophil cationic protein, was shown to be a ribonuclease with direct antiviral activity [39, 40]. The

eosinophil cationic protein is also involved in asthmatic airway inflammation [41] and toxic to tracheal epithelium [42]. Furthermore ECR has antiparasitic and cytotoxic properties and it was proposed for the use in anticancer therapy [43]. Thus it may be that nucleolytic activity of allergens can be involved in the activation of allergic inflammation but evidence on whether and how this could happen is still missing. Our experiments indicated that the allergenic response of Phl p 5 in terms of histamine releasability can be reduced by RNase inhibitors. But this effect seems to depend on the ability of the inhibitor to block IgE-binding epitopes and not on the loss of enzyme activity [44]. A mutant of Phl p 5b with no nucleolytic capacity was demonstrated to release as much histamine from basophils as the wild type (data not shown). Similar results have been found for phospholipase A2 (PLA) which as a nonactive mutant did not lose any histamine-releasing activity [45]. Interestingly, PLA showed induction of Th2 cytokines when correctly folded and enzymatically inactive, but when PLA was denaturated it preferentially stimulated Th1 cytokines [46]. The direction of the cytokine response was dependent on the way of antigen presentation either by B cells (correctly folded = Th2) or by monocytes (denaturated = Th1). Here it is only recognition of a stable allergen and not biological function which influences the balance of immune response towards allergenic molecules.

In summary, as long as the biological activity of an allergen is preserved, the 3-D structural integrity and the IgE-binding capacity of the molecule are kept stable (fig. 1). Under these conditions the biological activity of an allergen will mainly be involved in the process of unspecific induction of the inflammatory response or will augment the immunological shift towards an allergic reaction. The functional activity of allergens may therefore play a part among other factors such as infective agents, chemical substances, hormones, genetic variabilities and other adjuvants. This implicates that the best way to get rid of an allergy is still allergen avoidance or maybe the blockage or destruction of allergens before their encounter with the immune system. So-called 'IgE knockout mutants' of allergens with still conserved T cell reactivity might be promising for the improvement of allergen immunotherapy in the future [47]. To establish such strategies knowledge of specific structural features and functional activities of allergenic molecules will be very helpful.

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