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CORRESPONDENCE/REBUTTAL

Comment on Digestibility of Food Allergens and Nonallergenic Proteins in Simulated Gastric Fluid and Simulated Intestinal Fluid—A Comparative Study

Sir: Recently, T. J. Fu et al. (2002) conducted a study to evaluate the usefulness of digestive stability as a criterion for the assessment of the potential allergenicity of novel proteins including those introduced into foods through agricultural biotechnology. This paper dealt with a topic that is noteworthy in terms of the safety assessment of foods produced through agricultural biotechnology. Unfortunately, this study was not, in my view, a fair assessment of protein digestibility as a predictor of the allergenic potential of a novel protein.

As background, the use of protein digestibility or pepsin resistance has been advocated by several noteworthy organizations (the 1996 ISLI−IFBC decision tree, the 1996 FAO/WHO consultation, the 2000 FAO/WHO consultation, the 2001 FAO/WHO consultation on allergenicity assessment of GM foods, and the 2002 Codex ad hoc task force on safety assessment of biotechnology) as part of the allergenicity assessment of novel proteins (FAO/WHO, 1996; FAO/WHO, 2000, 2001, 2002; Metcalfe et al., 1996). These recommendations suggest that pepsin resistance should be used together with other criteria including sequence homology in the assessment. No single test including pepsin resistance is recognized as completely predictive of a protein's allergenic potential. In my view, the data from Fu et al. (2002) are not sufficient to warrant abandonment of pepsin resistance as one of several criteria in such assessments.

Fu et al. (2002) conclude that the digestive stability of proteins does not correlate well with allergenicity. However, I think that this basic conclusion may be erroneous on the basis of several factors.

(1) Fu et al. (2002) provide data that contradicts previous data published in the literature (Astwood et al., 1996). The key distinction seems to be in the detection of stable fragments of the protein, which some investigators detected but Fu et al. (2002) did not. These differences may be attributable to methodology. Fu et al. (2002) used an incredible pepsin to protein ratio of 10:1, which may have promoted more complete proteolysis more quickly. Fu et al. (2002) also used a less sensitive protein staining method (Coomassie, not colloidal, blue) that may have affected visualization of any existing firagments.

(2) Fu et al. (2002) can also be criticized on the basis of the selection of allergens and nonallergens used for this comparison. The data supporting the allergenicity of some of the selected allergens and nonallergens are weak. Papain and bromelain, for example, are not well described as ingestion allergens but are mostly known as occupational respiratory allergens (Baur et al., 1982; Lachowsky and Lopez, 2001). Actinidin primarily causes oral allergy syndrome, which is consistent with its rapid digestion (Besler et al., 2000). For nonallergens, I would not have chosen proteins that would be identified in sequence homology screens as potential allergens because digestive stability should never be used in isolation. Thus, the tropomyosins and lectins are rather poor choices.

(3) In the Fu et al. (2002) experiments, the true nonallergens are not stable (i.e., beef, pork, and chicken tropomyosin, rubisco, cytochrome *c*, PFK, sucrose synthase, and human lactalbumin). The other selected nonallergens such as proteinase inhibitors and lectins are good candidate allergens (based on sequence homology).

In the evaluation of the soundness of the Fu et al. (2002) conclusions, it is imperative to have clarity about what is and what is not an allergen. The arguments posed by Fu et al. (2002) fail badly in this important area.

In a perfect world (if this test were completely predictive), all nonallergens would be rapidly hydrolyzed. That appears to be true for the noncontroversial nonallergens selected by Fu et al. (2002) as noted above. However, should all stable proteins be allergens? I have never thought that this should be the case. In addition to digestive stability, such proteins must have the ability to stimulate the immune system in a particular way. I am not surprised that Fu et al. (2002) identified some stable proteins that were not allergens.

(5) Fu et al. (2002) stress the importance of the degree of allergenicity of a protein expressed as percent allergenicity. I only wish that it were possible to make such quantitative predictions on the basis of existing clinical evidence. This is very, very shaky in my opinion. Much of the data in the literature are dependent on IgE binding with sera from specifically selected patients. That is not the same as documenting that the protein is a true symptom-producing allergen for all of those individuals. Lactoperoxidase is a good example. It is mentioned as a milk allergen in only one study (Baldo, 1984) in all of the large volume of papers on milk allergy. All patients with IgE binding to lactoperoxidase also had significant IgE binding to other more prominent milk proteins, such as casein and lactoglobulin. So, is lactoperoxidase a clinically significant allergen or a laboratory curiousity? It is certainly not a proven

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allergen and should not be assigned a percent allergenicity equivalent to lactoglobulin and casein as Fu et al. (2002) did.

(6) Fu et al. (2002) used a 10× ratio of pepsin to protein; they used more pepsin that other investigators did. A 10× level is arguably ridiculous because in vivo protein would always exceed pepsin. The results presented by Fu et al. (2002) are, in my opinion, heavily dependent on this high ratio. In fact, they prove this in the results presented in Table 3 of their paper, but they do not extend these results to the entire list of proteins evaluated. Although scientists may disagree on the proper ratio, a standardized pepsin resistance assay is needed that is standardized in terms of the pepsin to protein ratio, pH, etc. The amount of pepsin should be based on enzyme activity and not on weight.

(7) Finally, pepsin resistance even under standardized conditions must be taken in a broader context. It is only one part of the allergenicity assessment and should be considered along with sequence homology searches and any other relevant data that exist. However, the relative abundance of the protein in the food is probably another factor to be considered together with pepsin resistance. Fu et al. (2002) determined that the major allergen in potato was labile to pepsin. However, patatin represents 20−40% of the total protein of potatoes, and a single serving of French fries can contain 5 g of patatin. Thus, abundance may influence the dose of patatin that survives digestion. The factors of pepsin resistance and comparative abundance of the protein should perhaps be considered together in the assessment.

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