

Allergen specific monoclonal antibodies: new tools for the management of allergic disease

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In the 20 years since the discovery of IgE (16, 17, 20, 21) there have been major advances in our understanding of immediate hypersensitivity. These include detailed knowledge of (i) the immunologic events controlling IgE synthesis, culminating in the molecular cloning of IgE binding factors (18, 23, 28, 30); (ii) the mechanisms controlling mediator release from mast cells and basophils (19); (iii) the biochemistry of newly formed mediators (33); (iv) the immunoregulatory interactions between T cells, mast cells, eosinophils and basophils and their role in both immediate and delayed reactions (2, 4, 14, 31). While these advances in basic research hold exciting prospects for future immunologic intervention to prevent allergic disease, for the present, allergists will continue to rely on improved diagnostic and therapeutic allergen extracts and improved pharmaceuticals for patient management. However, with the development of hybridoma technology and the production of monoclonal antibodies (Mab) (24), new immunologic tools are now available which can be used both in allergy research and in the clinical management of allergic symptoms (rhinitis, hayfever, asthma and atopic dermatitis).

In basic research, Mab can be used to investigate the repertoire of antigenic sites on allergen molecules, as site specific probes to investigate the epitope specificity of IgE and IgE antibody responses, and to study idiotypic

regulation of the IgE response. However, this review will focus on the two areas in which allergen specific Mab can directly benefit allergy diagnosis and patient care. First, in improving the quality of allergen extracts used for diagnosis and treatment, through allergen standardization. Second, in assessing the relationship between environmental allergen exposure and the onset of allergic symptoms, and in developing improved methods of allergen avoidance and control. In both cases, highly sensitive immunoassays are required to quantitate specific allergen levels, and there are clearly several advantages (sensitivity, specificity and reproducibility) in using Mab based assays. Furthermore, absolute allergen quantitation is important in defining extracts used in clinical studies, e.g. the best guide to the strength of the ragweed extracts used successfully in the Baltimore immunotherapy studies of the 1960's is their Antigen E (*Amb a I*) content. This paper describes the application of Mab immunoassays to quantitate three major house dust allergens: the dust mite allergens, *Der p I* and *Der f I* (5, 15), and the cat allergen, *Fel d I* (27). On a worldwide basis, house dust allergens (mite, cat, dog, cockroach and fungi) are perhaps the most common causes of perennial rhinitis and asthma. Better standardization of these allergens and better assessments of indoor allergen exposure using Mab could significantly improve the management of these important allergic diseases.

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Abbreviations used: Mab: Monoclonal antibody; *Der p I*: a major 24,000 MW allergen of *D. pteronyssinus*; *Der f I*: a major 24,000 MW allergen of *D. farinae*; *Fel d I*: the dimeric 38,000 MW cat salivary allergen; RAST: radioallergosorbent test; RIA: radioimmunoassay; IUIS: International Union of Immunological Societies; WHO: World Health Organization.

Monoclonal antibodies and the development of two-site immunoassays

Within the last 2-3 years, Mab have been developed to several major inhalant allergens, including *Amb a I*, *Lol p I*, *Der p I* and *Fel d I* (Table 1). Monoclonal antibodies directed against a number of distinct non-overlapping epitopes on each allergen have been produced and they can be used in pairs to develop two-site immunoassays. One Mab is coupled to a solid phase, usually a plastic microtiter well, and then incubated with allergen extract. Bound allergen is detected using a second Mab, of different epitope specificity, which is labelled with ^{125}I , biotin or an appropriate enzyme. The quantity of specific allergen, e.g. *Der p I*, in the extract is directly related to the amount of labelled Mab bound and is determined using a control curve of a reference with known allergen concentration, e.g. the *D. pteronyssinus* reference NIBSC 82/518 (12, 13). Table 2 shows the Mab and reference standards used in assays for *Der p I*, *Der f I* and *Fel d I*. The advantages of these assays are that they are highly sensitive (down to 0.5 ng/ml *Der p I* or 0.5 mU/ml *Fel d I*), are technically straightforward and rapid (results obtained within a few

Table 2

Monoclonal antibodies and reference extracts used in immunoassays for mite and cat allergens¹

Allergen	Solid phase Mab ²	^{125}I labelled Mab	Reference ³ standard
<i>Der p I</i>	10B9	5H8 C12	<i>D. pteronyssinus</i> NIBSC 82/518
<i>Der f I</i>	6A8	4C1	<i>D. farinae</i> UVA
<i>Fel d I</i>	Fd-2	Fd-1	NIH Cat E3

¹ Mab to *Der p I* and *Der f I* have been described in detail previously (5, 6, 15): 10B9 and 5H8 C12 are *Der p I* specific; 6A8 is *Der f I* specific; and 4C1 reacts with a cross-reacting epitope on both allergens. The *Fel d I* specific Mab were produced by Dr. Rob Aalberse, CLB, Amsterdam.

² Plastic microtiter walls are used for both mite assays. The *Fel d I* assays used CNBr activated paper discs.

³ NIBSC 82/518 is the WHO/IUIS *D. pteronyssinus* reference (29, 30) which has an assigned potency of 1×10^5 IU/ml and contains 12.5 µg/ml *Der p I*. *D. farinae* UVA is an in-house standard which was substandardized from the WHO *D. pteronyssinus* reference by antigen P₁ Equivalent assay and contains 1.4 mg/ml *Der f I*. NIH Cat E3 is the Office of Biologics Research and Review reference (OBRB, Bethesda, MD) and contains 10.5 units *Fel d I*/ml (1 unit is approximately 4µg).

Table 1

Allergen specific monoclonal antibodies produced in different laboratories

Source	Allergen*	Epitopes defined	Laboratory
House dust mites	<i>Der p I</i>	5	Chapman et al. (6)
			Aalberse (in preparation)
			Lind, Horne & Lowenstein (unpublished)
			Wu et al. (40)
			Chapman & Platts-Mills, Heymann (5, 7, 15)
Grass pollen	<i>Der f I</i>	2	Aalberse (in preparation)
	<i>Der p II</i>	1	Heymann et al. (in preparation)
	<i>Der f III</i>	1	
	<i>Lol p I</i>	> 6	Esch & Klapper (11)
	<i>Par J I</i>	2	Kahn & Marsh (22)
Ragweed pollen			Corbi et al. (10)
	<i>Amb a I</i>	1	Kriliš et al. (26)
		3	Olsen & Klapper (32)
Cat dander	<i>Fel d I</i>	2	Aalberse (in preparation)
Alternaria	<i>Alt a I</i>	1	Agarwal et al. (1)

* Allergen nomenclature is that currently recommended by the WHO (29).

hours) and can readily be used to assay large number of samples (a major advantage for epidemiological studies of indoor allergen exposure). Both the monoclonal mite and cat allergen assays have been validated against other techniques. Assays for *Der p* I and *Der f* I in mite and house dust extracts correlate with allergen levels determined by an inhibition radioimmunoassay (RIA) which measures both allergens ("Antigen P₁ equivalent") (8, 35). There is also a very good quantitative correlation between the Mab assay for *Fel d* I and double antibody inhibition RIA using polyclonal rabbit anti-cat antibodies and ¹²⁵I labelled allergen ($r = 0.91$, $P < 0.001$) (9).

Standardization of allergen extracts

The aim of standardization is to improve the quality and consistency of extracts used for allergy diagnosis and immunotherapy. This is achieved by precisely defining the numbers and quantities of specific allergens in an extract and by developing standard procedures for comparing the potency of one extract with another. To this end, the International Union of Immunological Societies (IUIS) has begun a program of allergen standardization to select candidate extracts to present to the World Health Organization for approval as International Standard Preparations. Three extracts, *D. pteronyssinus*, short ragweed and timothy grass, have so far been approved and several others, e.g. cat, dog, Bermuda grass and birch pollen are awaiting approval. These standards have an assigned potency in International Units and can be used as a "yardstick" against which the potency of extracts marketed by commercial manufacturers or used by research laboratories can be compared. In practice, this means that allergists in different areas or countries can directly compare the strengths of extracts they use for skin tests (or *in vitro* tests) and for treatment, in a common unitage.

It is evident that allergen quantitation and the ability to maintain precise methods for measuring allergens over several years are the keys to successful standardization. The use of Mab as primary reagents for allergen standardization

was first suggested 4–5 years ago (3, 25). However, it is only within the last 2 years that a sufficient number of Mab directed against major allergens has been produced to make this a realistic possibility (Table 1). The main use of Mab is in immunoassays to measure the content of major allergen(s) in an extract. Major allergen assays are the only quantitative methods which do not depend on using human subjects (inherently variable!) in either skin testing or the preparation of serum pools for RAST inhibition. While skin testing is useful in demonstrating biological activity, it is at best semi-quantitative and the results can be biased by the patient population tested. Likewise, RAST inhibition results vary with the composition of IgE antibodies in the allergen serum pool. Existing techniques for measuring specific allergens, e.g. radioimmunoassays and immunoelectrophoretic techniques, depend on polyclonal antisera which are usually limited in supply, are heterogeneous and difficult to reproduce, and often require immunoabsorption. Monoclonal immunoassays have several important advantages over these techniques: 1) their specificity can be defined and remains constant; 2) they do not require purified allergen; 3) the Mab can readily be produced in gram quantities and made widely available; 4) the Mab can be consistently reproduced over several years (we have continued to produce the same anti *Der p* I Mab from the clones we isolated 3 years ago).

Examples of the use of Mab assays to compare the *Der p* I and *Der f* I content of *D. pteronyssinus* and *D. farinae* extracts prepared by a commercial manufacturer are shown in Figs. 1A and 1B. The dilution curves in each assay are parallel. The *D. farinae* extracts were prepared under similar conditions and varied by < 2-fold in *Der f* I content (18–36 µg/ml). The *D. pteronyssinus* extracts were prepared experimentally to compare different extraction procedures and varied in *Der p* I content from 5–60 µg/ml. These assays have been used to compare nine commercially available mite extracts and showed that levels of *Der p* I and *Der f* I ranged from 10–80 µg/ml in *D. pteronyssinus* and *D. farinae* extracts, respectively (8). Each

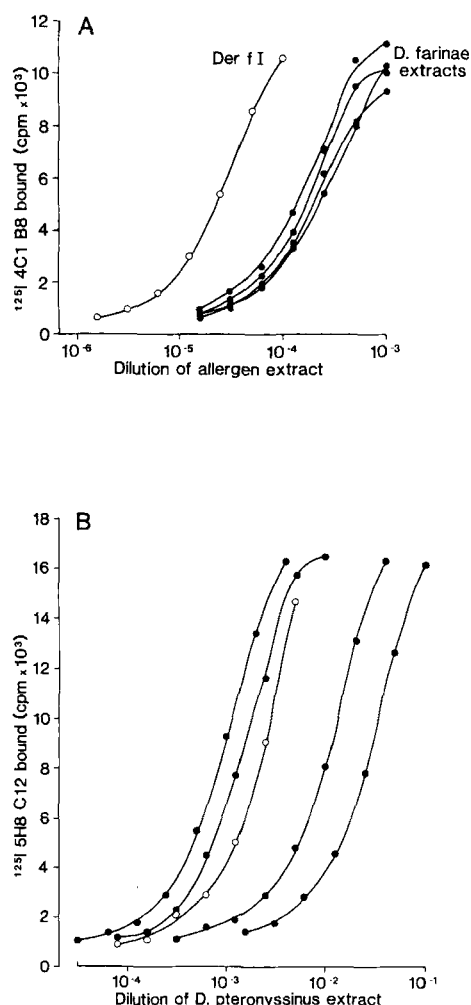


Fig. 1. Use of monoclonal immunoassays in allergen standardization. The quantities of Der f I or Der p I were compared in commercially prepared *D. farinae* (Fig. 1A) or *D. pteronyssinus* (Fig. 1B) extracts, respectively. The dilution curves for four *D. farinae* extracts were parallel to a pure preparation of Der f I (o, Fig. 1A) and those of *D. pteronyssinus* extracts were parallel to the WHO/IUIS *D. pteronyssinus* reference NIBSC 82/518 (o, Fig. 1B).

assay is "species specific" and does not detect either allergen in extracts of other mite species (*T. putrescentiae* and *A. siro*).

In similar studies, the Mab assay for *Fel d* I has been used to compare cat allergen levels in eight commercial cat, dog and house dust

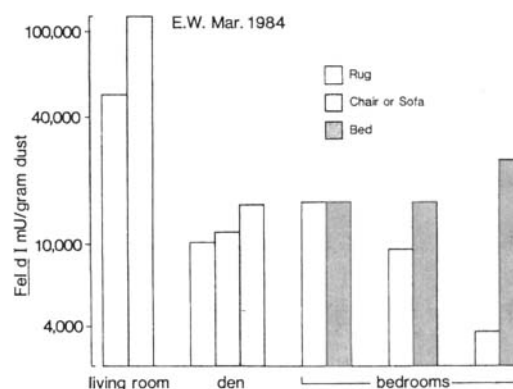


Fig. 2. Use of the monoclonal immunoassay for *Fel d* I to compare cat allergen levels in dust from different sites (rug, chair or sofa, or bed) in different rooms of an asthmatic patient's house. Allergen levels in the living room were 5-10 fold higher than in the den (converted basement) or bedrooms.

extracts (9). The *Fel d* I content varied markedly in both cat (0.6-30 U/ml) and house dust extracts (< 0.01-3.0 U/ml) and some dust extracts contained more *Fel d* I than cat dander extracts. Thus patients skin tested with these extracts would give positive immediate reactions to "house dust" even if they were exclusively sensitive to cats. None of the dog extracts contained *Fel d* I and neither did a panel of other commercial animal epithelium extracts (mouse, rat, gerbil, guinea pig, horse, cow, etc.), again confirming the exquisite specificity of the Mab assays.

These results leave little doubt that Mab immunoassays can provide absolute quantitation of specific allergen levels, and present evidence suggests that the reagents will be stable and reproducible for many years. Monoclonal assays for most of the allergens listed in Table 1 could readily be adopted by national standardization agencies and could easily be developed for other allergens, particularly if the allergen has been purified. The main question is whether assays for one or two allergens can reflect the "total potency" of an extract. This depends on the composition of the source material, the efficiency of extraction procedures and whether or

not the proportional relationship between major allergen(s) and the other allergens in the extract remains constant. For pollen extracts, where the pollen source can be > 95% homogeneous, measurement of major allergens (e.g. *Amb a* I) appears to be a good assessment of overall potency. This is also true for extracts in which a single allergen appears to account for most of the allergenic activity, e.g. *Fel d* I in cat extracts. The main problems arise where the ratio of major allergens varies, in extracts made from different source materials. The best example being comparisons of dust mite extracts prepared from mite bodies with those prepared from whole mite cultures. These extracts appear to contain different proportions of the major allergens (*Der p* I and *Der p* II, or *Der f* I and *Der f* II), and measurements of each allergen may be necessary to give the best assessment of overall allergenic activity.

Quantitative assessment of indoor allergen exposure

Pollen counts have been used for many years to measure airborne pollen allergen levels and to predict the severity of symptom days for patients with hayfever. These counts are broadcast or published in the press and when the counts are high hayfever sufferers are advised either to stay indoors or to carry appropriate medication. Although house dust is the primary source of allergen exposure within the home (where patients are exposed 16–20 h/day, year round), simple methods for measuring dust allergen exposure are not available. Moreover, this is a difficult problem because there are several different allergenic components in dust (e.g. mites, animal danders, insects, moulds and fungal spores) and the distribution of these allergens varies from house to house and in different parts of the country. Most dust allergen particles would not be distinguished on a simple slide count (although a recent report describes visual identification of cat dander particles) (39) and some, e.g. mite fecal particles, do not remain airborne for more than 20–30 min. (36). Dust mite allergen exposure can be assessed by counting mite bodies, but this is time consuming, requires detailed knowledge of

mite taxonomy and does not always reflect mite allergen levels. Recent year-round studies in Charlottesville have shown that mite allergen levels remain high for several months after the seasonal increase in mite numbers (37).

Immunochemical assays are the only practical methods for assessing indoor allergen levels. The advantages of using Mab immunoassays compared to RAST inhibition and other assays have been discussed above. Two additional points need to be stressed concerning indoor allergen exposure. First, absolute quantitation is essential, so that exposure to allergens from the same species (e.g. *Der p* I, *Der p* II) or from different species (mite, cat, cockroach) can be directly compared. Second, it is important to have an assay which can routinely handle large numbers of samples in clinical laboratories. Monoclonal assays are widely used in clinical laboratories in other immunodiagnostic tests, and similar technology could be used for measurements of allergen exposure in the investigation of allergic diseases.

Monoclonal assays for *Der p* I and *Der f* I have been used to compare the levels of these allergens in 30 dust samples collected in either Charlottesville, VA or London, UK (8). The combined levels of *Der p* I and *Der f* I ranged from < 4–200 µg/g dust and showed a very good quantitative correlation with Antigen P₁ equivalent levels determined by inhibition RIA ($r = 0.86$, $P < 0.001$). In addition, the Mab assay results correlated both with mite counts ($n = 15$, $r = 0.89$, $P < 0.001$) and with assessments of total mite allergen content by RAST inhibition ($n = 21$, $r = 0.77$, $P < 0.001$). Because the assays are species specific, they can be used to estimate the proportions of *D. pteronyssinus* and *D. farinae* in dust samples, without the need to do differential mite counts. On the other hand, the present assays would underestimate the allergen content of dust samples containing *D. microceras* or *Euroglyphus maynei* because they do not detect the homologous allergens from these species. Although *D. microceras* and *E. maynei* are occasionally found in high numbers, they are generally uncommon and it is debatable whether measuring allergen produced by these mites would have broad clinical application.

The *Fel d I* assay has been used to compare cat allergen levels in dust samples collected in the Charlottesville year-round study (from Feb '84 - Feb '85) (37). In six houses with cats, *Fel d I* levels were very high (10-200 U/g dust) but showed no evidence of seasonal variation and no correlation with either grass pollen or mite allergen levels (9). In five houses without cats, *Fel d I* was detected, but the levels were very low (< 1 U/g dust). Dust samples were obtained from several sites in each house and analysis of the range of *Fel d I* levels at various sites in an asthmatic patient's house, which contained a single cat, are shown in Fig. 2. These results demonstrate that the quantities of *Fel d I* in dust samples from the same house can vary by at least 10-fold and emphasize the need for assaying multiple samples. Direct comparisons of *Der p I*/*Der f I* and *Fel d I* levels (assuming 1 Unit *Fel d I* = 4 μ g) suggest that the quantities of *Fel d I* in dust can be several-fold higher than mite allergens. In keeping with this, we have been able to purify mg amounts of *Fel d I* from potent house dust extracts (50 U/ml *Fel d I*) in a single step by monoclonal affinity chromatography (9).

Clinically, knowing the quantities of specific allergens in a patient's house can be important in assessing whether allergen avoidance would be appropriate for patient management. The history of allergen avoidance in the treatment of allergic disease dates back to the 1920's, when Storm van Leeuwen demonstrated clinical improvement in asthmatic patients admitted to an "allergen free chamber" (38). More recently, significant reductions in bronchial hyperactivity in asthmatics have been demonstrated following prolonged allergen avoidance (34). To use allergen avoidance objectively, a level of indoor allergen exposure which is likely to be significant in causing allergic symptoms needs to be established (analogous to a "high" pollen count). Studies on seasonal variation in mite allergen levels suggest that 10 μ g/g *Der p I*/*Der f I* should be regarded as equivalent to a high pollen count (> 300 grains/m³) and that the aim should be to reduce these levels to < 0.2 μ g/g to achieve "allergen-free" conditions (34, 37). Our preliminary results on *Fel d I* levels

suggest that > 10 U/g is significant and should be reduced to < 1 U/g to achieve the level in cat-free homes. Using Mab assays, it will be possible to establish clinically significant levels for a panel of common dust allergens, to assess the distribution of these allergens within the home and to develop successful strategies for allergen avoidance.

Monoclonal antibodies - future prospects

Monoclonal immunoassays to measure household allergens have only been developed within the last 2 years, but their many advantages and applications in measuring dust allergens are already apparent. The limitations of the present assays lie mainly with the epitope specificity of the Mab. A single assay for *Der p I*/*Der f I* could be developed if more cross-reacting Mab were available, and technical improvements in the *Fel d I* assay could be achieved with Mab to different non-overlapping epitopes. (To date only 2 *Fel d I* specific Mab have been produced). Monoclonal antibodies to other mite allergens (*Der f II*, MW 12,500 and *Der f III*, MW 29,000) are already being produced and over the next 5 years the production of Mab to several more dust allergens (e.g. cockroach, dog, and possibly some fungi) can be anticipated.

A good example of the use of Mab assays in clinical studies is the National Institutes of Allergy and Infectious Diseases (USA) collaborative study of dust mite immunotherapy in asthma. This study is using *D. pteronyssinus* and *D. farinae* extracts which are being standardized relative to IUIS and OBRR mite references using Mab assays for *Der p I* and *Der f I*, as well as RAST inhibition. Furthermore, the study is being carried out in several allergy centers across the US (New Orleans, San Diego, Cincinnati, Galveston) and dust samples from patients' houses in each area are being analysed by Mab RIA for mite allergens. Studies of this type will provide considerable data about the epidemiology of mite asthma and whether or not clinical symptoms correlate with particular levels of mite allergen exposure. Monoclonal antibodies provide the tools with which these

studies can logically be extended to measure other allergens in dust, and also to develop better techniques for quantitating airborne allergen exposure. The relationship between the quantity and size of dust allergens that become airborne and the development of allergic symptoms remains poorly understood. Clinical studies would be greatly improved by better quantitation of allergens carried on different sized particles, and the high sensitivity of Mab assays has obvious application in this area.

In conclusion, the development of allergen specific, monoclonal immunoassays should significantly improve the management of allergic diseases by providing standardized extracts for diagnosis and immunotherapy and by providing objective assessments of environmental allergen exposure. The development of simple colorimetric or fluorimetric enzyme assays will mean that allergen analysis will no longer be restricted to specialized research laboratories, and physicians will be able to apply these techniques to the management needs of individual patients. Finally, although this review has considered only those areas directly relevant to patient care, in the long term, basic research on allergen structure, receptors and control of the IgE response using Mab should facilitate the development of more rational methods for the prevention and treatment of allergic diseases.

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