

Correspondence

Is cross-reactivity a real or an imaginary concept?

To the Editor:

I am writing in response to an erratum (J Allergy Clin Immunol 1996;98:999) of an abstract of which I was the senior author, which was submitted for the 1994 AAAAI meeting.¹ The abstract identified, by molecular cloning, a novel cross-reactive allergen, referred to as KBG51, found in Kentucky bluegrass (KBG) pollen. The allergenicity of KBG51 in the form of glutathione-S-transferase (GST)-KBG51 fusion protein was tested by ELISA with sera of 48 patients who were highly allergic to grass pollens. The reactivity of GST-KBG51 was originally estimated at "about 60% of grass allergic patients." In the erratum Drs. Schon, Luo, and Ni estimated instead that 20% (10 of 48) of sera were positive without providing the rationale for their interpretation of the data.

The estimate of 60% reactivity was arrived at as follows. The 48 serum samples came from Norwegian patients who were allergic to grass pollens as determined by clinical history and RAST. The sera had been screened to be unreactive to GST antigen. To establish an ELISA cutoff value, 10 serum samples, negative to KBG extract, were assayed with GST-KBG51 on the same plate. Three of 10 sera exhibited high optical density (OD) values, which I considered to be spurious. These results were therefore not included in the analysis because: (1) extensive cross-reactivity is known among grass allergens; (2) sera showing no reactivity with grass pollen extracts are occasionally reactive with recombinant allergens; (3) the patients were pollen allergic, although results were negative with KBG extract on a previous ELISA experiment; (4) Western blot analysis indicated extensive cross-reactivity of KBG51 with components of pollens from other grasses, trees, and weeds, suggesting that among this group of KBG-negative allergic sera, the possibility of positive reactions with KBG51 existed; (5) the OD values of these 10 samples were not normally distributed, suggesting that a parametric statistic (i.e., mean and SD) could not be computed before rejecting the outliers; and (6) these three samples could be defined as positive by a common sense criterion often used in clinical medicine (>200%). The rejection of these three samples was statistically justified because they represented OD values well above the mean (i.e., >mean + 4 SD).

Using the remaining seven determinations, which were normally distributed, I calculated their mean + 2 SD to establish the ELISA cutoff. With this ELISA cutoff value, 27 of the 48 sera, 56.25%, or "about 60%" were found to be positive. An important consideration was that I took into account cross-reactivity of KBG51 antigen while considering reactivity of this antigen with human sera. Because this was a controversial issue before the publication of the erratum, experts in the field of allergy and immunology were contacted at the time. All said that the cross-reactivity should be taken into account. Three of these experts have examined the data and found my interpretation of the data reasonable.

The erratum, as published by Drs. Schon, Luo, and Ni, suggests that the percent reactivity of GST-51 was overstated. The percentage reactivity of an allergen with human sera, however, depends on the sampling population, that is, whether it is (1) randomly sampled from an unselected population, (2) obtained from an unselected allergic population, or (3) selected from a specifically grass-allergic population. For major grass allergens (e.g., group I allergens), the reactivity to grass is estimated at about 20%, 50%, or more than 90%, respectively, in each of the above groups. KBG51 (group IV allergens) reactivity was examined by using a selected group of sera from patients highly allergic to grass pollens. Therefore a finding of about 60% reactivity (instead of >90%) suggests that it is not a major allergen.

The percent reactivity reported in the abstract was a preliminary result. At the 1994 AAAAI Meeting, additional ELISA results were reported with sera from allergic individuals who live in various geographic locations worldwide (a total of 1000 serum samples including the 48 sera). The data presented showed that the percent reactivity to this antigen in allergic populations ranged from 0% to 44%, and in specifically grass-allergic populations, from 14% to 90%. Variation in percent reactivity depended on the geographic location from which the sera were collected.

As stated in the erratum, from the ELISA with the allergic population (1000 sera), Drs. Schon, Luo, and Ni inferred that there was about 24% reactivity with GST-KBG51 antigen; it is therefore improbable that the reactivity of the same protein with the sera of 48 patients who were highly allergic to grass pollens is less than 20%. Furthermore, the percent reactivity deduced by me for the GST-KBG51 recombinant protein from this data is comparable to the percent reactivity of the corresponding natural allergens (group IV allergens), that is, 50% to 60% of the grass-allergic population.

Drs. Schon, Luo, and Ni also refer to a second abstract published in the proceedings of the 1994 Stockholm International Congress of Allergology and Clinical Immunology and presented at the Meeting.² In this abstract a statement about the reactivity of the sera was made, which was an unintentional error. The statement should have read: "analysis by ELISA using sera of about 1000 allergic individuals showed that about 51% of sera possessed IgE Abs specific to KBG pollen extract and about 40% of these KBG positive sera possessed IgE Abs to rCRAL51 (a synonym then used for the GST-KBG51, because of its highly crossreactive property) protein"; that is, about 25% of sera of this allergic population contained antibodies to rCRAL51. The data were presented at the Stockholm Meeting in a *correctly* interpreted form, as stated above for the AAAAI Meeting. These data have not been published elsewhere. The corrected and now accurate statement is included in a patent application on KBG51.

The erratum by Drs. Schon, Luo, and Ni may raise the issue of whether cross-reactivity should be taken into account when one is determining reactivity of an antigen with human sera. In other words, is cross-reactivity a real or an imaginary concept? A plethora of published evidence has demonstrated extensive cross-reactivities that exist among diverse aeroallergens. The molecular basis of cross-reactivity has been elucidated at the level of B- and T-cell epitopes. Because human beings are exposed to a diverse array of inhalant and ingested antigens and because the immune system mostly encounters the antigens in the "processed" form, it is my opinion that reactivity is reactivity, be it caused by the original antigen or a cross-reacting antigen. Hence, in determining reactivity one has little choice but to take into account the cross-reactivity.

Shyam S. Mohapatra, PhD
University of South Florida College of Medicine
Departments of Internal Medicine
and Medical Microbiology and Immunology
Division of Allergy and Immunology
c/o VA Hospital, 13000 Bruce B Downs Blvd.
Tampa, FL 33612

REFERENCES

1. Luo M, Ni H, Schon AH, Mohapatra SS. Identification of a novel highly cross reactive allergen in Kentucky bluegrass pollen [abstract]. J Allergy Clin Immunol 1994;93:373.
2. Mohapatra SS, Luo M, Ni H. Identification of a new highly cross-reactive allergen in plant pollens. Allergy Clin Immunol News Supp 1994;2:73.

1/8/80973