Dear Editor

Detection of Sugar Alcohol-Specific IgE

This is in reference to a recent letter on a pediatric case of erythritol-induced anaphylaxis published in Allergology International by Shirao et al. The authors have performed a careful and detailed study of this rare case, and have confirmed erythritol-induced anaphylaxis by positive skin prick test (SPT), double-blind, placebo-controlled food challenge (DBPCFC) and basophil-activation test (BAT). Further, they have mentioned that they were unable to detect erythritol-specific IgE in the allergic subject’s serum. However, since BAT for the allergic subject was clearly positive for erythritol, Shirao et al. have concluded that BAT for CD203c expression is a simple and useful method for identifying rare offending allergens in adverse reactions to foods.

Several years ago, a similar situation was encountered during the identification of the low molecular weight allergen in an adult case of erythritol allergy to pomegranate (Punica granatum) and cultivated mushroom (white button mushroom; Agaricus bisporus) wherein mannitol was identified as the allergen based on positive SPT of food extracts and isolated mannitol from pomegranate and mushroom extracts; pomegranate allergy was confirmed by DBPCFC using apple juice (contains sorbitol, but not mannitol) as the control and the vehicle for food challenge. In these two studies also, mannitol-specific IgE could not be detected in the allergic subject’s serum. The main reason appears to be the lack of binding of the hydrophilic mannitol (or any other sugar alcohol) to the hydrophobic polystyrene surface of microtiter wells. Therefore, to circumvent this technical problem, mannitol groups were created on a carrier protein, and the protein conjugate bearing mannitol groups was used as the coating antigen in direct ELISA; specifically, mannitol groups were obtained on keyhole limpet hemocyanin (KLH) by reductive amination of D-mannose. This conjugate was also used for immobilization on to Sepharose CL-6B to obtain a hapten affinity chromatography matrix. The allergic subject who had anaphylaxis to pomegranate and mushroom also suffered from anaphylaxis to a chewable drug, Cisapid MPS® (active ingredients: cisapride monohydrate and methyl polysiloxane). The eluate from the hapten affinity chromatography of the allergic subject’s serum was used as the source of specific IgE, and thus it was convincingly demonstrated that the allergen in the chewable drug was the drug excipient mannitol based on positive results from ELISA and SPT of isolated mannitol from the chewable drug as well as component-resolved diagnosis. The reductive amination reaction of aldose sugars with proteins in vivo resulting in Schiff base formation appears to provide a basis for the mechanism of allergenicity and immunogenicity of sugar alcohols, and these aspects have been described in detail in the hypothesis proposed by Venkatesh and Hegde.

The utility of erythritol-KLH-Sepharose CL-6B affinity matrix for the isolation of IgG antibodies specific to erythritol from rabbit polyclonal antiserum has been demonstrated further for the characterization of isolated erythritol-specific IgG antibodies; similarly, other hapten (sugar alcohol) affinity chromatography matrices have been prepared and used for the isolation and characterization of mannitol- and xylitol-specific IgG antibodies from rabbit polyclonal antisera. The content of these sugar alcohols in natural foods and some processed foods as well as the reported allergic reactions to sugar alcohols have been reviewed recently. Erythritol (E968), xylitol (E967), mannitol (E421) and sorbitol (E420), generally referred to as ‘sugar alcohols’ or ‘polyols’, are commonly used as reduced-calorie bulk sweeteners in the preparation of processed foods, and hence are considered as food additives. The characteristics of these sugar alcohols and their utilization as reduced-calorie bulk sweeteners in the preparation of processed foods have been well covered in a comprehensive treatise.

A flow chart depicting the preparation and isolation of IgG antibodies specific to sugar alcohols is shown in Figure 1. Since IgG antibodies specific to a particular sugar alcohol can be isolated from rabbit specific antisera, it follows that the hapten affinity chromatography matrix can be utilized for the purification of the particular sugar alcohol-specific IgE from allergic serum sensitized to a sugar alcohol, followed by direct ELISA using the corresponding sugar alcohol-protein conjugate as the coating antigen. The method is simple and easy to use for analysis of sugar alcohol-specific IgE from allergic human serum; moreover, the affinity chromatographic matrix can be stored in the presence of a suitable preservative and protease inhibitors at 4°C for a long time, and is re-usable. A column bed volume of 2 mL is sufficient to process 4-6 mL of normal or allergic human serum.

In view of the allergic reactions reported to erythritol, mannitol and xylitol (reviewed in reference 9) including the recent child case of erythritol-induced anaphylaxis, it is probable that more cases of allergic reactions to these sugar alcohols may likely occur in the future among the consumers due to the widespread consumption of processed foods containing sugar alcohols. In addition to SPT, BAT and food challenge tests, a confirmatory ELISA for the presence of sugar alcohol-specific IgE in the allergic serum should prove highly valuable. Once the causative allergen has been identified as a small molecule, affinity purification of sugar alcohol-specific IgE from allergic human serum on sugar alcohol-KLH-Sepharose...
**Fig. 1** Flow chart for the generation of sugar alcohol-specific IgG antibodies. Sugar can be any monosaccharide. Antibodies specific to D-mannitol, meso-erythritol and D-xylitol have been generated using the scheme outlined in this flow chart [Reproduced with permission from reference 9; ©2010, Nova Science Publishers]. Indirect competitive ELISA has been developed for the quantitation of erythritol and xylitol in foods using sugar alcohol-specific IgG antibodies.\(^9\) It should be noted here that the sugar alcohol-KLH-Sepharose CL-6B affinity matrix is of use in the detection of sugar alcohol-specific IgE from allergic human serum, as has been demonstrated in the case of mannitol-specific IgE.\(^4\)

<table>
<thead>
<tr>
<th>Carrier protein (BSA)</th>
<th>+</th>
<th>Sugar (D-Mannose/D-Xylose/D-Erythrose)</th>
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</thead>
<tbody>
<tr>
<td>Reductive amination</td>
<td>↓</td>
<td>NaBH₃CN pH 8, 37°C</td>
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<tr>
<td>Sugar alcohol-protein (BSA) conjugate (immunogen)</td>
<td>↓</td>
<td>Immunization of rabbits</td>
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<td>Collection of polyclonal antiserum</td>
<td>↓</td>
<td>Hapten-affinity purification on sugar alcohol-KLH-Sepharose-CL-6B affinity matrix</td>
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<td>Sugar alcohol-specific antibodies</td>
<td>↓</td>
<td>Immunoassays for specific sugar alcohols in biological substances</td>
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**REFERENCES**