Short Communication

Measurement of antigen-specific antibodies in human serum and saliva by multiple antigen simultaneous test

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ABSTRACT

Expression of antigen-specific serum IgE and IgA, and saliva IgA were measured in four volunteers using a Multiple Antigen Simultaneous Test (MAST). Two donors, expressing allergen-specific IgE in serum, expressed antigen-specific serum IgA strongly, but saliva IgA weakly. The other two donors, not expressing serum IgE, expressed serum IgA weakly and saliva IgA strongly. These results suggest a possibility that secretory IgA plays an important role in the prevention of food allergy. To clarify the role of IgA, further investigation on allergic patients is necessary.

Key words: allergy, IgA, IgE, MAST, saliva

INTRODUCTION

Recently, food allergy has become a serious health problem not only for children but also for adults.1,2 Some types of food allergy are mediated by allergen-specific IgE bound to mast cells or basophils.1 The binding of allergen to the membrane-bound IgE induces degranulation of these cells and releases inflammatory mediators such as histamine, platelet activating factors and leukotrienes. Although the serum IgE levels of healthy individuals are very low, they often elevate markedly in allergic patients.3 Thus, the measurement of total and allergen-specific serum IgE is used for the diagnosis of food allergy. Though serum IgE plays an important role in the development of food allergy, other classes of antibodies such as IgA also affect the process. Secretory IgA inhibits the absorption of allergens from the small intestine.4 Therefore, secretory IgA may inhibit the incidence of food allergy. However, information on the expression of allergen-specific IgA is very limited. It is known that IgA is secreted not only in intestinal secretions, but in saliva, tears and colostrum.4,5 We therefore tried to apply a multiple antigen simultaneous test (MAST) for IgE determination to the measurement of allergen-specific IgA.

METHODS

Serum and saliva samples were collected from four volunteer students (2 males and 2 females, 23–26 years old). One volunteer had been diagnosed as being allergic to soybean (MS) and one had been diagnosed as having atopic dermatitis (MT). The others (MI and KY) have never been diagnosed as having had an allergic disease and had no subjective symptoms. Informed consent was obtained from all volunteer students.

For the measurement of antigen-specific serum IgE, reactions were done according to the standard method recommended by the manufacturers (Mast Immunosystems, Mountain View, CA, USA). In brief, MASTette Test Chambers were filled with serum and allowed to stand at room temperature for 16 h. Peroxidase (POD)-conjugated anti-IgE supplied with the kit was diluted with dilution buffer and introduced to the test chambers before being allowed to react at room temperature for 4 h. After incubation with the substrate solution supplied with the kit, the test chambers were set in a supplied Photocassette and exposed to a Polaroid film. Chemiluminescent intensity of bands corresponding to each antigen was read using a supplied densitometer.

Then, the MAST method was applied to the measurement of antigen-specific IgA using a POD-conjugated anti-human IgA (Dakopatts, Glostrup, Denmark) in place of POD-conjugated anti-IgE supplied with the kit was diluted with dilution buffer and introduced to the test chambers before being allowed to react at room temperature for 4 h. After incubation with the substrate solution supplied with the kit, the test chambers were set in a supplied Photocassette and exposed to a Polaroid film. Chemiluminescent intensity of bands corresponding to each antigen was read using a supplied densitometer.

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according to the method reported by Fritsche and Bonzon. When serum or saliva samples were diluted with 0.1% fish gelatin, negative control level decreased more quickly compared with the samples diluted with the buffer appended for POD-conjugate dilution. Thus, 0.1% fish gelatin for sample dilution was used in this study. The optimum dilution ratio to decrease the negative control level and to obtain the highest positive signals was 100 times for serum IgA and 25 times for saliva IgA.

**DISCUSSION**

Although we used fish gelatin for sample dilution because of its low antigenicity, it has recently been reported that some individuals are sensitive to bovine gelatin injected with vaccines and most of them have bovine gelatin-specific IgE. However, only two of 11 patients showed allergic symptoms after eating gelatin-containing candies. This suggests that the allergenicity of orally administered gelatin is much weaker than that of other allergens.
Figure 1 shows the expression of allergen-specific antibodies measured by the MAST method. One volunteer (MS) expressed serum IgE strongly to Japanese cedar and weakly to house dust, egg white, milk and soybean. Volunteer MT expressed IgE to mite and cat dander, but the others, MI and KY, did not. The two donors expressing allergen-specific IgE showed strong and widespread expression of serum IgA and weak expression of saliva IgA. On the other hand, the two IgE-negative donors showed limited expression of antigen-specific serum IgA, except for cat dander, milk and beef, and stronger expression of antigen-specific saliva IgA.

Most saliva IgA is dimeric and derived from the mucosal immune system, while serum IgA is largely monomeric and derived from plasma cells in the bone marrow. The difference between expression of serum and saliva IgA suggests that their production or secretion is regulated independently.

Secretory IgA of mucosal tissues such as the small intestine and respiratory tract is known to be the first barrier against various pathogens such as bacteria, viruses and food antigens. In the case of IgA production, IgA plasma cells primed at intestine migrate via the systemic circulation to secrete IgA in saliva and tears. To understand the activity of the gut-associated lymphoid tissue, we measured the expression of antigen-specific IgA in saliva and observed a negative relationship between the expression of serum IgE and saliva IgA. Deficiency or poor expression of IgA in infants is related to the development of food allergy. These results suggest that secretory IgA plays an important role in the prevention of food allergy. However, because of the small number of cases in our study, further study with a large number of patients is necessary to clarify the role of secretory IgA.

We showed here that the MAST system was useful for determination of allergen-specific IgA as well as IgE. Studies of antigen-specific IgA expression in allergic patients using this system will contribute to the elucidation of the mechanism of allergy development.

**REFERENCES**