Editorial

Science from a black box

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IgE breeds IgE. We have suspected for more than 20 years that the largest risk factor for making allergen-specific IgE is to have allergen-specific IgE. This might sound like a description of immunologic memory, but it is not. In contrast to immunologic memory, which typically generates more antibody of the same specificity, in an expanding IgE response the newly produced IgE is very likely of a different specificity than the initial IgE: a different epitope on the same allergen or an epitope on a different allergen (epitope spreading). This is supported by the recent claim of a correlation between multiplicity of epitope recognition and total IgE levels.[1] For a discussion on possible mechanisms involved, see Aalberse and Platts-Mills.[2]

If "IgE breeds IgE" proves to be a major factor for IgE production, how does IgE production start in the first place? This question leads to the "initiator" allergen concept: allergens that induce an IgE response in a naive subject. Are allergens not all equal? Does a special category of more dangerous allergens exist that should be our main target for prevention? In a detailed serologic study of subjects participating in the German Multicentre Allergy Study birth cohort, Hatzler et al[3] investigated which allergen qualified most as an initiator allergen. They selected 126 children with seasonal allergic rhinoconjunctivitis before age 13 years and with IgE to grass. The winner as a potential initiator allergen was Phl p 1, which was present in 95 (75%) of 126 of the first positive serum samples. In 33 of these, it was the only grass allergen that was recognized. This was substantially more than for Phl p 5, with 46 positive results in a first positive sample and in just 4 cases the only positive reaction. Phl p 4 scored relatively high, but this response is difficult to interpret because of glycan-dependent cross-reactivity with vegetable foods.

The study is a good example of the power of commercially available test kits. However, the use of test kits raises some questions that need to be addressed. It is disturbingly common to find scientific publications referring in imprecise terms to such a test kit (often limited to the name of the manufacturer) with the sentence "used according to the manufacturer's instructions." In the present article the tests for IgE to allergenic components were performed with the ISAC allergen microarray (Phadia, Uppsala, Sweden). There is a reference to the manufacturer's instructions, but no indication of where to find these. It does not take much imagination to see the problems of a scientist reading this article a few years after publication who finds either that the test kit has disappeared or that the composition and instructions have changed over time. In the present case the Web site of the manufacturer[4] was found to provide a "Directions for use" document, stating that this document was issued in 2008 and revised in 2009. The 2008 document was no longer available at the Web site. This is a relatively trivial problem with a simple solution. The manufacturer should provide a version code of the kit (as was done in the present case but is not mentioned in the article), as well as of the instructions, in combination with a link to a Web site that would hold this information accessible for at least 10 years.

The next issue concerns the components of the test kit. More often than not, relevant details of some of the components will not be revealed to the user. For the allergen microarray, it is important for the reader to have information on the allergens used. It should be possible to identify differences in the reagents compared with other articles using nominally the same test kit. If a poorly performing allergen has been replaced by a better-performing allergen, such a change should be clear to the reader. At the lowest level of information, this could be done by using version numbers of the kit indicating minor or major adjustments. This would be helpful and should be easy to implement.

However, there is a much more fundamental issue at stake. The cornerstone of empiric science is that the results are made public in such a way that a peer scientist is able to understand the procedure and (in principle) to reproduce the experiment. Lack of availability and standardization of critical reagents used to be a problem. Technical developments, such as recombinant technologies and mAbs, have removed some of these hurdles. We now have many well-defined reagents shared by many scientists. However, mostly because of proprietary reasons, an increasing number of reagents are obtained as a "black box." A black box has been defined as "a device, system or object which can be viewed solely in terms of its input, output and transfer characteristics without any knowledge of its internal workings."[5]

What about reagents such as purified allergens on a microarray? It might be considered a major undertaking to make all the relevant information publicly available for well over a hundred proteins. However, devising an identifier system for recombinantly expressed proteins analogous to the digital object identification system[6] would already be a major step forward. This identifier should be version specific, preferably in a way that distinguishes between minor and major changes in the production process. Important bits of information could be linked to this identifier, such as the amino acid sequence of the expressed protein, the expression system, differences with natural proteins (eg, glycosylation, hydroxyprolines, and proteolytic processing), and aggregation state under nondenaturing conditions. Eventually, we will need a serologic comparison between the natural

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and recombinant proteins. This will take much effort, particularly for proteins with low expression as natural proteins. However, without this information, it would be unwise to make the move from natural to recombinant allergens.

How does all this reflect on the conclusion of the article by Hatzler et al[3] regarding the importance of Phl p 1 as an initiator allergen? Although 75% positive results clearly supports the major status of grass group 1 allergens, the 25% negative results are intriguing. There is potentially a technical issue that might explain some of these negative test results. Phl p 1 on the ISAC array is a recombinant protein. It has been reported that rPhl p 1 was not considered a suitable candidate as a reference protein for the CREATE project because of low IgE reactivity,[7] but this might well have been a different recombinant protein. Compared with protein expressed in a bacterial expression system, eukaryotic expression of Phl p 1 has been found to yield a protein with much better IgE reactivity.[8] Even bacterial expression might provide a protein with good immunologic reactivity.[9]

The literature reference for information on the recombinant proteins does not contain information on the expression system, purity or immune reactivity of each of these proteins individually. It is also not specified on the manufacturer's Web site.[4] "Tricks of the trade" have been around for a long time, but were (supposed to be) revealed upon scientific publication. Companies are understandably reluctant to release information that might compromise their commercial position. Yet I do want to know whether 25% of the children with pollen allergy really start their allergic career with an allergen different from grass group 1. The microarray manufacturer knows the answer, but I do not. This might not be the most important scientific question, but it could serve to make the point that the current situation is unsatisfactory. We scientists are becoming increasingly faced with "black boxes." Scientists, journal editors, and companies should start talking about how to deal with this situation.

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