

Evaluation of allergen exposure

Sten Dreborg, MD, PhD Lerum, Sweden

Key words: *Alternaria* species, home environments, mold, environmental allergens

In this issue of the Journal, Salo et al¹ report results on the exposure to *Alternaria* species antigens using samples from the National Survey of Lead and Allergens in Housing.² The article brings up some problems with this and similar studies, which should be discussed.

STUDY DESIGN

The design² of the study makes it possible to draw conclusions on the presence of *Alternaria* species antigen on a nationwide basis and whether, for example, exposure to *Alternaria* species antigens differ between major regions, but it is not possible to draw any conclusion regarding the possible exposure in smaller regions or areas. Thus the article gives only an overview of the total *Alternaria* species antigen exposure but no detailed information on *Alternaria* species allergen exposure, which is practically useful to practicing allergists.

The trial is cross-sectional. In such studies, secondary results cannot be used for anything but setting up new hypotheses (Fig 1). Before any firm conclusion can be drawn, the hypothesis set up on the basis of results from a cross-sectional study must be confirmed in a prospective trial that, if suitable and possible, should be interventional.

The conclusion by the authors that the presence in the home of dehumidifiers contributes to a higher level of *Alternaria* species antigen in the house can be explained by the humidity in the machine, which might favor mold growth.

On the other hand, I cannot find any biologically plausible explanation why smoking would contribute to higher levels of *Alternaria* species antigens. Such conclusions must be interpreted with great caution. Randomization of smoking habits is not possible. Then there is a high risk that there is an indirect instead of direct

Abbreviation used

IUIS: International Union of Immunological Societies

relationship between smoking and the presence of *Alternaria* species antigen. The hypothesis that tobacco smoking increases exposure to *Alternaria* species antigen has no plausible explanation. Therefore the conclusion that tobacco smoking increases *Alternaria* species antigen levels cannot be accepted without further trials designed to investigate this proposal.

For the purpose of investigating mold allergen levels, samples were taken during the summer, autumn, and winter (ie, during periods with quite different climatic conditions). This is not optimal, and the design could influence the results. I think samples should be taken during a limited period of time with defined indoor conditions and with information on the type of air conditioning used in the home because contaminated central or window air conditioners might favor mold growth. The most useful information about the contribution of the indoor environment to mold exposure would be obtained if samples were taken in the winter when doors and windows are closed, at least in the northern United States. The design² of the study¹ will also influence the relevance of the levels in American homes of cat and dog allergens³ and mite allergens⁴ published in this Journal.

EXPOSURE TO ALTERNARIA SPECIES: ANTIGEN OR ALLERGEN?

The investigators used a polyclonal antibody against total *Alternaria* species antigen and not an mAb or polyclonal antibody against a defined *Alternaria* species allergen. This issue is very important. As pointed out by Aalberse and van Ree,⁵ it is essential to "pick your antibodies with care." I would like to add the following: Also pick your antigen-allergen standard and antigen for production of antibodies with care.

To have a practical value, results from studies must be relevant (ie, "predict what we want to know"⁵). That means that the exposure to *Alternaria* species allergen is most relevant, whereas exposure to *Alternaria* species antigen is of less value.

Received for publication June 15, 2005; accepted for publication June 20, 2005.

Reprint requests: Sten Dreborg, MD, PhD, Alingsåsvägen 11, SE-443 35 Lerum, Sweden. E-mail: sten.dreborg@telia.com.

J Allergy Clin Immunol 2005;116:620-2.

0091-6749/\$30.00

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doi:10.1016/j.jaci.2005.06.027

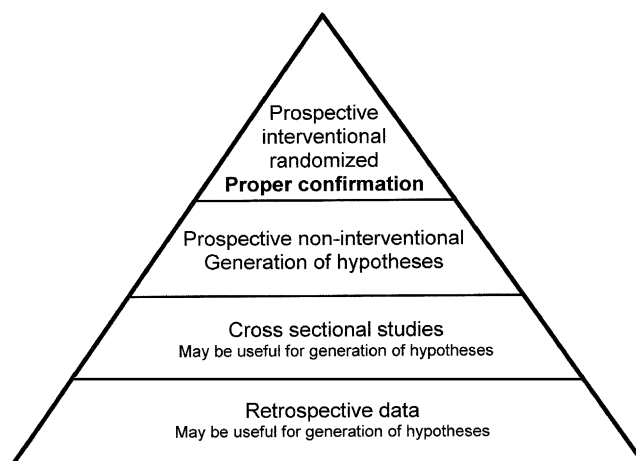


FIG 1. The relevance of different types of clinical studies for documentation. Modified with permission from Dreborg S. Dietary prevention of allergic diseases in infants and small children. *Pediatr Food Allergy Gastroenterol Nutr.* 2004;6(2):4-11.

The problem with the study by Salo et al,¹ with respect to the antigen-allergen assay, is 2-fold: the antigen-allergen and the antibody.

Antigen-allergen

Extracts of allergenic source materials (eg, the mold *Alternaria alternata*) contain a number of proteins capable of inducing an antibody response in humans and animals. Some of these antigens cause IgE production in humans and are then named allergens.

Mold extracts are complex mixtures of proteins. Mold extracts from the same species vary in allergenic and antigenic composition. Molds grown on different media from different sources produce partly different antigens and allergens. In fact, even cultures of the same strain grown on slightly different media, differing only regarding the concentration of glucose, have different composition if extracted at the same time.⁶ There were also marked differences between samples taken from *Aspergillus* species cultures at different time points.⁶ Even multiple cultures of the same strain frozen and thawed and then grown on the same medium and at the same temperature do not give the same result.^{7,8}

The International Union of Immunological Societies (IUIS) subgroup on allergen standardization established an international reference standard of *Alternaria* species extract.⁹ The international standard was established nearly 20 years ago. It was characterized with methods that allowed identification of allergens but not variants of them. Thus variations in glycosylation, isoforms, and the presence of fragments was not taken into account. Today the IUIS *Alternaria* species standard can be characterized by using modern techniques. The IUIS standard preparation or any other well-defined and well-documented extract that is calibrated against the IUIS standard, kept freeze-dried, and frozen at low temperature with the lowest possible water content and that ensures stability should be used as the standard.

Salo et al¹ used a manufacturer's *Alternaria* species extract as the standard but did not document calibration of the manufacturer's extract against the IUIS standard.

Most assays for determination of the allergen content in the environment are based on a major allergen as the standard. The major allergen can either be purified naturally occurring allergen or a recombinant protein that, in some cases, such as Bet v 1, is one of many isoforms. By using such an isoform as the standard, it is only possible to determine the presence of that isoform, fragments of it, or cross-reacting proteins in extracts or dust samples.

The best standard for assays for determination of indoor allergens is a defined purified allergen or a defined recombinant allergen made available to all other researchers. The purified antigen-allergen can be purified by using mAbs¹⁰ or polyclonal antibodies¹¹ and affinity chromatography.

Antibodies

The antibody used by Salo et al¹ was produced by rabbits immunized with an *Alternaria alternata* extract with many undefined antigens present. Because the standard was a mixed *Alternaria* species extract and the antibodies were produced by rabbits, the presence of the total amount of *Alternaria* species antigens was determined by using this assay.

Batches of rabbit antibodies always differ to some extent. Different rabbits produce different amounts of antibodies with different affinity. All rabbits do not necessarily produce antibodies against all epitopes in the allergen extract or purified allergen. Furthermore, the composition of the antiserum differs between samples taken from the same rabbit at different times. These facts contribute to the difficulty to reproduce and use the results by Salo et al.¹ In 2 earlier publications by the same group,^{3,4} it is stated in the Methods section that mAbs were used. However, in fact, polyclonal rabbit antibodies were

used to detect the major allergen of dog dander, Can f 1. The results with an *Alternaria* species antigen assay,¹ polyclonal rabbit antibodies versus an affinity-purified allergen¹¹ or mAbs versus a purified or recombinant allergen,¹⁰ are not comparable. This is an issue that must be discussed.

Polyclonal antibodies against a single purified, naturally occurring allergen, as used by Schou et al,¹¹ will detect all possible variants of the naturally occurring allergen. The authors also showed that the standard curves, using the IUIS international standard and the purified Can f 1 as the standard, were parallel. They also showed that the polyclonal anti-Can f 1 antibody did not detect other allergens present in dog extract. However, the assay has the drawback that the polyclonal rabbit antibodies are difficult to reproduce. Thus the repeatability of the results is dependant on the size of the anti-Can f 1 polyclonal rabbit antiserum pool and its stability.

In most assays used for estimating allergen exposure in the environment, mAbs are used. Monoclonal antibodies can be produced for ever; that is, results obtained in one study can be compared with those in previous and later studies using the same antibody and the same well-defined purified or recombinant allergen as the standard.

On the other hand, mAbs do not react with all possible variants of major allergens and therefore do not result in the "correct exposure" to the major allergen, and the data are still less representative for the total exposure to all allergens of the species investigated. However, until we know more about the variability of allergens and the clinical importance of isoforms and fragments, assays for allergen detection should use mAbs against major allergens in combination with defined purified^{10,11} or recombinant allergens.

However, there are limitations also with mAbs. Aalberse and van Ree⁵ have recently reviewed the problems involved with mAbs used for detection of allergens.

SUMMARY

It will be practically impossible to compare the results of the study by Salo et al¹ with those of studies using similar antibodies produced by other rabbits immunized with other *Alternaria* species extracts. Furthermore, the

study by Salo et al¹ gives some information on the presence of *Alternaria* species antigen in American homes. However, it is not possible to compare the degree of exposure to *Alternaria* species antigen with that of cockroach,¹² mite,¹³ or pet^{10,11} allergens.

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