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Recombinant Proteins and Peptides as Diagnostic and Therapeutic Reagents for Arthropod Allergies

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Abstract: Domestic arthropods are chief sources of potent allergens that trigger sensitization and stimulate IgE-mediated allergies. Diagnosis and immunotherapy of arthropod allergies rely on the use of natural allergen extracts which are associated with low specificity and efficacy, the risk of anaphylactic reactions, and the extended period of treatment. Most of the problems associated with natural allergen extracts for allergy diagnosis and immunotherapy can be circumvented with the use of recombinant allergens and peptides. Recombinant allergens are recently developed for microarray-based multi-allergen tests which provide component-resolved diagnosis (CRD) of the patient's sensitization profile. Moreover, recombinant protein technology and peptide chemistry have been used to construct isoallergens, allergen mutants, allergoids, T and B cell peptides, hypoallergens, and mimotopes with reduced allergenicity but enhanced immunogenicity for allergen-specific immunotherapy (SIT) and vaccination. The basics of recombinant arthropod allergen technology are in place providing a lucid future for the advancement of diagnosis and immunotherapy of arthropod allergies.

Keywords: Allergy, recombinant allergens, arthropod allergens, hypoallergens, diagnostic reagents, therapeutic reagents.

INTRODUCTION

The worldwide incidence of allergic diseases has risen dramatically over the last few decades, an increase that has been described as a "21st century epidemic" [1-2]. With about 25-30% of the population being afflicted, allergic disorders are among the most common causes of chronic illness [3]. Allergic diseases are major contributors to morbidity and mortality of the world population. In addition to being annoying, debilitating, or even fatal, allergic diseases cause substantial losses to public health and economy [4]. These maladies are characterized by over-reactivity of the immune system initiated by mechanisms caused by allergens which do not themselves cause disease in healthy individuals [5]. Immunoglobulin E (IgE) is the specific agent responsible for the observed clinical features of allergies such as airway hyper-responsiveness, goblet cell metaplasia, mucous overproduction, mucosal edema, and other inflammatory reactions [6]. Interaction between an allergen and allergen-specific IgE antibodies on mast cells and basophils induces the release of inflammatory mediators which manifest as asthma, allergic rhinitis, or atopic dermatitis symptoms.

Allergens from arthropods such as insects, house dust mites (HDMs), and crustaceans are potent immunogens that trigger sensitization and stimulate IgE-mediated allergies [7]. Arthropod allergens are diverse group of proteins that exhibit catalytic, regulatory, and structural functions [8]. The ability of arthropod allergens to elicit cellular and molecular immune response is similar to other types of allergens found in animal dander, pollens, fungal spores and foods. Humans come in contact with arthropod allergens through stinging

insects, skin contact or inhalation of insect and HDM secretions, or even ingestion of crustaceans and arthropod-infested foods.

The increasing prevalence of allergic diseases, together with their substantial burden on healthcare cost and economy and the associated load on the lifestyle of the suffering individual, have launched the pursuit on more efficient means of treatment besides the usual medication options of allergen avoidance, use of corticosteroids, anti-histamines, and sympathomimetics [9-10]. The advent of advanced technologies in proteomics and genomics has exploited the molecular pathogenesis of allergic diseases to remodel currently existing and design novel and to useful diagnostic and therapeutic options that helps not only in alleviating symptoms but provides long-term remedy [11].

Over the past two decades, the techniques of molecular cloning and recombinant protein expression had provided an avenue to develop pure and chemically well-defined allergens for allergen-specific immunotherapy and improve diagnostic test and profiling. The utilization of recombinant allergens in managing allergic disorders is highly favored over natural allergen extracts because of their specificity, long-term effect, and reduced side-effects [12]. This minireview presents current knowledge with respect to the use of recombinant proteins and peptides in improving diagnosis and treatment of allergy caused by arthropod allergens. Recombinant immunogens from insects, mites and crustaceans that are currently used in immunotherapy and diagnosis as well as the future challenges and drawbacks of these technologies are also presented.

ARTHROPOD ALLERGIES

The pervasive subsistence and the significant infestation of insects and mites in the household make them important

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sources of indoor allergens and risk factors for allergic disorders. Humans are continuously exposed, and thus sensitized, to most of these arthropod allergens causing different forms of arthropod allergies including allergic asthma, allergic rhinitis and atopic dermatitis. Insect-derived particles, like excreta, hairs, salivary secretions, and exoskeletons are common sources of aeroallergens in homes. House fly (*Musca domestica*), mosquitoes (*Aedes* sp., *Culex* sp., and *Anopheles* sp.), cat and dog fleas (*Ctenocephalides felis*), and cockroaches (*Blattella* sp., *Periplaneta* sp., *Blatta* sp. and *Supella* sp.) contribute mainly in this group of immunogens [7]. Nonbiting chironomid midges (*Chironomus thummi thummi*), confused flour beetle (*Tribolium confusum*), and rice weevils (*Sitophilus granarius*) frequently found in flour, cereals, and dried fruits are likewise shown to give positive skin reactions when natural and purified extracts of these insects were used. Some individuals develop severe allergic reactions to venoms released by stinging insects like apids (honey bees and bumble bees), vesmids (hornets, wasps, and yellow jackets), and formicids (fire and harvester ants). Identified allergens in these insects are usually enzymes involved in glycosaminoglycan degradation and substrate phosphorylation [7]. Several species of dust mites (*Dermatophagoides* sp., *Euroglyphus* sp., and *Blomia* sp.) are common in homes worldwide and therefore, are potent allergen sources [13].

The widespread human consumption of different crustaceans like shrimps (*Penaeus aztecus*, *Metapenaeus ensis*, *Penaeus indicus*), lobsters (*Panulirus homarus*, *Panulirus stimpsony*, *Homarus americanus*), crabs (*Charybdis feriatus*, *Scylla serrata*, *Callinectes sapidus*) and other seafoods are often accompanied by exposure and potential risk of allergic reactions. Hypersensitivity reaction to allergens from seafoods is one of the most common forms of food allergies [14]. Multiple sensitizations from varied shellfish sources have been reported and these often lead to the development of urticaria, angioedema, laryngospasm, asthma, and life-threatening anaphylaxis [15].

Allergy to different arthropods has been extensively demonstrated in various studies with the identification and quantification of specific IgEs. Identification of shrimp allergen-specific IgE antibodies has been demonstrated by prick skin test and the radio-allergosorbent test (RAST) [16-17]. Production of specific IgEs probably account for the observed immediate hypersensitivity reactions upon exposure or ingestion of shellfish in some allergic persons. Significantly high cross-reactivity between shrimp and crab allergens has been shown in a competitive IgE inhibition assay [18]. Allergy to crustaceans is not limited to consumption of seafood. Exposure to allergens in the fishing and seafood processing industry also results to production of allergen-specific IgEs [19].

Allergic reactions to cat fleas, *Ctenocephalides felis*, has been demonstrated by radio-allergosorbent test using spent and unspent culture media of adult fleas where 33.6% of patients tested showed positive allergen-specific IgEs [20]. In a separate study, 9% of the patients were RAST positive only against the cat flea [21]. Multiple allergens with molecular weights ranging from 34-60 kDa were identified as IgE-binding proteins from house flea extracts [20]. The al-

lergenic proteins from flea extracts potentially include debris from shed exoskeletons and body parts, fecal matter, molting enzymes, salivary secretions, and larval / pupal bodies.

The house fly *Musca domestica* is also a potential source of allergens that could sensitize and induce allergic reactions in atopic individuals. Western blots with housefly extracts revealed IgE-binding to allergen bands of 70, 50, and 16 kDa [22]. Exposure to housefly-derived aeroallergens including excreta, disintegrated bodies and shed hairs could induce respiratory allergy and conjunctivitis [23]. A common 37 kD allergen was shown in six different extracts from 6 different species of flies to exhibit IgE reactivity but species-specific allergens were likewise observed [24].

Hypersensitivity to mosquito bites is known to be an allergic reaction. The molecular and immunological mechanisms involved in mosquito bite allergy include IgE-, IgG- and T lymphocyte-mediated hypersensitivities [25]. Although mosquito bites often involve Type I immediate hypersensitivity, some cases of anaphylactic reactions were also reported. Systemic anaphylaxis from mosquito bites in 2 patients was associated with positive reactions to *Aedes aegypti* and *Culex pipiens* [26]. In both cases, immunotherapy using whole body mosquito extracts was performed. Components of the mosquito saliva are commonly implicated as an allergen source. A mosquito salivary allergen with a molecular weight of 37 kD binds IgE in 43% of patients tested with positive saliva-IgE to *A. aegypti* [27].

Cockroaches are important source of indoor allergens for patients with allergic airway diseases, urticaria and atopic dermatitis. Cockroach allergy is generally associated with more severe perennial symptoms and high levels of total IgE [28]. Exposure resulting from ingestion or through the inhalation of materials derived from cockroaches in airborne dust leads to sensitization. Cockroach allergens are derived from several sources, such as salivary secretions, fecal material, secretions, cast skins, debris, and dead bodies. The most common and clinically important cockroach species include *Blattella germanica*, *Periplaneta americana*, and *Blatta orientalis*. Allergen extracts derived from cockroaches elicit IgE reactivity of up to 50% among atopic individuals [29].

HDM allergy, like most forms of allergic diseases, is an IgE-mediated disorder. HDM have been well recognized to play an important role in the pathogenesis of allergic diseases. HDM-specific IgE antibody levels correlate significantly with the mite allergen contents found in the dusts in the homes of mite-allergic patients. *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis* are few of the important HDM species. Despite the discovery of at least 19 groups of HDM allergens, the major allergens belonging to groups 1 and 2 remain the most clinically important allergens with an IgE reactivity between 80-100% [13].

RECOMBINANT ARTHROPOD ALLERGENS

The technique of molecular cloning and recombinant protein expression had provided an avenue to develop pure and chemically well-defined allergens for both diagnosis and immunotherapy. With genetic engineering, highly specific and purified recombinant arthropod allergens have been gen-

erated which offers various advantages compared to crude arthropod allergen extracts. High-level expression of recombinant arthropod allergens is important to obtain sufficient quantities of protein for structural, functional and immunological studies [30]. The use of recombinant arthropod allergens offers the possibility to make quantitative diagnoses with standardized allergens [31]. In contrast to extract-based diagnosis, the use of recombinant allergens allows the precise measurement of specific IgE level to a particular allergen thus precisely identifying the disease-eliciting allergens for each patient [32]. With the use of a panel of recombinant allergens (with comparable epitope complexity as that of crude extracts) for diagnosis, it is possible to identify the complex reactivity profiles (allergogram) of allergic patients, a type of allergy diagnosis called "component-resolved diagnosis" (CRD) [33]. With the precise information about a patient's sensitization profile, a component-resolved immunotherapy with the use of modified recombinant allergens could be administered [32]. Recombinant and genetically engineered allergen molecules with reduced allergenic activity (hypoallergenic allergens) will provide alternative reagents for immunotherapy [33]. With recombinant allergen expression, innovative new strategies for allergen immunotherapy like peptide-based vaccines; nucleotide-conjugated vaccines that promote Th1 responses; and prophylactic allergen vaccines could be developed [34].

Over the past decade, a wide array of arthropod allergens have been cloned and expressed as recombinant proteins (Table 1). To date most of the recombinant arthropod allergens are expressed in the *Escherichia coli* system. Recombinant allergens expressed in *E. coli* as fusion proteins offer the advantages of ease of manipulation, short time of expression, ease in obtaining large quantities of recombinant proteins, and the availability of fast and efficient purification systems. Despite these advantages, however, there remain several disadvantages associated using this expression system. In some cases, production of GST-fusion protein remains unsatisfactory due to the inability of the GST to be cleaved off from the fusion protein, which may result to undesirable biological activity. Other disadvantages of recombinant allergens produced from *E. coli* include low yield for insoluble proteins and the possibility of endotoxin contamination cannot be completely eliminated thus can probably induced endotoxic or septic shock via platelet-activating factor (PAF) – mediated mechanism [146]. Bacterial endotoxins such as lipopolysaccharides (LPS) have been reported to act as natural adjuvants to allergic reactions by binding to surface receptors of immune cells such as macrophages and dendritic cells that secrete pro-inflammatory cytokines [1-2]. Endotoxins may be present in allergen vaccines and thus may bring about health risk upon administration of allergen immunotherapy [3]. The lack of post-translational modifications which are important to confer allergenicity and antigenicity in some allergens is likewise a major pitfall of the microbial system.

As an alternative to prokaryotic system, eukaryotic systems like *Saccharomyces cerevisiae*, *Aspergillus oryzae*, *Pichia pastoris*, insect cells, and mammalian cells have been used for recombinant arthropod allergens [31,35]. The use of eukaryotic systems for the expression of arthropod allergens offer several advantages including the production of endo-

toxin-free recombinant allergens thus reducing the risk of anaphylactic shock when used in humans; availability of a number of vectors for both cytoplasmic and secretory expression of proteins; production of recombinant allergens with posttranslational modifications; and high level of protein expression.

Production of recombinant arthropod allergens started with the group 1 allergens from the HDM species *Dermatophoides pteronyssinus*. Recombinant Der p 1 expressed in *E. coli* [36] and *Saccharomyces cerevisiae* [37] showed reduced IgE-binding compared to the natural allergen. Recombinant Der p 1 containing the proenzyme sequence with high IgE-binding comparable to the natural allergen has been successfully expressed in *Drosophila* cells [38], mammalian cells (CHO-K1 cells) [39] and *Pichia pastoris* [40]. Similarly, recombinant group 1 allergen Der f 1 from *Dermatophagoides farinae* has been generated in *E. coli* [41], *Aspergillus oryzae*, [42], *Pichia pastoris* [43], baculovirus expression system [44], and Sf9 cells [45]. On the other hand, group 1 allergen from the house dust mite species *Blomia tropicalis*, recombinant Blo t 1, was expressed as a histidine-fusion protein in *E. coli* [46] and as a secretory protein in *Pichia pastoris* [47]. Following the group 1 allergens in HDMs, 19 other groups from different species of HDMs have been cloned, expressed and characterized [48]. Recombinant allergens from other arthropods such as insects and crustaceans are likewise steadily increasing over the years as shown in Table 1.

RECOMBINANT ARTHROPOD ALLERGENS AS DIAGNOSTIC REAGENTS

Arthropod allergies, like any other allergic diseases, are characterized by elevated levels of allergen-specific serum IgE. Thus far, the detection of IgE levels, as a biomarker for allergic diseases is the most widely accepted diagnostic procedure mainly because it is a non-invasive method. Evaluation and diagnosis of atopy and allergic diseases are invaluable in the management and proper treatment of these conditions. Almost all diagnostic procedures for atopic disorders begin with a comprehensive inquiry on the medical history and physical examination of the patient. When these assessments suggest allergy, several specific measurements are performed including serum level of total IgE, determination of allergen-specific IgE antibody, i.e. skin-prick test (SPT) and radioallergosorbent test (RAST), nasal or bronchial provocation using specific allergens for the former or histamines, metacholine, and propranolol for the latter, and tests which measure peripheral blood eosinophil and serum eosinophil cation protein (ECP). Although widely used by medical practitioners, natural allergen extracts used in most of these diagnostic procedures contain a mixture of both allergenic and non-allergenic components. Thus, false positive and false negative results and non-specificity with respect to the exact allergen affects the reliability and sensitivity of the procedures [49].

With the continuously increasing number of allergen sequences being reported, more and more recombinant allergens can be generated. Through recombinant DNA technology, it is now possible to assemble a panel of recombinant allergens comprising of an epitope complexity comparable to

Table 1. Summary of Recombinant Arthropod Allergens

Arthropod	Allergen	Biologic Function	Mw (kd)	Type of Modification	Ref.
Cat flea (<i>Ctenocephalides felis</i>)	Cte f 2	Serine protease	31	Recombinant molecule	[72]
Yellow jacket (<i>Vespula vulgaris</i>)	Ves v 2	Unknown	43	Recombinant molecule	[73]
	Ves v 5	Unknown	23	Recombinant molecule	[74]
Yellow jacket (<i>Vespula germanica</i>)	Ves g 5	Unknown	23	Recombinant molecule	[75]
Paper Wasp (<i>Pollistes annularis</i>)	Pol a 5	Unknown	23	Recombinant molecule	[76]
Yellow jacket (<i>Vespula vulgaris</i>) and Paper Wasp (<i>Polistes annularis</i>)	Ag 5s (Ves v 5 & Pol a 5)	Venom proteins	23	Hybrid molecules	[77]
Mosquitoes (<i>Aedes aegypti</i>)	Aed a 1	Salivary proteins	68	Recombinant molecule	[78]
	Aed a 2	Salivary proteins	37	Recombinant molecule	[78]
White-face hornet (<i>Dolichovespula maculata</i>)	Dol m 5	Venom protein	23	Recombinant molecule	[79]
German cockroach (<i>Blatella germanica</i>)	Bla g 1	Unknown	43	Recombinant molecule	[80]
	Bla g 2	Binding protein	36	Recombinant molecule	[81]
	Bla g 4	Lipocalin (calyeyin)	18	Recombinant molecule	[82]
	Bla g 5	Glutathione-S-transferase	23	Recombinant molecule	[83]
American cockroach (<i>Periplaneta Americana</i>)	Per a 1	Unknown	20-25	Deletion mutant	[84]
	Per a 3	Arylphorin-like	72-78	Recombinant molecule	[85]
	Per a 7	Tropomyosin	33	Recombinant molecule	[86]
Nonbiting midges (<i>Chironomus thummi</i>)	Chi t -	Tropomysin	33	Recombinant molecule	[87]
Western bloodsucking conenose (<i>Triatoma protracta</i>)	Tria p 1	Lipocalins	19	Recombinant molecule	[88]
Red fire ant (<i>Solenopsis invicta</i>)	Sol i 3	Unknown	24	Recombinant molecule	[89]
Pine processionary caterpillar (<i>Thaumetopoea pityocampa</i>)	Tha p 1	IgE-binding	15	Recombinant molecule	[90]
Indian meal moth (<i>Plodia interpunctella</i>)	Plo i 1	Arginine kinase	40	Recombinant molecule	[91]
Honey bee (<i>Apis mellifera</i>)	Api m 1	Phospholipase A2 (PLA)	16-20	Recombinant molecule	[92]
	Api m 2	Hyaluronidase	44	Recombinant molecule	[93]
	Api m 3	Acid phosphatase	49	Recombinant molecule	[94]
	PLA- derived peptides	Phospholipase A2	16-20	T cell peptides	[95]
	Api m 1 mutant	Unknown	16-20	Recombinant hypoallergenic derivative	[96]
	Api m 1 + Api m 2	Unknown	58	Hybrid molecules- fusion protein	[97]

(Table 1) contd....

Arthropod	Allergen	Biologic Function	Mw (kd)	Type of Modification	Ref.
House dust mite (<i>Dermatophagoides pteronyssinus</i>)	Der p 1	Cysteine protease	25	Recombinant molecule, T cell peptide	[36, 98]
	Der p 2	Niemann-Pick C2 homologue	14	Site-directed mutant	[99]
	Der p 3	Trypsin	25	Protein	[100]
	Der p 4	Alpha-amylase	57	Recombinant molecule	[101, 102]
	Der p 5	IgE-binding	15	Recombinant molecule	[103]
	Der p 6	Chymotrypsin	25	Recombinant molecule	[104]
	Der p 7	Unknown	23.7	Recombinant molecule	[105]
	Der p 8	Glutathione-S-transferase	25.6	Recombinant molecule	[106]
	Der p 10	Tropomyosin	36	Recombinant molecule	[107]
	Der p 11	Paramyosin	103	Recombinant molecule	[108]
	Der p 14	Vitellogenin-apolipoprotein like	177	Recombinant molecule	[109]
House dust mite (<i>Dermatophagoides farinae</i>)	Der f 1	Cysteine protease	25	Recombinant molecule	[110]
	Der f 2	Niemann-Pick C2 homologue	96	Recombinant molecule, Site-directed mutant; allergen fragments	[111]
	Der f 3	Trypsin	25	Recombinant molecule	[112]
	Der f 6	Chymotrypsin	25	Recombinant molecule	[113]
	Der f 7	Unknown	25	Recombinant molecule	[114]
	Der f 10	Tropomyosin	37	Recombinant molecule	[115]
	Der f 11	Paramyosin	98	Recombinant molecule, allergen peptides	[116]
	Der f 14	Vitellogenin-apolipoprotein like	177	Recombinant molecule	[117]
	Der f 15	Chitinase	62.5	Recombinant molecule	[118]
	Der f 16	Gelsolin	55	Recombinant molecule	[119]
	Der f 17	Calcium binding EF Protein	30	Recombinant molecule	[120]
House dust mite (<i>Blomia tropicalis</i>)	Blo t 1	Cysteine protease	25	Recombinant molecule	[121]
	Blo t 3	Trypsin	25	Recombinant molecule	[122]
	Blo t 5	Unknown	14	Recombinant molecule	[123]
	Blo t 10	Tropomyosin	35	Recombinant molecule	[124]
	Blo t 11	Paramyosin	102	Recombinant molecule	[125]
	Blo t 12	Unknown	14	Recombinant molecule	[126]
	Blo t 13	Fatty acid-binding	14.8	Recombinant molecule	[127]
	Blo t 19	Antimicrobial peptide	7	Recombinant peptide	[128]

(Table 1) contd....

Arthropod	Allergen	Biologic Function	Mw (kd)	Type of Modification	Ref.
House dust mite (<i>Glycyphagus domesticus</i>)	Gly d 2	Secreted protein	13.7	Recombinant molecule	[129]
House dust mite (<i>Euroglyphus maynei</i>)	Eur m 1	Cysteine protease	25	Recombinant molecule	[130]
	Eur m 2	Niemann-Pick C2 homologue	14	Recombinant molecule	[130]
	Eur m 3	Trypsin	25	Recombinant molecule	[31]
	Eur m 4	Alpha-amylase	57	Recombinant molecule	[101]
	Eur m 14	Vitellogenin-apolipoprotein like	177	Recombinant molecule	[131]
Storage mite (<i>Lepidoglyphus destructor</i>)	Lep d 2 (formerly Lep d 1)	Niemann-Pick C2 homologue	14	Recombinant molecule	[132]
	Lep d 5	Unknown	15	Recombinant molecule	[133]
	Lep d 7	Unknown	25+	Recombinant molecule	[133]
	Lep d 10	Tropomyosin	35	Recombinant molecule	[134]
	Lep d 13	Unknown	14.6	Recombinant molecule	[133]
Storage mite (<i>Acarus siro</i>)	Aca s 13	Fatty acid-binding protein	15	Recombinant molecule	[135]
Mold mite (<i>Tyrophagus putrescentiae</i>)	Tyr p 2	Unknown	16	Recombinant molecule	[136]
	Alpha-tubulin (?)	Tubulin	50	Recombinant molecule	[137]
	Tyr p 13	Fatty-acid binding	14.6	Recombinant molecule	[138]
Sheep scab mite (<i>Psoroptes ovis</i>)	Pso o 1	Cysteine proteinase		Recombinant molecule	[139]
Brown shrimp (<i>Penaeus aztecus</i>)	Pen a 1	Tropomyosin	36	Recombinant molecule	[140]
	VR9-1 (mutant Pen a 1)	Tropomyosin	36	Recombinant molecule	[141]
Crab (<i>Charybdis feratus</i>)	Cha f 1	Tropomyosin	30.4	Recombinant molecule	[142]
Atlantic lobster (<i>Homarus americanus</i>)	Hom a 1	Tropomyosin	32.9	Recombinant molecule	[143]
Spiny lobster (<i>Panulirus stimpsoni</i>)	Pan s 1	Tropomyosin	34	Recombinant molecule	[144]
Greasyback Shrimp (<i>Metapenaeus ensis</i>)	Met e 1	Tropomyosin	31.7	Recombinant molecule	[145]

that found in crude allergen extracts. The availability of recombinant allergens opens new opportunities for a more refined allergy diagnosis thus providing opportunity to select optimal therapeutic strategies [32]. The use of recombinant mite allergens offers the possibility to make quantitative diagnoses with standardized allergens. Purified and well characterized recombinant allergens can be used to allow the determination of the individual's IgE reactivity profile [50]. This component-resolved diagnosis (CRD) can be performed by multi-allergen assay on allergen microarray chips which provides a comprehensive, highly specific, and precise diagnosis [51]. CRD can also tell whether a patient is co-

sensitized or cross-sensitized to a particular allergen [52]. Recombinant allergen-based immunoassays can also be used to monitor and detect new sensitizations in extract-based immunotherapy and this would allow the treatment to be stopped before the new sensitizations become clinically relevant. Recombinant marker allergens existing only in certain allergen source can be included in the allergen chips to detect one specific allergen or several allergens from which the patient is sensitized [32]. Furthermore, marker allergens can be utilized to quantitate immunological effects of SIT by determining IgG responses.

Recently, high-throughput and chip-based microarrayed recombinant allergens for diagnosis of allergic diseases had been proposed [49, 53]. This multi-allergen test offers the advantage of determining and monitoring the complex sensitization profiles of allergic patients with the use of small amounts of blood serum.

ENGINEERED ARTHROPOD ALLERGENS FOR IMMUNOTHERAPY

Type I allergies are primed, mediated, directed, and driven by protein molecules existing in the immune system and the environment of the afflicted individuals. Manipulating this biomolecular nature of allergic response is therefore a clever way of mitigating allergy symptoms. Environmental allergens and immune proteins, like cytokines and IgE itself, are engineered to increase immunogenicity, reactivity, and distinctiveness compared to the classic chemotherapy which generally lacks these advantages [54].

Allergen-specific immunotherapy (SIT) is a treatment modality which involves repeated administration of allergen extracts to patients with IgE-mediated allergy [55]. It has been shown to be highly effective in allergic rhinoconjunctivitis and allergic asthma. Traditional protein-based allergen immunotherapy using crude allergen extract however has limited scope of efficacy. Problems associated with SIT include difficulty in standardization, initiation of neoteric IgE specificities, severe side effects, and endotoxin contamination [56-57]. The issues concerning SIT can be solved by using pure and standardized recombinant allergen specifically tailored to avert IgE-crosslinking on immune cells and capable of eliciting T cell and IgG-mediated responses. It is essential therefore that the allergen structure must be elucidated first prior to application for therapy and diagnosis. The specific 3D conformation of the peptide or amino acid residues recognized by IgE must be addressed when designing recombinant allergen [58]. Likewise, it is important that the modified allergen carries structural motifs necessary for T cell recognition (T cell epitopes) and for initiation of IgG antibodies reactive with the natural allergen (blocking antibodies) [59].

The term 'hypoallergenic allergen derivatives' or hypoallergens describe these types of recombinant allergens since they possessed reduced allergenicity but preserved T cell epitopes and immunogenicity [60]. Hypoallergens bind reduced quantities of IgE and which may preferentially target antigen-presenting cells (APCs) that induce Th1 type responses [59]. Genetic engineering of allergens modifies the structure of the target protein to alter its function or properties. With the increasing number of allergen complementary DNA (cDNA) sequences available in database, one can precisely and effectively manipulate the gene to produce the recombinant hypoallergens. Genetic modifications of allergens comprise of site-directed and deletion mutations, fragmentation, oligomerization, and hybridization to produce chimeric or hybrid protein [59]. Recombinant Der p 2 with site-directed mutagenesis on residues involved in the formation of disulfide bonds resulted in reduced IgE binding [61]. A 10-, 40-, and 100-fold reduction of IgE binding were observed with the removal of disulfide bonds between C8-C119, C21-C27 and C73-C78, respectively. Reduced IgE-binding on mutagenized recombinant Der p 2 has been ob-

served in other studies [62]. Similarly, recombinant Der f 2 with disrupted disulfide bonds resulted in reduced IgE binding [63] but with retained T-cell epitopes essential for immunotherapy and ability to stimulate T-cell proliferation [64].

Following structural analysis and epitope mapping, a recombinant allergen can be constructed to specifically carry amino acid modifications or deletions on the sequence corresponding to IgE-binding epitopes [59]. American cockroach Per a 1 is an example of recombinant allergen prepared in this manner [65]. Allergen hybrids and shuffled molecules can also be designed to carry two or more allergens of interest, homologous protein antigens, or unrelated allergens from the same species [66]. Allergen chimeras are prepared by disintegrating genes of two or more related allergens followed by indiscriminate re-combination and amplification. The resulting chimeric allergens are proven to have increased immunogenicity and reduced allergenicity. Some of the examples of promising chimeras are the yellow jacket venom-paper wasp (Ver v 5-Pol a 5); birch, hazel and alder pollens; and timothy grass pollen allergens (Phl p 1, 2, 5, & 6) [59].

Mimotope technology, synthetic peptides, allergen-derived B cell peptides, and the use of recombinant DNA molecules correspondingly provide means of creating recombinant allergens preeminently for vaccination. Mimotopes are random peptides isolated from phage library using allergen-specific antibodies. These hapten-like molecules mime IgE binding sites of allergens and therefore capable of inhibiting antibodies against the IgE epitopes [52]. B cell epitopes can likewise be utilized to induce IgG antibody which lacks allergenic activity and act as blocking antibody [60, 67].

Chemical modifications of allergens produced by recombinant DNA technology are being done apart from genetic exploitation. Glutaraldehyde or paraformaldehyde can be used to initiate oligomerization of allergens to produce allergoids [68]. Maleic acid anhydride can be utilized to yield maleylated allergens with increased immunogenicity towards the Th1 type immune responses [66]. Protein or immunostimulatory oligodeoxynucleotides (ISS-ODN) moieties are added to recombinant allergen to mask allergenic determinants and thus, interfere with cross-linking of IgE on mast cells and basophils [59, 69]. ODN's also act as an adjuvant which can prime Th1 responses via TLR9-dependent NF- κ B signaling in a preclinical allergy- and cancer-specific immunotherapy [147].

The effectiveness of the methods for reducing allergenicity of allergens and production of recombinant proteins will depend not only on the success of modifications made but also on the steps carried out to come up with the purified product (Fig. 1). Various heterologous systems are currently used and evaluated for the production of recombinant allergens. Among which are microbial systems, yeast and mammalian cell cultures [70]. Plant bio-factories are also an attractive option because of versatility and added advantages [70-71]. After production of the allergen, it must be characterized structurally and immunologically, and its purity and safety determined, prior to preclinical and clinical evaluation (Fig. 1).

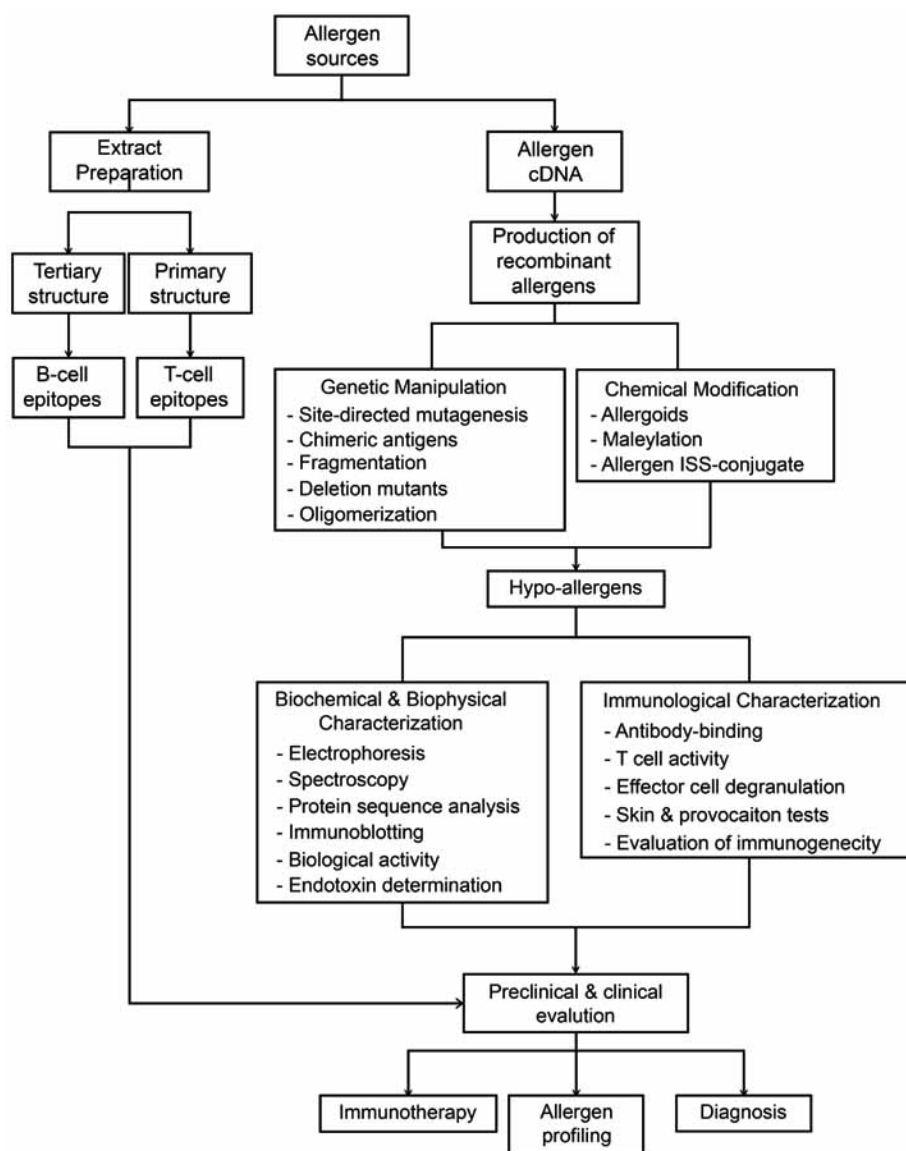


Figure 1. Development of diagnostic and therapeutic reagents for allergic diseases.

WHAT LIES AHEAD IN ARTHROPOD ALLERGY DIAGNOSIS AND IMMUNOTHERAPY?

The utilization of recombinant arthropod allergens for routine diagnosis and therapy requires that accurate production, manufacture, and quality control processes be developed. Allergen-specific therapy based on a component-resolved diagnosis appears feasible in the near future for several reasons. First, an increasing number of functional recombinant allergens is now available that allows the precise determination of the patients sensitization pattern (allergen profile/allergogram) for subsequent specific immunotherapy. The goal to mimic the profile IgE reactivity observed in allergen extracts with a panel of recombinant allergens is not far fetched. Second, because of extensive cross-reactivities, it may be possible to select a few recombinant model allergens that bind a high percentage of specific IgE for the usual immunotherapy regime. Finally, a number of antigen-specific therapy concepts have been developed re-

cently, which may improve current forms of immunotherapy. In the near future, production of recombinant allergens as defined molecular entities makes it feasible to develop prophylactic allergen vaccines, a much needed technology for the increasing prevalence of allergic diseases.

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