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ORIGINAL CONTRIBUTION

Another Anti-allergic Mechanism: Antibody IgE Deglycosylation Induced by a Substance Extracted from Human Urine

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Enzymically-deglycosylated antibody IgE lost its allergic activity in mouse systemic anaphylaxis, though the IgE kept its antibody activity. IgE antibody obtained from mice treated with a substance extracted from human urine was deglycosylated. This IgE also lost the allergic activity on the systemic anaphylaxis but kept its antibody activity. These findings strongly suggest that glycosylation of IgE has a close relation to the binding of the Fc receptor, and that humans have another anti-allergic mechanism: in vivo *IgE antibody deglycosylation induced by the substance.*

INTRODUCTION

A substance extracted from human urine with a urea precipitation method has been shown to have anti-allergic activity in both human and mouse and is used for treatment of allergic diseases in Japan. This substance does not affect the production of antibody IgE, and the anti-allergic activity is not found immediately after the treatment [1]. This substance has been recognized as a polypeptide with the molecular weight of 1 K Da, but, in spite of many previous efforts, the amino acid sequence has not been currently clarified.

In allergic reactions, IgE antibody binds to the receptors of mast cells, and the mast cells secrete chemotactic factors which induce allergic symptoms. Granato and Neeser have reported that enzymically-deglycosylated IgE retains antigen-binding activity but loses its ability to bind to the mast cell receptors [2].

Here we hypothesize that the urine-derived substance decreases glycosylation of IgE antibody, and this deglycosylation decreases the allergic reactions of both of human and mouse. In this paper we report effects of the enzymically-deglycosylated mouse IgE antibody on the allergic reactions and investigate the effects of the urine-derived substance on IgE antibody deglycosylation.

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[c] *Abbreviations:* PS, physiological saline; RT, room temperature.
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MATERIALS AND METHODS

Animals

Male ddY mice (SLC Co., Tokyo, Japan), 8 weeks old, were used. They were housed in groups of five in plastic cages (338 x 140 x 225 mm) with free access to food (F2 : Funabashi Co., Tokyo, Japan) and water. The animal room was kept at 21 to 25°C with 50 to 60 percent humidity and illumination from 7:00 to 19:00. The following experiments were performed from 15:00 to 17:00. All of the experiments were approved by the Ethics Committee for Animal Experiments of Akita University School of Medicine, Japan.

Animal preparations

Animal preparations were performed as previously reported [1] with some modifications. Briefly, 200 mice were intraperitoneally injected with 10 mg/kg of ovalbumin (OA; Sigma Co., St. Louis, MA, USA) prepared with physiological saline (PS)[b]. Seven days after the injection, 80 mice were immunized intraperitoneally by injection of 3 mg/kg of OA; another 40 mice with 3 mg/kg of OA and 1 mg/kg of the substance (obtained from Nihon Kayaku Co., Tokyo, Japan) prepared with PS; another 40 mice with 3 mg/kg of OA and 10 mg/kg of the substance; and the other 40 mice with 3 mg/kg of OA and 100 mg/kg of the substance. Three days after the second injections, serum was individually collected from these mice.

Purification of the antibody IgE

The anti-OA IgE was purified from the serum using an affinity column bound with OA and an affinity column bound with anti-mouse IgE rat IgG (Southern Biotechnology, Inc., Birmingham, AL, USA). Furthermore, to exclude contamination with IgG, the IgE was further purified using an affinity column bound with anti-mouse IgG sheep IgG (Rockland, Gilbertsville, PA, USA). The purity of the IgE was confirmed with the use of SDS-PAGE.

Enzymic-deglycosylation of the antibody IgE

Ten mg of the purified anti-OA IgE obtained from mice treated with only OA was prepared with citrate-acetate buffer pH 4.0 and incubated at 37°C for 24 hours with 10 mU of Glycopeptidase A (EC 3, 5, 1, 52; Seikagaku Co., Tokyo, Japan). The solution was desalted, and the anti-OA IgE was purified using the affinity column bound with anti-mouse IgE rat IgG.

Glycosylation of the anti-OA IgE

Antibody IgEs purified as above and the enzymically-treated antibody IgE were prepared at 1 mg/ml with PS. One hundred µl of each of the IgE solutions was poured into wells of a 96-well plastic plate (Sumitomo Bakelite Co., Tokyo, Japan). After two hours at room temperature (RT). the wells were washed three times with washing solution (WS: PS containing 1 mM tris-HCl pH 7.4 and 0.005 percent Tween 20 from Wako Co., Osaka, Japan). As a blocking solution, 330 µl of 5 percent bovine serum albumin (Sigma) was poured into the wells and was left for 30 minutes at RT. Then, the wells were washed three times with WS. Then 100 µl of biotinized lectins prepared at 2 µg/ml with PS was poured into the wells. Each of the biotinized lectins used in the present study specifically recognizes the terminal structure of IgE sugar chains (DSA; Galβ1-4GlcNAc, AAL; Fucα1-2Gal or SSA; Sialα2-6Gal, Hohnen Co., Tokyo, Japan). After incubation for one hour at RT, the wells were washed four times with WS and then 100 µl of horseradish peroxidase-avidin (Hohnen) prepared at 0.1 µg/ml with PS was poured into the wells. After incubation for 30 minutes at RT, the wells was washed five times with WS. Color was developed with the use of the coloring kit (Sumitomo Bakelite). The light absorbance of the colored product

was measured at the dual wavelengths of 450 and 655 nm. These procedures were performed following the lectin-ELISA method previously described [3].

Antibody activities of the IgEs

One hundred μl of each of the IgE solutions prepared as above was poured into a well previously coated with 10 percent OA in a 96-well plastic plate (Sumitomo Bakelite). After two hours incubation at RT, the wells were washed three times with WS as described above. One hundred μl of horseradish-peroxidase conjugated anti-mouse IgE rat IgG (Southern Biotechnology) prepared to 2 μg/ml with PS was poured into the wells. After incubation for one hour at RT, the wells were washed four times with WS, and color was developed with the use of the coloring kit. The light absorbance of the colored product was measured at the dual wavelengths of 450 and 655 nm.

Activity of the antibody IgE on mouse systemic anaphylaxis

Antibody IgE-inducing systemic anaphylaxis experiments were previously reported [4]. The detection of the allergic activities of the IgEs was performed as follows. Briefly, the anti-OA IgE of the mice treated with OA only, that of the mice treated also with 100 mg/kg of the substance and the enzymically-deglycosylated anti-OA IgE were individually injected intraperitoneally into 10 intact mice at the dose of 0.7 mg/kg; as a control, 100 μl of PS was injected to the another 10 intact mice. One hour after these injections, the thickness of the right rear footpad of these mice was individually measured. Twenty-three hours after this, these mice were injected with 1 mg/kg of OA in 100 μl of PS containing 0.5 percent Evans Blue Dye (Sigma) through their tail vein. Twenty-five minutes after the injection, the body temperature was measured using a rectal thermometer (Terumo Co., Tokyo, Japan) for two minutes. One hour after the injection, the thickness of the right rear footpad was also measured, and at the same time, the leakage of the dye was observed in the ear skin.

RESULTS

Table 1 indicates the deglycosylations of the anti-OA IgEs. The reactivities of the terminal sugar chain structures of the enzymically-treated IgE were not found (Table 1, upper). Those of IgEs obtained from the mice treated with the substance were decreased in a dose-dependent way (Table 1, lower).

The enzymatic removal of sugar chains did not affect the binding activity to OA of the IgE antibody (Table 2, upper). Furthermore, the IgE deglycosylated by the urine-derived substance also showed comparable antibody activity (Table 2, lower).

The mice treated with the anti-OA IgE with intact sugar chains (collected from mice treated with OA only) showed the distinctive systemic anaphylaxis, which was indicated by decrease of the body temperature, swelling of the footpad and dye leakage in the ear skin. The mice treated with the anti-OA IgE lacking the sugar chains (collected from mice treated with 100 mg/kg of the substance) or the enzymically-deglycosylated IgE did not show the symptoms of systemic anaphylaxis, nor did the control mice (Table 3).

DISCUSSION

The sugar chain structures of IgE are in the Fc portion of the molecule, the portion that binds to the Fc receptor of cells mediating allergic reactions. Granato and Neeser have already reported that IgE-glycosylation relates to the receptor-binding but not to the antibody activity [2]. The present findings clearly indicate that the glycosylation of antibody IgE has a close relation to the activity inducing allergic reactions, which strongly suggest that the

Table 1. Reactivities of terminal sugar chain structures in anti-OA IgE.

	Light absorbance at 450 and 655 nm Terminal sugar chain structure		
Anti-OA IgE	**Galβ1-4Glc**	**Fucα1-3Gal**	**Sialα2-6Gal**
Intact	0.108	0.073	0.068
Enzymically-treated	0.019	0.007	0.007
Anti-OA IgE obtained from mice treated with the substance			
1 mg/kg	0.084	0.058	0.058
10 mg/kg	0.078	0.052	0.052
100 mg/kg	0.028	0.008	0.006
Blank control: 5% BSA	0.003	0.003	0.001

Values in this table indicate the reactivity of 1 mg/ml of the IgE solutions.
OA, ovalbumin; BSA, bovine serum albumin.

Table 2. Antibody activities of the anti-OA IgEs.

	Light absorbance at 450 and 655 nm
Anti-OA IgE	**Anti-OA reactivity**
With sugar chains, intact	0.144
No sugar chain, enzymically-treated	0.148
Anti-OA IgE obtained from mice treated with the substance	
1 mg/kg	0.151
10 mg/kg	0.150
100 mg/kg	0.152
Blank control: 10% OA	0.001

Values in this table indicate the reactivity of 1 mg/ml of the IgE solutions.
OA, ovalbumin.

sugar chain structures modify the biological activities of the IgE. Since treatment with the glycopeptidase does not change the amino acid sequence of the IgE, the sugar chain structures may affect the hyper-structure of the Fc portion of the IgE. But, the mechanism of the effect on the Fc receptor binding is not yet clear.

Allergic reactions are important in recognizing harmful circumstances, but excessive allergic responses can lead to injury. Not only mouse but also humans regulate and block some aspects of the excessive immune responses. In the present studies, the IgE antibody obtained from mice treated with a substance extracted from human urine decreased the allergic activity of IgE antibody via deglycosylation. This appears to be an example of one of the blocking systems.

Table 3. Systemic anaphylaxis induced by the anti-OA IgE.

Anti-OA IgE	Anaphylaxis symptoms		
	Rectal temperature (ºC)	Swelling of the footpad (mm)	Number of mice showing dye leakage
With sugar chains, intact	35.8 ± 0.32*	0.50 ± 0.10*	10
No sugar chain, enzymically treated	39.2 ± 0.19	0.02 ± 0.03	0
Anti-OA IgE obtained from mice treated with the substance			
100 mg/kg	39.1 ± 0.20	0.02 ± 0.02	0
Control: IgE obtained from the mice not immunized with OA	38.9 ± 0.22	0.02 ± 0.01	0

Values in this table indicates the mean ± SE of the rectal temperatures and the swelling of the footpad (the differences of the second measured and the first measured) in the 10 mice.
*$p < 0.01$ compared to the control (Mann-Whitney U test).
OA, ovalbumin; PS, physiological saline.

The amino acid sequence of the substance is an important aspect of the characterization of the urine-derived substance. We tried to determine the amino acid sequence of this substance using a protein sequencer, but the substance did not attach to the detecting membrane. This observation strongly suggests that the substance may have a very hydrophilic structure, perhaps resulting from the presence of sugar chains. The previous report indicated that the amino acids composing the substance are serine, threonine and asparagine [1]. These amino acids are potential sites of linkage of sugar chains. We speculate that the humoral anti-allergic polypeptide, the precursor of the urine-derived substance, is glycosylated and excreted in urine. Regretfully, the amino acid sequence of the precursor has not been clarified at the present time.

The present findings suggest another aspect of human allergic reactions. Intensity of allergic reaction differs among individual patients. Part of this difference may be based on IgE antibody glycosylation.

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