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Application of Multiple Antigen Simultaneous Test to the Measurement of Allergen-specific IgA and IgG in Human Sera and Saliva

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Multiple antigen simultaneous test (MAST) kit using for the measurement of allergen-specific human serum IgE was applied for the measurement of antigen-specific IgA and IgG in human serum and saliva. In measurement of IgA and IgG, an appropriate dilution by fish gelatin was necessary, 5^2 times for saliva IgA, 10^2 times for serum IgA and 10^3 times for serum IgG. When the expression of antigen-specific serum IgE, IgG and IgA, and saliva IgA in four volunteers were studied using the MAST method, a positive relationship was observed in the expression of serum IgE and serum IgA, and a negative relationship in the expression of serum IgE and saliva IgA. These results suggest possibilities that the expression of serum and saliva IgA are regulated differently and secretory IgA plays a putative role in the amelioration of food allergy.

INTRODUCTION

Recently, food allergy has become a serious health problem not only for children but also for adults. Type I allergy plays a crucial role in the induction of some types of food allergies mediated by allergen-specific IgE bound to mast cells or basophils (Metcalfe, 1991). The binding of allergen to the membrane-bound IgE induces degranulation of these cells and release of inflammatory mediators such as histamine, platelet activating factor and leukotrienes. Although the serum IgE level of healthy individuals is very low, it often markedly elevates in allergic patients (Björkstén *et al.*, 1983) Thus, measurement of total and allergen-specific serum IgE is prerequisite for diagnosis of food allergy. To detect allergen-specific IgE, radioallergosorbent test (RAST) is adopted most frequently (Adler *et al.*, 1991; Williams *et al.*, 1992). Multiple antigen simultaneous test (MAST) is also available (Miller *et al.*, 1984; Brown *et al.*, 1985; Agata *et al.*, 1993).

In addition to IgE, other classes of antibodies affect the allergic reactions. Secretory IgA (sIgA) presents in intestinal fluid, saliva, and tears so on (Shorter and Tomasi, 1982; Watanabe *et al.*, 1984). Intestinal sIgA inhibits the absorption of allergens from the small intestine (Shorter and Tomasi, 1982). Some types of IgG also inhibit the allergic reaction through the competition with IgE (Aalberse *et al.*, 1993). However, the information on the expression of allergen-specific IgA and IgG is in human serum and secretory fluids limited. In the present paper, we applied the MAST system for the measurement of

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allergen-specific IgA and IgG in human serum and saliva to obtain the further information on Ig expression. The preliminary part of this study was reported previously (Takasugi *et al.*, 1996).

MATERIALS AND METHODS

Materials

Peroxidase (POD)-conjugated anti-human IgA and IgG were purchased from Dakopatts (Glostrup, Denmark) and MAST kit from Hitachi Chemical Co. (Hitachi, Japan). Fish gelatin was purchased from Sigma Chemical Co. (St. Louis, MO) and 0.1% fish gelatin dissolved in phosphate buffered saline containing 0.05% Tween 20 (TPBS) was used for antibody or sample dilution.

Serum and Saliva samples were collected from 4 volunteer students (two males and two females, 21–26 year olds) in our department. Informed consent was obtained from all donors.

Measurement of antigen-specific immunoglobulins by MAST

Serum or saliva samples were diluted either with the dilution buffer attached to the MAST kit or with 0.1% fish gelatin dissolved in TPBS, except for the case of IgE measurement where samples were not diluted. The reaction was conducted according to the method recommended by the manufactures. Samples were filled in MAST pette Test Chamber and stood for 16 hr at room temperature. For measurement of IgE, POD-conjugated anti-human IgE attached to the kit was diluted with the attached buffer or with 0.1% fish gelatin dissolved in TPBS to know the effect of fish gelatin on measurement by MAST method. Then it was added to the Test Chamber and reacted at room temperature for 4 hr.

In the cases of IgA and IgG, POD-conjugated anti-human IgA and IgG diluted with the attached buffer were used instead. Test Chamber was washed 3 times with the attached washing solution between each step. After reacting with the substrate solution, the Test Chamber were set in an attached cassette and exposed to a Polaroid film. Chemiluminescence intensity of bands corresponding to each antigen was read using the attached densitometer and expressed in volts.

RESULT

Effect of fish gelatin on measurement of allergen-specific IgE

To avoid nonspecific adsorption of antibodies, blocking with milk proteins or gelatin is routinely adopted to enzyme-linked immunosorbent assay (ELISA), and these blocking solutions are usually used for dilution of samples or antibodies (Takasugi *et al.*, 1996; Yamada *et al.*, 1989). Since the expression of IgA and IgG was much higher than that of IgE, sample dilution is inevitable for measurement of allergen-specific IgA and IgG. Thus we examined the effect of blocking solution used in ELISA on the determination of allergen-specific antibodies using the MAST kit.

To examine the effect of blocking solution on IgE measurement, POD-conjugated anti-human IgE was diluted 50 times with the dilution buffer or fish gelatin, which has

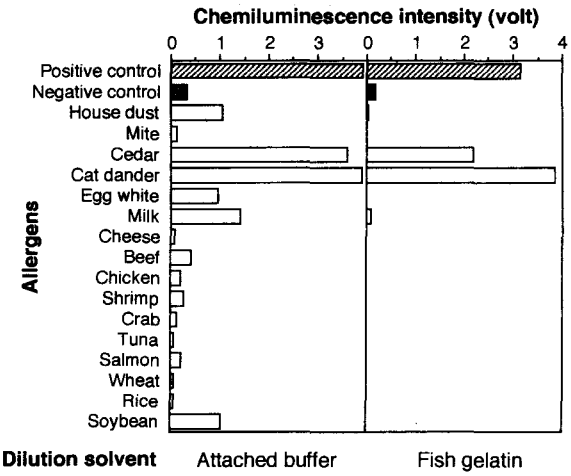

Fig. 1. Effect of fish gelatin on measurement of antigen-specific IgE in human serum.
POD-conjugated anti-human IgE attached to the kit was diluted with the attached buffer or fish gelatin and serum samples were not diluted.

been used for measurement of food allergen-specific antibodies using ELISA (Yamada *et al.*, 1991). As shown in Fig. 1, positive signals were given against house dust, cedar, cat dander, egg white, milk and soybean when the anti-human IgE was diluted with attached buffer. When the antibody was diluted with fish gelatin, the intensities of all signals including those of the positive control were markedly decreased and signals for house dust, egg white, milk and soybean were almost disappeared. This means that the dilution of anti-human IgE with fish gelatin is inappropriate for IgE measurement.

Effect of sample dilution on measurement of allergen-specific IgA and IgG

Then, the MAST kit was applied to the measurement of allergen-specific IgA and IgG using POD-conjugated anti-human IgA and IgG. Since the MAST system gives the relative Ig content corrected by the negative control value which does not fix any antigens, the background signal must be as low as possible for accurate analysis. When the saliva sample was sequentially diluted by 5 times, the negative control value decreased markedly after dilution of 5^2 times with fish gelatin and after 5^3 times dilution with the attached buffer (Fig. 2). Signal intensities for antigen were strongest in the sample diluted 5^2 times with fish gelatin, and then decreased markedly in the samples diluted further. After 5^2 times dilution with fish gelatin, signal intensities for house dust, cedar, mite, cat dander, egg white, shrimp, crab and wheat were exceeded voltage of 1.0 as chemiluminescence. When sample was diluted with attached buffer, signal intensities were much lower than those of the sample diluted with fish gelatin. Since a similar relationship was observed in serum IgA and IgG measurement, we diluted saliva and

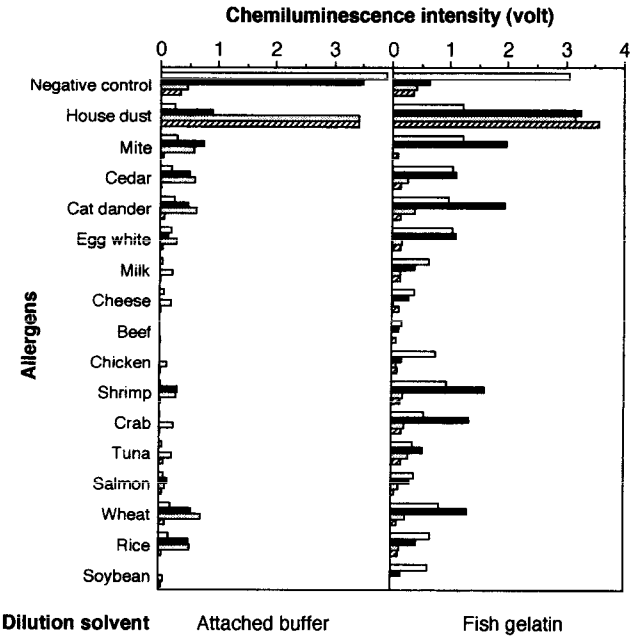

Fig. 2. Effect of sample dilution with fish gelatin on measurement of IgA in human saliva.
Samples were diluted 5 (□), 5^2 (■), 5^3 (▨) and 5^4 (▩) times with the attached buffer or fish gelatin, and used for measurement of antigen-specific saliva IgA.

serum samples with fish gelatin thereafter.

Figure 3 shows the relationship between the dilution ratio and signal intensities for negative control and two positive signals for house dust and crab in samples diluted with fish gelatin. The intensity of house dust-specific saliva IgA was high at more than 5 times dilution, but that of crab-specific saliva IgA was highest at 5^2 times dilution. Since signal intensities for most allergens were highest at 5^2 times dilution, we diluted saliva sample 5^2 times with fish gelatin thereafter.

In the case of IgA, the serum samples were sequentially diluted by 10 times because of its higher IgA content. The negative control value markedly decreased after 10^2 times dilution with fish gelatin and after 10^3 times dilution with the attached buffer for serum IgA. When samples were diluted with the buffer, signal intensities for allergens except for house dust were much lower than those of sample diluted with fish gelatin (data not shown). As shown in Fig. 3, signal intensity for house dust decreased slowly with sample dilution and that for crab decreased more rapidly. According to the above results we diluted serum samples 10^2 times with fish gelatin to detect allergen-specific IgA with a low background level.

In the case of serum IgG, the negative control value markedly decreased after 10^3 times dilution with fish gelatin and after 10^4 times dilution with attached buffer (data not

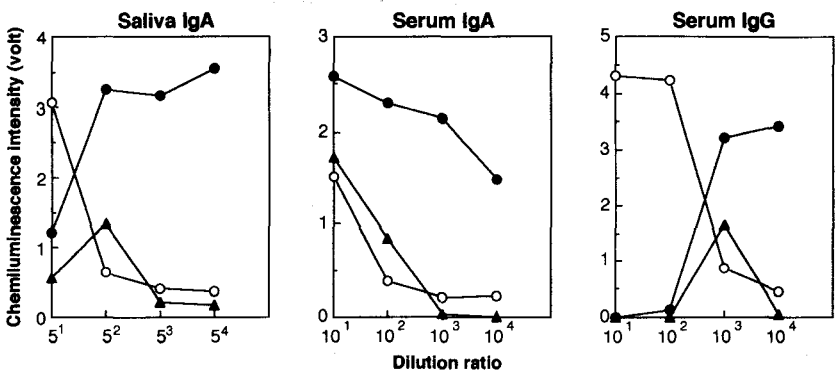

Fig. 3. Relationship between sample dilution and signal intensities by application of MAST. Saliva and serum samples were diluted with fish gelatin and allergen-specific IgA and IgG were measured using a MAST kit. Negative control (○), house dust-specific antibody (●), and crab-specific antibody (▲).

shown). As shown in Fig. 3, signal for house dust was detected when sample was diluted 10^3 and 10^4 times with fish gelatin, and signal for crab, only after 10^3 times dilution. Thus, sera were diluted 10^3 times with fish gelatin for IgG measurement.

Comparison of allergen-specific antibodies in human sera and saliva

Allergen-specific IgE was measured by the method recommended by the manufactures, and allergen-specific IgA and IgG were measured by the condition as described above. To compare the expression of allergen-specific antibodies, the expression of allergen-specific IgG is shown in Fig. 4 together with that reported previously (Takasugi *et al.*, 1996). Donor A strongly expressed serum IgE against cedar and cat dander, and weakly against house dust, egg white, milk and soybean. Donor B also expressed IgE against mite and cat dander, while donors C and D did not.

In the case of saliva IgA, all donors strongly expressed house dust-specific IgA, but the expression of IgA specific to other allergens, especially to food allergens varied extensively. Donor A, who expressed allergen-specific IgE most extensively, gave the poorest expression of saliva IgA. Donor B also expressed very low levels of allergen-specific saliva IgA, but donors C and D expressed saliva IgA more strongly.

In contrast, donors A and B expressed allergen-specific serum IgA more extensively than donors C and D. The latters strongly expressed serum IgA against some limited allergens such as cat dander, milk and beef, while the intensities of serum IgA against other allergens were much weaker.

In the case of serum IgG, difference among donors was much smaller than that observed in serum IgE, saliva IgA and serum IgA. Serum IgG specific to egg white, milk and cheese was expressed more strongly than that specific to other allergens in all four donors.

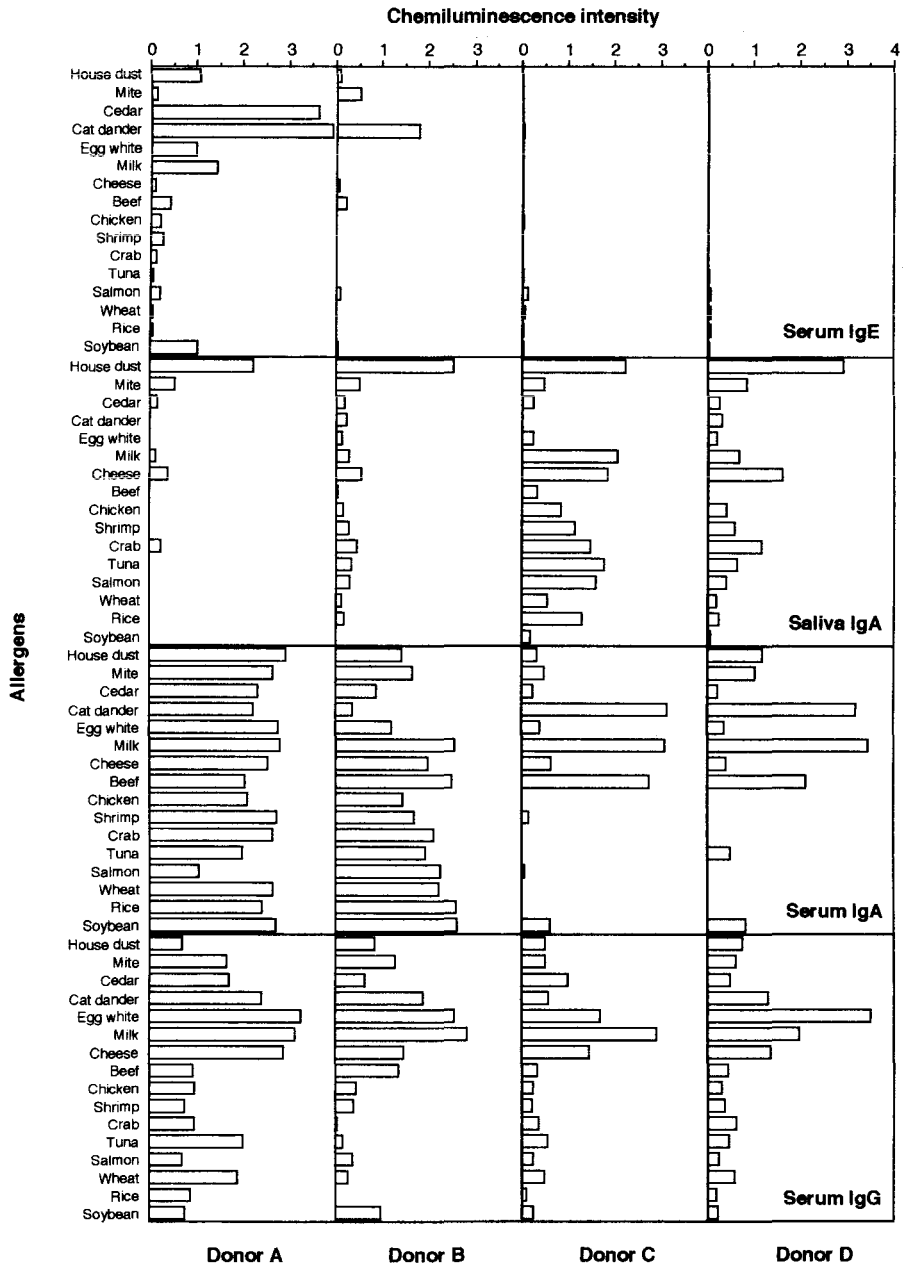

Fig. 4. Expression of antigen-specific antibodies in human serum and saliva.
The data for serum IgE, IgA and saliva IgA were the duplication of our preliminary report (Takasugi *et al.*, 1996).

DISCUSSION

In diagnosis of food allergy, RAST and its modified methods are extensively used for measurement of allergen-specific IgE (Williams *et al.*, 1992). In addition, several non isotopic alternates of RAST have been reported (Adler *et al.*, 1991; Brown *et al.*, 1985; Lee *et al.*, 1988). Among them, MAST is a convenient method for simultaneous measurement of IgE against multiple allergens (Miller *et al.*, 1984; Brown *et al.*, 1985; Agata *et al.*, 1993). We applied here the commercial MAST kit for measurement of IgA and IgG which modify allergic reaction in addition to IgE.

In the assays using antibodies such as ELISA (Engvall and Perlmann, 1971), blocking with bovine serum albumin or a milk protein preparation is essential to decrease nonspecific adsorption of antibodies (Yamada *et al.*, 1989; Yamada *et al.*, 1991). Since these bovine proteins are allergens themselves to be studied, alternate blocking agents with low allergenicity are necessary. Fish gelatin is one of such alternates (Yamada *et al.*, 1996; Fritsché and Bonzon, 1990). When fish gelatin was used for dilution of POD-conjugated anti-human IgE, however, the signal intensities of test samples were much lower than those of samples diluted with the attached buffer. Since the decline of positive signal intensities is undesirable for diagnosis of allergy, fish gelatin should not be used for IgE measurement.

Since IgA and IgG are expressed more intensively than IgE, sample dilution is inevitable for measurements of antigen-specific IgA and IgG. Fish gelatin gave higher signal intensities than the attached buffer. The signal intensities were highly dependent on sample concentration, and the highest signal intensities were obtained after dilution with fish gelatin 5^2 times for saliva IgA, 10^2 times for serum IgA and 10^3 times for serum IgG.

When the MAST was applied to the measurement of antigen-specific IgA, IgE and IgG, a positive relationship was observed between the expression of serum IgE and IgA, while a negative relationship between serum IgE and saliva IgA. Two donors having serum IgE expressed serum IgA strongly and extensively, while saliva IgA weakly and limitedly. In other two donors without serum IgE, an inverse relationship was observed in the IgA expression. Most of saliva IgA is dimmeric and derived from common mucosal immune system, while serum IgA is largely monomeric and derived from plasma cells in the bone marrow (Wolf *et al.*, 1994). In addition, the effects of a gluten-free diet on the concentrations of serum and saliva IgA specific to gliadin were different in patients with celiac disease and dermatitis herpetiformi (Patinen *et al.*, 1995). These results suggest that production or secretion of the two sources of IgA 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 (Shorter and Tomasi, 1982). In the case of IgA production, IgA plasma cells primed at the intestine migrate via the systemic circulation to secret IgA in saliva, tears and colostrum (Jackson *et al.*, 1981; Visakorpi, 1982). To know 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 expressions of serum IgE and saliva IgA. IgA deficiency or its malexpression in infants are related to the development of food allergy (Visakorpi, 1982; Sloper *et al.*, 1981; Kaufman and Hobbs, 1970, Noma *et al.*, 1996). These observations indicate that secretory IgA plays a crucial role in the

prevention of food allergy.

In the present study, we demonstrated that the MAST system was useful for determination of allergen-specific antibodies. The results suggest a regulatory role of the production of IgA, especially to secretory IgA in the development of food allergy. Although further studies with a large number of patients are necessary, measurement of antigen-specific antibodies may provide useful information on the intestinal situations in relation to food allergy.

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