

EDITORIAL

The characterization of allergens

We do not, in 1996, have standardized extracts because it is impossible to standardize allergen extracts. There is difficulty in defining standardization [1]. Moreover, the term 'major allergen' is so entrenched in the literature that it would be difficult not to use a term that possibly serves little purpose and has, as yet, no agreed definition [2]. The WHO/IUIS committee have defined the nomenclature for allergens [3]. A 'major' allergen is 'a substance originating from an appropriate allergen source material that is always present in an extract of that source material and on respirable particles (or ingested food). Most often this will be a protein, it will induce an IgE response in more than 50% of a sample of patients who are exposed to the allergen source material and who have clinical symptoms on contact with that source of material'.

Such a definition may be agreed by some [4,5], but not everyone [2,6]. Patients clinically are very different, so that for some the reaction of a minor allergen is their major allergic response. Already there seems to be a concept that perhaps a major allergen is a marker protein in that it possesses over 80% of the activity of the crude or purified extract and 80% of allergenic individuals respond; or should we agree that the figure is not 80% but 50% [3]? There are problems over whether strengths should be based on quantitative skin tests in allergic patients *in vivo* or IgE binding *in vitro*. There are now purified standardized major allergens for a few of the clinically common allergens, but soon it seems likely that there may be two or more major allergens from one source. Moreover, if D p I, the major allergen of dust mite, is removed from a mite extract, this has little effect on the overall potency [7].

Noon used wt/vol for making his Timothy grass extracts [8]. He boiled his extract for 15 min and kept the pH at 7. Under these conditions the phleum extract was stable. If he had boiled it for longer or if the water had not been very freshly distilled, he would have destroyed the activity.

In a recent publication [9], the American Academy of Allergy, Asthma and Immunology (AAAAI) and the American College of Allergy, Asthma and Immunology (ACAAI) jointly prepared a critical review concerning the technology to be used in allergy diagnostic tests. One section of this report is on allergen standardization. It is pointed out that although immunoassays utilizing both immunodiffusion and immuno-absorption are highly reproducible, their usefulness is limited because of the great variability of allergenic epitopes in extracts employed in clinical practice. Most allergens, however, are still identified and labelled by weight/volume or protein nitrogen units (PNU).

Much advance has recently been made in the measurement by immunoassay of allergenic mediators, but particularly in the detection of low levels of histamine. Histamine is only elevated transiently in the serum but tryptase serum concentrations can persist for 2 or 4 hours after a systemic reaction and its measurement can be useful diagnostically in an allergic reaction. The eosinophil cell is paramount in allergic inflammation, so it may be that the activation markers of eosinophils such as the eosinophilic cationic protein and major basic protein may become further tests for evaluating allergic responses.

In America, because of the lack of standardized methods for determining the relative potency and bio-equivalent doses of allergenic extracts as well as establishing patient sensitivity, the FDA Laboratory of Allergic Products has developed defined methods of procedures for calculating these parameters [10]. Both prick/puncture and intracutaneous methods have been used for standardization [11,12,13,14]. In Europe there is a newly published position paper of the EAACI sub-committee on allergen standardization and skin tests [15]. It is stated that when IS (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.

No one system of labelling extracts has yet been agreed. Manufacturers should produce more product information, such as wt/vol, PNU, major and minor allergen content and biological potency. But if potency units are given using biological allergy units (BAU), we can assume that two extracts from different companies each labelled 10 000 BAU/ml are interchangeable.

Theoretically a standard extract must contain defined and consistent amounts of all major and minor allergens and each allergen must be biologically potent. It must be remembered that potency is very variable in sensitized subjects so that an allergen extract labelled in terms of potency, if it lacks one or several allergens, would be potent in some individuals but not others. While there are internationally recognized reference preparations for a few extracts, the manufacturers of allergenic extracts tend to develop their own references. One such reference has recently been described [16] in which the extracts are standardized by basophil histamine release, which is considered an additional useful *in vitro* test for allergenic extract standardization. At present the allergenic extracts are standardized by using ID₅₀EAL (intradermal dilution for 50 mm sum of erythema determines the allergenic unit) skin test technique to assign allergy units to reference

preparations. New batches of extracts are compared with the reference by RAST inhibition or other *in vitro* techniques. It was found that basophil histamine release, skin tests and RAST correlated well ($P < 0.01$).

Standardization by itself does not necessarily mean that the extracts that are used for skin testing are at the appropriate concentration. In this issue there is a description of how to obtain the optimal diagnostic concentration (ODC). Only one allergen was studied [17]. The authors are worried that a standard extract used for skin testing does not necessarily ensure it is an appropriate concentration for accurate diagnosis. They measured wheal diameter and the data were submitted to ROC analysis (receiver operating characteristic) [18]. These analyses provided ranges of concentrations in units based on *in vitro* immunological assays, over which diagnosis is most reliable for any particular skin wheal size. Using such a method the diagnostic extracts are labelled as diagnostic units (DU). The final formulations tell the user that the product is at the most appropriate concentration for diagnosis. A 3 mm wheal diameter cut-off was chosen as an appropriate size for routine diagnosis.

The problems when carrying out skin testing using the skin prick test (SPT) procedure are multi-factorial. There are many devices used which do not all produce similar results, since it is impossible to quantify the exact amount of the injected material by the SPT. Attempts have been made to standardize the SPT [19]. Some investigations have preferred using the intradermal method for testing and standardization of allergen extracts [20], but fatalities can occur [21]. Some allergists still use house dust extracts for skin testing. House dust is a mixture of allergens from a variety of sources such as animals, insects, moulds, pollens, mites, etc. House dust may contain microbial toxins such as endotoxins and mycotoxins. It is not surprising that no unique allergen in house dust has been identified.

Allergen standardization

Standardized food extracts are not available though many food allergens have been well characterized. Commercially available food extracts may produce false negative results because of alterations of the relevant allergens by their preparation, especially because many plant-derived food allergens are heat labile and testing with the appropriate fresh fruit or vegetable is necessary using the prick by prick method. Storage, cooking or the digestive process may produce changes of food allergen so that skin or immunological testing give false negative results. The unheated enzyme added to flour alpha-amylase (Asp 0 2) derived from *Aspergillus oryzae* is a potent sensitizer in the baking industry, but in bread, because it is heat labile, it rarely causes trouble to consumers [22]. In contrast, heating or

storing of food may be important in some food anaphylactic reactions. This was seen in an atopic girl who had anaphylaxis from aged or heated pecan nuts but could safely eat fresh pecans [23]. Raw peanuts sometimes also can be safely eaten by patients anaphylactically sensitive to peanuts which have been roasted. Many food allergies are destroyed by heating but it is as well to remember that Küstner of the classical Prausnitz-Küstner test was allergic to cooked fish but not to raw fish [24].

The isolation and characterization of allergens from moulds is beset with many difficulties. Even when working with a well-characterized strain, because of the inherent ability of the organism to vary in response to growth conditions, it may be almost impossible to grow two consecutive cultures with even similar antigenic contents [25,26]. Different isolates from the same species may exhibit different allergenic profiles. There are at least 17 different allergens in an extract of *Cladosporium herbarum* isolate [27]. Recombinant DNA techniques have been employed in molecular characterization of allergens which have resulted in availability of sequence data and biological functions of a number of allergenic proteins or polypeptides from a variety of sources. These include *Cladosporium*, grass, tree, weed, mites, cat and venoms [28]. Such studies give much hope for more efficacious skin testing and immunotherapy. With extracts of *cladosporium herbarum*, it has been found that the DNA clone encodes a 278 amino acid polypeptide homologous to the C-terminal of 70 KD heat shock protein (psp 70). This polypeptide possesses features common to other psp's 70.

A recent review article on defining allergens of mammalian origin [29] points to the difficulties of identification and subsequent purification of allergenic extracts, but there is hope that with the advent of rDNA and gene-cloning techniques a new approach to allergen research dawns. There are many factors which affect the stability of any allergenic extract, which are mixtures of labile and stable proteins. Temperature, dilution, diluent, type of allergen and protease antigens can all affect the strength of the extract. Human serum albumin (HSA) or Tween 80 improves the stability of allergenic extracts but phenol has the opposite effect. The effect of sucrose against the degrading effects of phenol is very evident in preliminary experiments [30] which suggests that 0.5 M sucrose may be considered as a substance for the development of more stable allergenic extracts.

Conclusion

Labelling an extract with a major allergen content is deceptive because such an extract may be missing minor allergens which may be major for some subjects. Nor can the extract be labelled with all the major and minor

allergens. 'Potency' is the relative ability of an allergenic extract to elicit a biological response. But an extract lacking one or more allergens will be potent for some individuals but not to others. Preparation of ideal standardized extracts is now possible if not yet attainable, so that by using standard molecular biology techniques major and minor allergens can be cloned, sequenced and tested for biological potency.

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