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Immunochemical and molecular methods for defining and measuring indoor allergens: in dust and air

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Much of our understanding of the role of indoor allergens in asthma derived initially from immunochemical studies on the identification and analysis of allergen molecules. The isolation of mite, cat, dog and cockroach allergens allowed the biochemical properties of these allergens to be defined and their allergenic importance to be established, using the purified allergens for skin testing and serum IgE antibody assays. The production of monoclonal antibodies (mAb) provided reliable and consistent markers for specific allergens and enabled improved purification and allergen detection tests to be developed (1). Since the application of molecular cloning techniques in the late 1980s, over 20 indoor allergens alone have been sequenced, in addition to a similar number of grass, weed, tree pollen and venom allergens. As a result, allergens constitute one of the most clearly defined groups of molecules in biomedical research (2). Recent studies have shown that IgE mediated sensitization to indoor allergens, principally dust mite, cat, dog and cockroach, is an important cause of asthma and an associated cause of perennial rhinitis and atopic dermatitis. This presentation focuses on recent progress and strategies for defining indoor allergens and on the applications of immunochemical and molecular technologies to the management of allergic disease.

Allergen cloning and sequencing strategies

A list of indoor allergens that fulfill the WHO/IUIS allergen nomenclature guidelines is shown in Table 1 (3). Many were originally defined on the basis of the biochemical and allergenic properties of the natural allergen e.g the Group 1 and Group 2 Dermatophagoides allergens and cat allergen, Fel d 1. In these cases, the availability of partial protein sequences facilitated the determination of the complete nucleotide sequence using either cloning or polymerase chain reaction (PCR) based techniques. Recently, however, the most common strategy employed is to screen cDNA expression libraries with human IgE antibodies and to sequence clones which show a high prevalence of IgE antibody binding (2, 3). This approach allows nucleotide and amino acid sequence to be rapidly obtained and is especially useful where there is little or no pre–existing data on the allergen. It is important to consider several approaches to allergen cloning and sequencing. For example, we have recently cloned two allergens directly from a B. germanica cDNA library by screening with IgE ab (Bla g 4, Bla g 5) (4). However, using the same approach, we were unable to isolate a Bla g 2 clone, and sequencing of this allergen required screening with high titer murine polyclonal antibody (5). N–terminal and internal protein sequences matched the nucleotide sequence of the full length cDNA clone, and provided further useful markers for confirming the Bla g 2 sequence. Cat allergen, Fel d 1, comprises two separate protein chains and provides another example of an allergen that could not be cloned from the cDNA – sequencing in this case being accomplished from the protein (> 95%) and by PCR (6). Ultimately, whatever sequencing strategy is adopted, the allergenic importance of the protein has to be established, by skin testing, serologic IgE ab assays, or histamine release. Ideally, the reactivity of natural and recombi-

Table 1. Structural and functional properties of indoor allergens

Source	Allergen[1]	MW	Function	Sequence[2]
House dust mite:				
Dermatophagoides spp.	Group 1	25 kd	Cysteine protease	cDNA
	Group 2	14 kd	Unknown	cDNA
	Group 3	~30 kd	Serine protease	Protein
	Der p 4	~60 kd	Amylase	Protein
	Der p 5	14 kd	Unknown	cDNA
	Der p 6	25 kd	Chymotrypsin	Protein
	Der p 7	22–28 kd	Unknown	cDNA
Euroglyphus maynei	Eur m 1	25 kd	Cysteine protease	PCR
Blomia tropicalis	Blo t 5	14 kd	Unknown	cDNA
Lepidoglyphus destructor	Lep d 1	14 kd	Unknown	None
Mammals:				
Felis domesticus	Fel d 1	36 kd	(uteroglobin)	PCR
Canis familiaris	Can f 1	25 kd	Unknown	cDNA
Mus musculus	Mus m 1	19 kd	Calycins, pheromone	cDNA
Rattus norvegicus	Rat n 1	19 kd	binding proteins	cDNA
Cockroach:				
Blattella germanica	Bla g 1	20–25 kd	Unknown	None
	Bla g 2	36 kd	Aspartic protease	cDNA
	Bla g 4	21 kd	Calycin	cDNA
	Bla g 5	36 kd	Glutathione transferase	cDNA
Periplaneta americana	Per a 1	20–25 kd	Unknown	None
	Per a 3	72–78 kd	Unknown	None
Fungi:				
Aspergillus fumigatus	Asp f 1	18 kd	Cytotoxin (mitogillin)	cDNA

[1] New nomenclature proposed by the WHO/IUIS sub-committee.
[2] Method given for full sequence determination, where available. However, protein sequences are incomplete, usually N-terminal or internal peptide sequences have been determined.

nant allergens should be quantitatively compared and this is essential if the recombinant allergen is to be used for diagnosis or treatment (2–7).

Homology searches of nucleotide and amino acid sequence databases, have allowed the biologic function of many allergens to be identified (Table 1), and where no significant homology exists the allergen function is usually unknown. Three dimensional allergen structures can also be predicted on the basis of known structures from protein families. The B. germanica allergen, Bla g 4, is a calycin (or lipocalin), and this family contains several proteins of known tertiary structure, including two major allergens, β-lactoglobulin and mouse and rat urinary proteins. Using homology based modelling techniques, it has been possible to construct two three dimensional models for Bla g 4, and these models can be used to design further structural experiments (4). Crystallographic studies of the mouse urinary allergen have shown that it is calycin which functions as a pheromone binding protein. There is good evidence that cockroach allergens are secreted or excreted and, by analogy, it is also possible that Bla g 4 has a pheromone binding function. Thus structural studies can provide important clues as to biologic function, and in turn, knowledge of the biology of allergens may lead to novel approaches to controlling their production.

Recombinant allergens/peptides and new forms of immunotherapy

The advent of recombinant allergens means that it is now possible to consider using these reagents for diagnosis and treatment of allergic disease. The rationale for using recombinant allergens for diagnosis is that expression systems can be used to produce one or two highly purified and homogeneous allergens, in essentially unlimited supply. For the allergist, diagnosis by skin testing or serum IgE ab assays would thus be based on one or two allergens, rather than a heterogeneous protein mixture. Clearly, this approach relies on the fact that allergens produced in bacteria, yeast or eukaryotic cells, have

Table 2. Indoor allergen measurement by mAb ELISA

Allergen	Capture[1] mAb	Second[2] antibody	Reference standards
Der p 1	5H8	4C1	NIBSC 82/518, UVA 93/03
Der f 1	6A8	4C1	CBER E1-Df, UVA 93/02
Group 2	1D8	7A1	CBER E1-Df or Dp; UVA 92/01, 92/02
Fel d 1	6F9	3E4	CBER Cat E3
Can f 1	Cf-1b	Rabbit IgG ab	NIBSC 84/685
Bla g 1	10A6	Rabbit IgG ab	UVA 93/04
Bla g 2	7C11	8F4	UVA 93/04
Asp f 1	4A6	Rabbit IgG ab	Affinity purified Asp f 1

[1] Used as 50% ammonium sulfate fractions of ascites and coated on microtiter plates at 1μg/well.
[2] Biotinylated mAb used at ~20ng/well. Rabbit secondary antibodies are detected using peroxidase labelled polyclonal goat anti rabbit IgG.

comparable immunologic reactivity to the natural protein. In general, recombinant grass or tree pollen allergens (e.g. birch pollen, Bet v 1 and Bet v 2) retain their antigenicity and the potential for these allergens to be used for diagnosis looks promising (2, 7, 8). For indoor allergens, the situation is less clear. Recombinant mite Group 2 allergens show comparable reactivity to the natural allergens for IgE ab, however, recombinant Group 1 allergens produced in bacteria or yeast are not as immunoreactive as native allergen (9–12). Fel d 1 is more complex because it comprises two polypeptide chains and N–linked carbohydrate (6, 13, 14). IgE ab binding to each recombinant chain can be demonstrated by RAST, but these chains do not contain all the epitopes present on native Fel d 1 (15, 16). The availability of recombinant allergens has facilitated analysis of the location of B cell epitopes by comparing binding to truncated forms of the molecules, or to peptide sequences, or to variants containing different amino acid substitutions. We have recently been using the latter approach (site directed mutagenesis) to generate Der p 2 variants with single amino acid substitutions and have demonstrated differences in MAb and IgE antibody binding to variants lacking particular disulfide bonds or "surface exposed" amino acid residues (17).

There are several applications of molecular biology in new treatment strategies. Traditional desensitization extracts using natural allergen extracts could progressively be replaced with a cocktail of a few selected recombinant allergens. The content and potency of the cocktails would be accurately defined so that patients would receive graded doses of recombinant allergens in much the same way that they receive current immunotherapy. This approach would exclude non–allergenic components present in natural extracts and would allow immunotherapy to be based on injections of known amounts of specific allergens. The success of this approach depends on whether immunotherapy with selected allergens is as effective as using the whole extract.

Perhaps a more exciting prospect that has emerged from work both on molecular biology and on T cell responses to allergens, is that of developing immunotherapies based on the down regulation of T cell responses (18–20). This is assumed to occur during conventional immunotherapy, but may be more efficiently achieved using synthetic peptides directed against known T cell epitopes to "desensitize" T cells. Strategies for downregulating allergen specific T cell responses using synthetic peptides include: i) induction of T cell anergy or unresponsiveness; ii) inhibition of interactions between T cell receptors and HLA Class II molecules; and iii) altering patterns of cytokine production, from T_H2 to T_H1. The advantages of peptide based vaccines are that they can be produced in gram quantities as a defined pharmaceutical and that they are less likely to cause adverse reactions. From a theoretical standpoint, our understanding of the repertoire of T cell epitopes on many allergens is incomplete. Current work suggests that multiple epitopes exist on most allergens and that these epitopes may be presented by several different HLA molecules. Defining which of these epitopes is important and how they should be incorporated into theraputic products is a daunting task. Nonetheless, considerable progress has been made in recent years and clinical trials of peptide based immunotherapeutic products for cat allergy are now underway.

Environmental allergen detection tests

The production of monoclonal antibody based ELISA tests for measurement of indoor allergens has played a key role in establishing the importance of allergen exposure as a cause of asthma, and offers exciting prospects for developing effective avoidance strategies for asthma management. Table 2 shows mAb combinations currently in use in our laboratory and several other groups have also produced mAb that are suitable for allergen immunoassays (1, 21–23). Exposure to indoor allergens is usually assessed by measuring allergen levels in 3–4 dust samples collected from appropriate "reservoirs" within the home. These include mattresses, bedding, carpets and soft furnishings (e.g. sofas) for mite and animal danders, and kitchens, for cockroach infestation. While there are several potential problems in assessing exposure using reservoir measurements (depending on sampling areas, collection devices and flow rates, and gross composition of dust), standardized sampling techniques and extraction proce-

Table 3. Proposed threshold values for indoor allergens

	Exposure leading to: IgE sensitization	Allergic symptoms
Dust mite:		
Group 1 allergen	>2μg/g	10μg/g**
Mite counts	>100 mites/g	>500 mites/g**
Guanine	>0.9mg/g	>3.0mg/g
Cat – Fel d 1	>8μg/g	?
	1–8μg/g	?
Dog – Can f 1	>10μg/g	?
Cockroach – Bla g 2	>2 units/g	?

** A level above which individuals who are going to develop symptoms will do so. This level increases the risk of acute asthma.

dures have evolved in recent years and have provided epidemiologic data on allergen exposure from many parts of the world. Based on these studies, it has been possible to propose risk levels of exposure for the development of IgE ab responses: for dust mite 2μg Group I allergen/g dust; cat, 1–8μg Fel d 1/g; and cockroach, 2U/g Bla g 2 Table 3). Levels of > 10μg/g mite Group 1 have also been proposed as a threshold above which most patients with asthma will experience symptom exacerbations (24, 25). Ideally, one would like to assess allergen exposure by measuring airborne levels and obtain estimates of the quantity of allergen inhaled by allergic patients. At present, the nature and size of some indoor allergens makes airborne measurements difficult to use for routine monitoring of exposure. Mite and cockroach allergens become airborne on particles > 10μ in diameter, which only remain in the air for 20–30 minutes following disturbance. Under undisturbed conditions, it is difficult to detect allergen and this usually requires prolonged sampling times (3–4 days) (26, 27). Although detection of airborne mite allergen on personal lapel monitors has been reported, this approach has not been adopted as a routine method of assessing exposure (28). Cat allergen occurs on < 2– > 10μ particles and can remain in the air for long periods (29). Dog allergen has also been detected in the air of houses containing dogs (30). Measurement of airborne cat allergen (Fel d 1) has been used in clinical studies to evaluate the dose of Fel d 1 that causes asthma attacks and is being used as a model for comparing the effects of allergen removal devices on reducing airborne allergen levels (31, 32).

In addition to epidemiologic studies, there are several important applications of mAb ELISA tests. Standardization of allergen extracts is increasingly being based on measurements of one or more specific allergens and this is most easily accomplished using mAb based assays (1, 21–23). Of great practical importance, and benefit to allergic patients, is the use of ELISA tests to evaluate the efficacy of avoidance procedures. This includes monitoring the effects of acaricidal or chemical treatments on allergen levels and assessing the effects of physical avoidance procedures on reducing allergen exposure (24, 25, 32). Many household products (cleaners, air filtration devices etc.) are marketed specifically to allergy patients with little or no evidence of their effects on allergens, either in the air or in dust samples. These products can now be objectively tested by manufacturers and this should lead to marked improvements in design and efficacy.

Conclusions

Advances in allergen immunochemistry and molecular biology have developed to the point where they are now being applied to the treatment of asthma and other allergic diseases. Innovative immunotherapeutic strategies are being investigated, alongside more systematic approaches to avoidance. Further applications of biotechnology will improve understanding of the basic mechanisms and management of allergic diseases caused by indoor allergens.

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