Correspondence

Reply

To the Editor:

Buchanan and Frick[1] raise some questions about our paper on thioredoxin allergenicity.[2] It is true that we have cloned thioredoxins and profilins using phage display technology[3]; however, this work demonstrated cross-reactivity between wheat and maize profilin, but not between thioredoxin and profilin, which represent completely different molecular structures. In contrast, in our work, we have demonstrated cross-reactivity between wheat, maize, and human thioredoxin *in vitro*.[2] We fully agree that detection of IgE-binding *in vitro*, although highly specific as shown by competition ELISA, does not demonstrate the allergenicity of a protein. However, recently we have also isolated thioredoxins as IgE-binding proteins from the mold *Aspergillus fumigatus* (Asp f 28 and Asp f 29), and from the yeast *Malassezia furfur* (Mala s 13).[4] This study shows that Mala s 13, Asp f 28, and Asp f 29 are recognized by serum IgE of *M furfur*-sensitized individuals in ELISA and Western blot analyses, and cross-reactivity has been confirmed in competition ELISAs. Moreover, Mala s 13 was able to induce strong positive skin prick tests in patients showing detectable serum IgE against Mala s 13, and both Mala s 13 and human thioredoxin elicited eczematous reactions in these patients in atopy patch tests, clearly showing the allergenicity of the proteins *in vivo*. The thioredoxins show between 41.9% and 49.5% sequence identity at the primary structure level, and the identical amino acids are scattered over the whole length of the primary structure. The solved 3-dimensional structure of Mala s 13 compared with the solved structure of human thioredoxin (A Limacher et al., unpublished data, 2006) reveals that shared identical amino acids distantly located at the primary structure level assemble to contiguous patches covering surface-accessible areas comparable to those buried by antigen-antibody interactions, thus providing a logical explanation for the observed cross-reactivity at the molecular level.

So far, we have not investigated the presence of thioredoxin in Western blot analyses of wheat extracts. However, an unequivocal identification of thioredoxin in extracts separated on SDS-PAGE gels without mAbs would be difficult because the wheat allergens α-amylase inhibitor 0.19 and CM17, β-purothionin, and even profilin show molecular weights in the same range as thioredoxin. Because of the relatively low resolution and the similar molecular weights of these allergens, they can hardly be resolved as single bands on SDS-PAGE. The content of thioredoxin in wheat extract should, however, be sufficient to detect the protein in Western blots with a mAb and to elicit type I skin reactions because the sensitivity of both methods lies in the picogram to nanogram range.

Of course, external addition of thioredoxin to wheat extract can contribute to the reduction of its allergenicity through reduction of disulfide bonds changing the 3-dimensional structure of allergens, because IgE-allergen interactions are exquisitely dependent on the 3-dimensional structure.[4] In contrast, sensitization to allergens in atopic individuals occurs through uptake via the mucosal pathway and presentation of antigenic peptides in the MHC class II context, a process that is independent from the enzymatic activity of the allergens.[5]

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