

REVIEW

What is a 'major' allergen?

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*Denn eben wo Begriffe fehlen
Da stellt ein Wort zur rechten Zeit sich ein.
Goethe: 'Faust'*

What Goethe made Mephistopheles say in the above motto applies to many fields of science including allergy, namely, that precisely when we fail to comprehend something we will just find a name for it. The term 'major allergen' has been floating around in the allergy literature for quite some time now and it seems that we have come to a point where everybody takes the expression for granted. It certainly appeals to our instinctive feelings for scientific law and order that there should be such things as major and minor allergens, and the classification has indeed become deeply entrenched in our nomenclature. In fact, the terminology has become so accepted that the proviso 'major allergen' has already slipped into legislative documents [1], guidelines for allergen nomenclature [2] and the newly published Position Paper of the EAACI Subcommittee on Allergen Standardization and Skin Tests [3]. In this last document the editor Dreborg makes no bones about it:

'When ISs (International Standards) are available, extracts should be calibrated against the corresponding IS and the potency given in IU (International Units)/ml with information on the method used for calibration. Furthermore, when possible, the content of the major allergen should also be given in the package insert.'

The designation is being specified in the same document and by the same author:

'These methods (. . . CRIE and immunoblotting) can also be used to define major allergens, i.e. the allergens which cause frequent and pronounced sensitization in human beings.'

It is precisely the last addition that sets off a train of thinking because, as far as I know, no one has ever yet identified the nature of the sensitizing allergen, nor would this be an easy thing to do. Leaving aside the loose qualification of a 'frequent and pronounced' physiological condition, there is no scientific basis so far for the inference that the sensitizing molecule(s) and the eliciting

allergen(s) are really quite the same. More important, reference to a major allergen on the label or insert of a pharmaceutical allergen product for use in humans presumes some added quality assurance with respect to diagnostic validity and therapeutic efficacy, for both of which there is no evidence. However, if the allergen manufacturing industry is going to be asked to define (the content of) a major allergen in a package insert, at the very least there should be no ambiguity as to what a major allergen really is.

One of the earliest specifications was provided in 1978 by Löwenstein:

'The term 'major allergen' is used for an antigen with which more than 50% of a selected group of patients (clinically allergic to the allergen) react in crossed radio-immuno-electrophoresis (CRIE), and against which half of these patients have specific IgE of high binding capacity' [4].

This is the rather ambiguous formulation of the laboratory scientist wrestling with an uncomfortable methodology which certainly does not permit the evaluation of the binding capacity, i.e. the affinity or avidity of antibodies of whatever isotype. In 1987 the same author issued a slightly modified version, whereby the second half of the definition was phrased:

'... and among which half of the patients have high levels of specific IgE' [5].

These two editions are not altogether identical: antibody affinity for allergens is not equitable with antibody concentration, they are in fact two independent parameters. But it is really in the most recent wording that I lost this author's meaning completely:

'The term "major allergens" is used for an antigen with which more than 50% of a selected group of patients (clinically allergic to the allergen) react in crossed radioimmuno-electrophoresis (CRIE) and against which half of these patients have specific IgE of high binding capacity "...". The number of defined allergens detected in most allergenic extracts is between 25% and 50% of the total number of detectable antigens, whereas there are only one to three major allergens. Increasing the number of individuals investigated, when identifying allergens, will yield an increased number of allergens. Consequently, any antigenic constituent may be regarded as a potential

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allergen, although its probability of binding IgE within a selected allergic population may be extremely low' [6].

After this exercise one tends to prefer the no-nonsense definition by another laboratory worker during a discussion at the 1990 Paul Ehrlich Seminar:

'If an allergen can be seen (. . . in CRIE) after 3 days of autoradiography in more than 50% of the patients, according to our definition, it is a major allergen' [7].

Naturally, this statement likewise refers to human sera assayed in a rabbit antiserum system fraught with technological pitfalls and cannot have been meant to carry any clinical connotation. In any event, in order to make this a workable definition and to apply CRIE for evaluating major allergens, a number of prerequisites would have to be fulfilled, e.g. specification of the inoculating allergen preparations, agreement on the immunization protocol, standardization and availability of the antiserum reagents, and validation of the procedures for identification and quantification. Other technologies like isoelectrofocusing (IEF) or immunoblotting may fare no better. In discussing IEF of the first international standard for timothy pollen allergenic extract, Gjesing *et al.* observed that:

'we are not, however, able to say whether absence of a band means that an important allergen is missing or whether it means that the extract is without a superfluous inactive protein' [8].

From 1978 onwards various contributions of laboratory scientists and of more medically oriented investigators did not quite help to clear up any misunderstandings. King [9] furnished the observation that:

'Allergens from each source can be divided into major and minor ones, based on their relative allergenic activities. Major allergens are highly active in the majority of allergic individuals tested, while minor allergens are poorly active. However, minor allergens can be as active as, or more so than, major allergens in some selected individuals.'

While this statement does not exactly put us on the road to the quantitative assessment of major allergens, the assertions by Aas [10] likewise provided nothing tangible in the way of what might be a major allergen and how it could be measured:

'Only one or a very few of the allergenic proteins or polypeptides present in a given extract act as denominators of the allergic reaction in all or most patients allergic to the matter. They are called "major allergens". In addition, the same extract may contain other allergenic proteins called "minor allergens", which are important for only a small number of patients or of little importance for all of them.

Most patients reacting to any of the minor allergens usually react also to the major allergens present in the material, but for some patients the minor allergen may be the most active one. The presence in different substances of similar minor (or rarely, also major) allergens accounts for varying degrees of crossreactivity.'

Already at an early stage Marsh [11] intervened in a scholarly attempt to get to grips with the problem:

'In the past, the terms "major" and "minor" have been used to describe the relative allergenic importance of different components isolated from crude allergen-containing extracts. Such terminology would seem to imply that a major allergen is more immunogenic and reactogenic than a minor allergen in all subjects displaying sensitivity to the crude extract, and/or that a greater percentage of the allergic subjects are appreciably more sensitive to the major than to the minor allergen. However, recent research has clearly shown that certain pollen components, generally of 5000–12000 kDa, are more reactive in some allergic subjects than the so-called major allergens. A further complication arises from the observation that different allergenic components (of pollens) are extracted at radically different rates. Thus, an apparent major allergen (e.g. antigen E or crude ragweed pollen) may not be the major causative agent in specific allergic disease if it is eluted relatively slowly before the allergen particle is swallowed. In order to clarify the problem of nomenclature, I propose that the term "major allergen" be used only to denote an identified highly purified component to which over 90% of clinically specifically allergic individuals react appreciably in high allergen dilution (e.g. produce a "two-plus" skin reaction following intradermal skin testing with the allergen at a concentration of 10^{-3} µg/ml or lower). This definition includes the possibility that a major allergen, defined on the basis of studies with complex crude allergenic extracts, may not be the principal agent actually provoking allergic symptoms in vivo, due to factors such as slow extraction from the allergenic matrix or rapid catabolism of the allergen.'

Meanwhile by the mid-80s practicing clinical allergists already had their own, though hazy interpretation of what they never asked for in the first place:

'As far as I understand it, a major allergen is defined as a single component from an extract which will produce an allergic response in over 50% of the individuals who react to the crude extract,'

is what Warner made of it, while Frankland was even less conspicuous:

'The term "major allergen" by definition means, I suppose, that 80% of the patients are reacting to it.'

Between the lines one senses that the matter did not really concern him much and that in his clinical practice he had no need for major allergens anyway [12].

The fact of the matter is that we now already have four distinct descriptions of a major allergen, each based on a different principle, viz. presumed physiology, tricky laboratory technology, dubious epidemiology, and intradermal tests. To this might be added the opinion of Platts-Mills who, in a footnote to a table of major and minor allergens proceeded to dilute the issue even further:

'Allergens have been judged to be major because a large proportion (>75%) of the patients allergic to the crude extract have IgE antibodies to that protein or because IgE antibody to that protein has been shown to represent a significant proportion of the total serum IgE' [13].

By bringing in the incidence of IgE antibodies and some perhaps significant, but non-quantified ratio between allergen-specific and total IgE, this author succeeded in adding one further dimension to the allergological uncertainty principles that we already had. Estimates vary of the proportion of IgE antibodies specific for the major component antigen E of ragweed pollen relative to total IgE in the sera of ragweed-allergic patients, but in one study the mean value achieved only 4.5% [14]. The way things are we would with the above pronouncement at any rate have a good case for earmarking each and every allergen as a major allergen. Indeed, in terms of frequency of specific IgE binding, i.e. in percentage of positive sera, Thomas, in a recent Editorial on mite allergens [15], dropped the criterion to an equally arbitrary 40%. I have seen no definitions so far, but with the advent of the (laboratory-dependent) monoclonal antibodies now coming into use, and the (cleverly designed) immunoassays based on them for measuring predominant individual components of allergenic extracts, one can easily see a host of new 'major' allergens coming up over the scientific horizon.

On reviewing the situation, one wonders what then is the justification for major allergens. Repeatedly quoted scientific papers on individual allergens reveal this remarkable paucity of essential data in the form of balance sheets for recovery and activity needed to isolate and identify a major biochemical principle. With rare exceptions each of the pertinent publications over the past two decades instead provides a strong motive for biochemists to abandon attempts at obtaining a single molecular species carrying the bulk of the biological activity, whichever way it is being measured. An early paper by Gleich *et al.* [16] shows in graphical display the RAST-inhibitory potencies of the 'major' antigens E and K and the 'minor' allergen Ra3 in (non-parallel) relation to the activities of whole ragweed pollen extracts. The *in*

vitro potency of the major antigen E (AgE or *Amb e 1*, if one so prefers) from this graph appears at least 10 times lower than that of the dialysed total proteins. Combined with the original data from King *et al.* [17], namely that AgE comprises 6% of the total pollen proteins, AgE at a conservative estimate represented only about 0.5% of the total activity in terms of specific IgE binding; for the second 'major' antigen K, less potent by a factor of 1000, the situation was infinitely worse. The way I have been trained this would make these components of ragweed pollen insignificant contributors to the allergenicity of whole pollen extract, and I would call them 'minor' allergens. However, until this day one should perhaps reserve one's opinion because proper balance sheets of biological activity based on quantitative skin tests in allergic patients *in vivo* or even IgE binding *in vitro* were not published. And yet, on the basis of absorptions with rabbit antiserum to AgE King *et al.* estimated that AgE represents over 90% of the total activity, a figure that should be viewed with considerable reservation in view of the pitfalls of the methodology, especially because it provides the only justification so far for certifying AgE as a 'major' allergen [17]. Nevertheless, during the Seventh Paul Ehrlich Seminar held recently in Frankfurt, Dr Liu voiced the position of the US Center for Biologics Evaluation and Research CBER as follows:

'A marker protein may be a major allergen (e.g. it possesses over 80% of the activity of the crude or purified extract and over 80% of allergic individuals respond) or an allergen that is present in all extracts in a consistent relationship to other components of the extract.'

Although numerical data and tabulated activity reports are unavailable for the (isolation of) major allergens *Der p 1* and *Fel d 1* of mites and cats, from the existing evidence and by any kind of approximation one invariably ends up with a consistent result: on a weight basis the major allergen is always less, or only fractionally more active than the full extract, while at the same time comprising only a small portion of the total dry weight. It was clearly demonstrated by Van der Zee *et al.* that the removal of the 'major' allergen *Der p 1* from a mite extract indeed had but little effect on the overall allergenic potency [18]. It had been laid on the line for house dust before, and it appears to be always the same: the whole is (much) more than the sum of its isolated constituents [19].

One is left with the impression that a major allergen is something that a particular investigator wishes it to be, within the constraints of the clinical, epidemiological, technological or economical parameters available to him. I have this singular feeling that maybe there is no such thing as a major allergen. Maybe we are just looking at qualitative and quantitative differences in immunogeni-

city among the (crossreacting) components of highly complex mixtures, but that allergenicity is something else again, e.g. adjuvanticity [20–22]. Nothing would of course be gained by reverting to my own assessment some 20 years ago, namely that 'all allergens are equal, but some allergens are more equal than others', although at least it keeps the door open for alternative and possibly non-immunological interpretations [23]. Perhaps the best idea at this moment would be for the Sub-Committee on Allergen Standardization and Skin Tests of the EAACI and/or the Allergen Nomenclature Committee of the IUIS to clarify the matter and reach a consensus on what a major allergen really is or should be.

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