

IMPROVEMENT IN THE THERAPEUTIC EFFICACY OF AN ASTHMA-INDUCING SEA  
SQUIRT ANTIGEN TERMED DIIIa BY ITS POLYMERIZATION IN  
HYPOSENSITIZATION OF SEA SQUIRT ALLERGY

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Polymerization using glutaraldehyde markedly improved the apparently low efficacy of an asthma-inducing sea squirt antigen, DIIIa (MW 9,980), in hyposensitization therapy on patients with sea squirt allergy. A product (poly-DIIIa-G) comparable to Gi-rep (MW 106,000) in MW-distribution showed high therapeutic efficacy comparable to the most effective therapeutic antigen, Ei-M, which was paralleled by a significant increase in the allergen-specific IgG titer in most of the successfully hyposensitized patients as assayed using Ei-M as the target antigen. Another product (poly-DIIIa-E) comparable to Ei-M (MW 22,800) in MW also showed a high but slightly lower therapeutic efficacy relative to poly-DIIIa-G with an apparent increase in the specific IgG titer in some patients. However, no significant change in the IgG titer was detected in most patients unsuccessfully treated with intact DIIIa. On the other hand, no significant change was detected in the IgE titer specific to the target antigen in the patients, except in a few cases where the change was independent of the therapeutic effect. The apparent correlation between the increase in the specific IgG titer and the enhancement of the therapeutic effect suggested that polymerization enhanced the immunogenicity of DIIIa and resulted in a significant improvement in the therapeutic efficacy through an additional induction of the specific IgG capable of competing, as a blocking antibody, with the specific IgE for an asthma-inducing antigen, like DIIIa, in patients treated with the polymerized antigens.

**Key words:** conjunctival reaction — epitope — hyposensitization — mucosal allergenicity — polymerization — skin reaction

## INTRODUCTION

Among the three glycoproteins, Gi-rep (MW 106,000)<sup>1)-3)</sup>, Ei-M (MW 22,800)<sup>1)-3)</sup>, and DIIIa (MW 9,980)<sup>4)-5)</sup>, isolated as antigens specific to sea squirt allergy, DIIIa is the only antigen capable of inducing conjunctival reactions and asthmatic attacks in addition to skin reactions in patients with sea squirt allergy. Although Gi-rep and Ei-M are also capable of inducing skin reactions, neither antigens has ever induced allergic reactions in mucosa such as the bronchia and conjunctiva.

In the preceding study, periodate oxidation eliminated not only mucosal allergenicity but also skin allergenicity from DIIIa, suggesting that allergenicities in both the mucosa and skin depended on the same epitope residing in the carbohydrate chains of DIIIa<sup>5)</sup>. On the other hand, proteolysis with pronase E and any treatment of chemical modifications of the carboxyl or amino groups eliminated mucosal allergenicity from DIIIa, whereas skin allergenicity was still conserved even after these treatments<sup>5)</sup>.

These observations suggested that expression of the allergic reaction in mucosa would be supported by a relatively small structural factor residing in the protein moiety of the antigen. Thus, the mucosal allergenicity-supporting factor was expected to contribute to the permeability of DIIIa into mucosa, enabling the carbohydrate

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**Abbreviations:** ELISA enzyme-linked immunosorbent assay; MW molecular weight; RIA radioimmunoassay; SD standard deviation

epitope to interact with the allergy-specific IgE antibody attached to mast cells distributed in mucosa.

Recently, patients with sea squirt allergy were subjected to hyposensitization with each of the three sea squirt antigens containing the allergy-specific carbohydrate epitope in common, and symptoms in 90% and 72% of the allergic patients were improved by therapy with Ei-M and Gi-rep, respectively, whereas successful cases were limited to only 36% in patients treated with DIIIa<sup>6)</sup>.

During the hyposensitization therapy, the patient's sera were examined for their titers of IgG and IgE antibodies against Ei-M as the most effective therapeutic antigen. It has been observed that the specific IgG titer was significantly elevated in most patients successfully treated with Ei-M or Gi-rep, while no such elevation of the specific IgG titer was detected in most patients unsuccessfully treated with DIIIa<sup>6)</sup>. On the other hand, no significant change in the specific IgE titer was detected in any patients, regardless of the therapeutic effect<sup>6)</sup>. From these observations, the effect of hyposensitization was ascribed to the therapy-induced specific IgG capable of competing, as a blocking antibody, with specific IgE for an asthma-inducing antigen, like DIIIa, containing the same allergy-specific epitope.

The content of the epitope responsible for the skin reaction in Gi-rep was about 13% that in Ei-M as estimated from the content of type  $\alpha$  epitope assayed in vitro as a marker of the allergy-specific epitope<sup>4)</sup>. Therefore, the slightly lower therapeutic efficacy of Gi-rep relative to Ei-M was ascribable to its low content of the specific epitope<sup>6)</sup>. However, as the content of the specific epitope in DIIIa was estimated to be comparable to that in Ei-M<sup>4)</sup>, it was expected that DIIIa would also induce the specific IgG to express the therapeutic efficacy comparable to Ei-M, which inducement was independent of the mucosal allergenicity-supporting factor<sup>5)</sup>. Nevertheless, DIIIa was significantly less effective in the induction of the specific IgG and in hyposensitization therapy<sup>6)</sup>.

These observations suggested that the efficacy of an antigen to induce the specific IgG was determined not only by the content of the allergy-specific epitope but also by its immunogenicity depending on its molecular properties, such as

MW. Therefore, in the present study, DIIIa was polymerized through glutaraldehyde to improve its immunogenicity, and the polymerized antigens were examined for their efficacy in the induction of the specific IgG as well as in hyposensitization therapy.

## MATERIALS AND METHODS

**Antigens** Antigen preparations termed Gi-rep, Ei-M<sup>1)-3)</sup> and DIIIa<sup>4)5)</sup> were acidic glycoproteins previously isolated from a sea squirt, *Styela plicata*. Of the three, DIIIa was characterized as the only antigen capable of inducing asthmatic attack and conjunctival reaction in patients with sea squirt allergy. Although the other two were also responsible for skin reactions, neither has ever induced allergic reactions in such mucosa. In the present study, DIIIa was polymerized through glutaraldehyde, and the product was fractionated into two preparations termed poly-DIIIa-G and poly-DIIIa-E on the basis of molecular size-distribution (Fig. 1).

**Subjects** The patients subjected to the present study were all sea squirt allergy sufferers. They were highly sensitive to the three sea squirt antigens as revealed by skin test against the antigens at concentrations of 10-100  $\mu$ g/ml. Typical asthmatic attacks were induced in the patients only when they engaged in oyster-shucking work. The symptoms improved spontaneously when they refrained from this work for a few days. Thus, the causality is quite clear between the symptom and the presumed allergen.

**Skin allergenicity** Similarly to the preceding study<sup>5)</sup>, each of the patients received 20  $\mu$ l of an antigen solution (100  $\mu$ g/ml in 0.9% NaCl) by subcutaneous injection. When erythema, appearing after 15 min, were <10, 10 to 20, 20 to 30, 30 to 40, and >40 mm in diameter, scores of 0, 0.5, 1, 2, and 3 were assigned, respectively. The allergenicity of a polymerized DIIIa sample was expressed as the ratio of the total scores obtained in 4-12 patients for the sample to that obtained for intact DIIIa in the same patients, i.e. (total score for the sample)/(total score for intact DIIIa). For example,

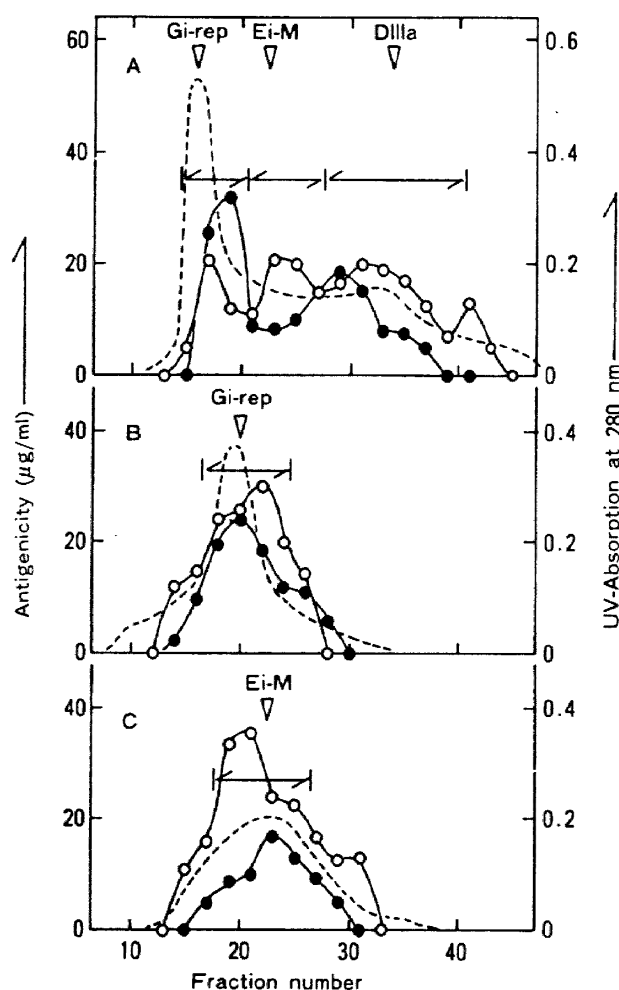


Fig. 1. Gel chromatographic fractionation of polymerized DIIIa. (A) The polymerization product from DIIIa (10 mg) was chromatographed on a column of Ultrogel AcA 54 ( $1.4 \times 100$  cm) with 1% NaCl into 3 ml-fractions. (B) The fractions Nos. 14-20 in Fig. 1A from five cycles of the fractionation were pooled and chromatographed on a column of Sepharose 6B ( $1.4 \times 100$  cm) with 1% NaCl into 3 ml-fractions, and the fractions Nos. 17-24 indicated were pooled as poly-DIIIa-G. (C) The fractions Nos. 21-24 in Fig. 1A from five cycles of the fractionation were pooled and chromatographed on a column of Ultrogel AcA 54 into 3 ml-fractions, and the fractions Nos. 18-26 were pooled as poly-DIIIa-E.  $\gamma$ -Activity (○) and  $\alpha$ -activity (●) were assayed by RIA, respectively. Gi-rep, Ei-M, and DIIIa with marks "▽" indicate the positions of their elution peaks as MW-standards, respectively.

when scores of 0, 1, 0, and 1 were obtained for a sample, and scores of 2, 3, 2, and 3 were obtained for intact DIIIa in patients a, b, c, and d, respectively, the ratio of the total score was calculated as

$(0+1+0+1)/(2+3+2+3)=2/10$  in the 4 patients ( $n=4$ ).

**Conjunctival allergenicity** Conjunctival reaction test were carried out in the same patients subjected to skin tests, similarly to the preceding study<sup>5</sup>. One eye in each of the patients was challenged with a drop of a polymerized DIIIa solution (1 mg/ml in 0.9% NaCl) and congestion of the conjunctiva was examined after 15 min. Simultaneously, the other eye was challenged with the intact DIIIa solution at the same concentration as a control. When there was no congestion, local, intermediate, or general congestion, scores of 0, 1, 2 and 3 were assigned, respectively. The allergenicity of the sample in the conjunctiva was then expressed by the ratio of the total score obtained for the sample to that obtained for the intact DIIIa in the same patients, in the same way that skin allergenicity was expressed.

**Antigenicity to rabbit anti-DIIIa serum** Two types of antigenicity were assayed in DIIIa and its polymerized products against the previously prepared rabbit anti-DIIIa serum (L 708)<sup>5</sup> by the same method of radioimmunoassay (RIA) as used in the preceding study<sup>5</sup>.

**$\gamma$ -Activity** The antigenicity depending on type  $\gamma$  epitope<sup>5</sup> as a specific marker of DIIIa was assayed from the inhibition of the binding of <sup>125</sup>I-labeled DIIIa to the rabbit antiserum, and was referred to as  $\gamma$ -activity.

**$\alpha$ -Activity** The antigenicity depending on type  $\alpha$  epitope<sup>1)-5)</sup> as a marker of the epitope responsible for skin reaction specific to sea squirt allergy was assayed from the inhibition of the binding of <sup>125</sup>I-labeled Gi-rep (containing type  $\alpha$  epitope alone<sup>1)-4)</sup>) to the same anti-DIIIa serum, and referred to as  $\alpha$ -activity.

**Titer of immunogen-specific IgG in immunized mice** A serum from a mouse (BALB/c) immunized with a given antigen was assayed for the IgG-titer specific to the antigen used as immunogen by enzyme-linked immunosorbent assay (ELISA) on the microtiter wells coated with the immunogen. The IgG trapped in the well was measured using a color development at 420 nm from 2,2'-azino-di (3-ethylbenzthiazoline sulphonyl)-

nate/H<sub>2</sub>O<sub>2</sub> with horseradish peroxidase-linked Protein A (Zymed, So. San Francisco) that bound to the immunoglobulins through a rabbit anti-mouse IgG + IgM + IgA (Zymed).

**Hyposensitization therapy** As described in the previous paper<sup>6)</sup>, each of the patients received subcutaneous injections of an antigen solution (100 µg/ml) once a week during the initial two months in increasing the dose from 10 µg up to 50 µg. Then, the injection intervals were prolonged gradually to once a month at 50 µg-dose to maintain the therapy.

When a patient required no symptomatic drug or only a small amount of the drug to engage in oyster-shucking work after the therapy, the hyposensitization was evaluated as "markedly effective" or "effective", respectively. Contrarily, when no significant reduction was observed in the drug-requirement, the hyposensitization was evaluated as "ineffective".

**Sera from the allergic patients** Serum was obtained monthly or bimonthly from each patient during the hyposensitization, and the sera were frozen and stored at -20°C until use.

**Titers of the allergy-specific IgG and IgE in patients** The titers of IgG and IgE antibodies in sera from the patients were assayed against Ei-M as the most effective therapeutic antigen, as in the previous study<sup>6)</sup>.

**Specific IgG** The IgG antibody specific to Ei-M was assayed by the same method of radioimmunoassay (RIA) as used in the previous study<sup>6)</sup>. The titer of the specific IgG was expressed by %binding of <sup>125</sup>I-labeled Ei-M to the serum IgG that was trapped on Protein A-Sepharose CL-4B (Pharmacia, Uppsala).

**Specific IgE** The IgE antibody specific to Ei-M was assayed by radioallergosorbent test (RAST) using paper disc-bound Ei-M as the allergosorbent. The titer of the specific IgE was expressed by %binding of <sup>125</sup>I-labeled anti-human IgE antibody (Pharmacia) to the serum IgE that was trapped on the allergosorbent.

**Materials for gel chromatography** Ultrogel AcA 54 (LKB, Bromma) and Sepharose 6B

(Pharmacia) were used for gel chromatography.

## RESULTS

**Polymerization of DIIIa** A 0.2 ml portion of 25% glutaraldehyde (ca. 0.78 mmol) was added dropwise to 50 mg of DIIIa (ca. 15 mmol of -NH<sub>2</sub><sup>5)</sup>) in 5 ml of 10 mM phosphate buffer, pH 7.0, containing 0.9% NaCl under constant stirring. After the reaction at 22°C for 2 hr, the reaction mixture was dialyzed against deionized water and freeze-dried as the polymerization product. Each one fifth portion of the product was chromatographed on an Ultrogel AcA 54 column and the fractions were examined for γ-activity (the antigenicity depending on type γ epitope<sup>5)</sup>) as a marker of DIIIa and α-activity (the antigenicity depending on type α epitope<sup>1)-5)</sup>) as a marker of the carbohydrate epitope responsible for skin reaction specific to sea squirt allergy), in addition to UV-absorption at 280 nm (Fig. 1A).

By referring the elution behavior of Gi-rep, the fractions Nos. 14-20 in Fig. 1A were pooled as the component comparable to Gi-rep (MW 106,000) in MW-distribution, and the pooled fraction was chromatographed again on a Sepharose 6B column (Fig. 1B). Fractions Nos. 17-24 in Fig. 1B were pooled, dialyzed and freeze-dried to yield 8 mg of a polymerized DIIIa preparation termed poly-DIIIa-G from 50 mg of DIIIa.

By referring the elution behavior of Ei-M, fraction Nos. 21-24 in Fig. 1A were also pooled as the component comparable to Ei-M (MW 22,800) in MW, and the pooled fraction was chromatographed again on an Ultrogel AcA 54 column (Fig. 1C). Fraction Nos. 18-26 in Fig. 1C were pooled, dialyzed and freeze-dried to yield 14 mg of another polymerized DIIIa preparation termed poly-DIIIa-E from 50 mg of DIIIa.

Examination of allergenicities in vivo showed that the conjunctival allergenicity specific to DIIIa was eliminated by the polymerization reaction, whereas the skin allergenicity was mostly conserved in both polymerized products (Table 1). On the other hand, examination of the antigenicities against the rabbit anti-DIIIa serum in vitro showed that α-activity as a marker of skin allergenicity increased significantly with the increase

Table 1 Allergenicities of polymerized DIIIa preparations

| Antigen      | Degree of polymerization | Allergenicity relative to DIIIa |             |
|--------------|--------------------------|---------------------------------|-------------|
|              |                          | In conjunctiva                  | In skin     |
| Poly-DIIIa-E | 2.3                      | 0/27(n=14)                      | 19/17(n=10) |
| Poly-DIIIa-G | 11                       | 0/29(n=13)                      | 12/13(n=9)  |

See MATERIALS AND METHODS for the method of assay and the expression of the allergenicities.

in molecular size of the products, whereas  $\gamma$ -activity specific to DIIIa was apparently reduced by the polymerization reaction (Table 2).

**Immunogenicity and toxicity in mice** Each mouse was immunized with 100  $\mu$ g of a polymerized antigen or intact DIIIa with the aid of Freund's complete adjuvant by peritoneal injection, followed by injection of the same amount of the antigen without adjuvant as a booster 3 weeks after the first immunization. The sera from the mice were examined by ELISA for the titer of IgG specific to the respective antigen used as immunogen, and it was observed that the induction of the specific IgG was significantly enhanced in parallel with the increase in MW of the immunogen (Fig. 2). This observation suggested that the polymerized antigens, as well as Ei-M and Gi-rep, could induce the allergy-specific IgG capable of acting as a blocking antibody in hyposensitization therapy on patients with sea squirt allergy<sup>6</sup>.

Before the polymerized antigens were used to hyposensitize the allergic patients, 4 mice were immunized every week by subcutaneous injections of 1.7  $\mu$ g-each (8.5  $\mu$ g/kg) of poly-DIIIa-G for 8 weeks. No significant pathological changes were detected in their livers, kidneys, or spleens, suggesting the preparation was harmless in the therapy, although the dose corresponded to 250 times that used to hyposensitize the patients.

**Hyposensitization therapy with polymerized DIIIa** Patients with sea squirt allergy were subjected to hyposensitization therapy with poly-DIIIa-G, poly-DIIIa-E, or intact DIIIa (Table 3).

Hyposensitization therapy with intact DIIIa was ineffective in 10 of 14 patients. The appar-

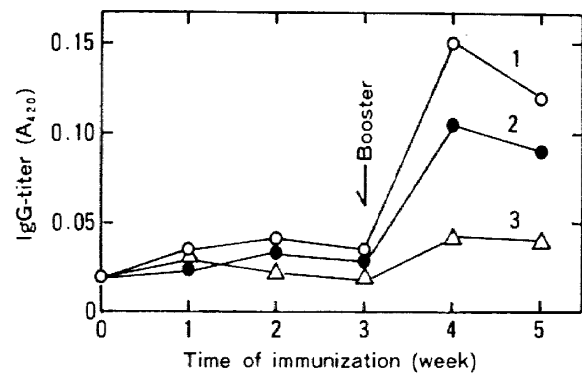


Fig. 2. Titers of IgG specific to the immunogens in immunized mice. Each mouse (BALB/c) was immunized with 100  $\mu$ g of polymerized or intact DIIIa with the aid of Freund's complete adjuvant by peritoneo-injection, followed by injection of the same antigen without adjuvant as a booster after 3 weeks from the initial immunization. The serum from a mouse was examined by ELISA for the IgG titer against the antigens used as immunogen. The numbers 1, 2, and 3 indicates the mice immunized with poly-DIIIa-G, poly-DIIIa-E, and intact DIIIa, respectively.

Table 2 Antigenicities of polymerized DIIIa preparations

| Antigen          | Degree of polymerization | Antigenicity                        |                                      |
|------------------|--------------------------|-------------------------------------|--------------------------------------|
|                  |                          | $\gamma$ -Activity as DIIIa (mg/mg) | $\alpha$ -Activity as Gi-rep (mg/mg) |
| DIIIa (MW 9,980) | 1                        | 1.0                                 | 0.011                                |
| Poly-DIIIa-E     | 2.3                      | 0.6                                 | 0.08                                 |
| Poly-DIIIa-G     | 11                       | 0.4                                 | 0.15                                 |

See MATERIALS AND METHODS for the method of assay and the expression of the antigenicities.

ently low therapeutic efficacy was consistent with that observed in the previous hyposensitization with DIIIa<sup>6</sup>. However, therapy with poly-DIIIa-E was effective in all 8 patients subjected to it. Furthermore, therapy with poly-DIIIa-G was markedly effective in 6 and effective in 8 of 14 patients subjected. The high therapeutic efficacy of the polymerized antigens was comparable to that observed in the previous hyposensitization with Ei-M<sup>6</sup>.

**Influence of hyposensitization of titers of specific IgG and IgE antibodies** As in the previous

Table 3 Influence of polymerization on therapeutic effect of DIIIa as the antigen for hyposensitization

| Antigen         | Degree of polymerization | Therapeutic effect |           |             |
|-----------------|--------------------------|--------------------|-----------|-------------|
|                 |                          | Markedly effective | Effective | Ineffective |
| DIIIa(MW 9,980) | 1                        | 1                  | 3         | 10          |
| Poly-DIIIa-E    | 2.3                      | 0                  | 8         | 0           |
| Poly-DIIIa-G    | 11                       | 6                  | 8         | 0           |

See MATERIALS AND METHODS for the evaluation of the effect in the hyposensitization therapy.

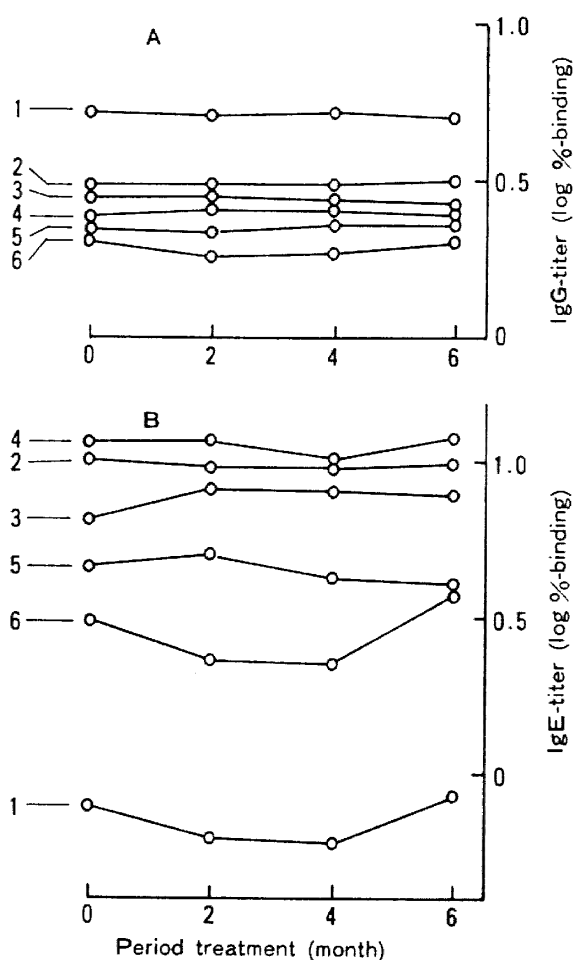


Fig. 3. Influence of hyposensitization therapy with DIIIa on titers of Ei-M-specific IgG (A) and IgE (B) in sera of allergic patients. The numbers on the left indicate individual patients who were unsuccessfully hypsensitized with DIIIa treatment.

study<sup>6)</sup>, the titers of IgG and IgE antibodies specific to Ei-M, the most effective therapeutic antigen<sup>6)</sup>, were assayed as the allergy-specific antibody titers in the sera of allergic patients treated with each of the polymerized antigens and

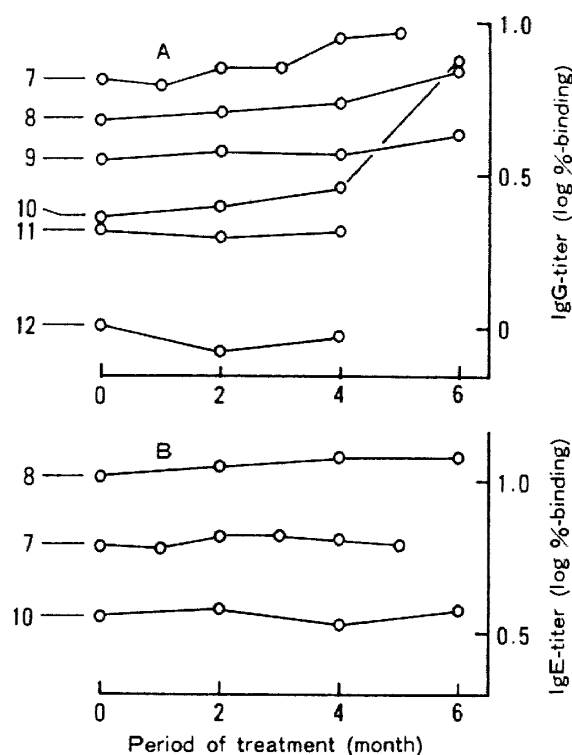


Fig. 4. Influence of hyposensitization therapy with poly-DIIIa-E on titers of Ei-M-specific IgG (A) and IgE (B) in sera of allergic patients. The numbers on the left indicate individual patients who were effectively hypsensitized with poly-DIIIa-E treatment.

intact DIIIa over the period of the hypsensitization therapy to examine the changes in the titers from the initial titer levels (Figs. 3~5). Since some patients dropped out of the 6 month hypsensitization, available sera were limited; thus, all of the available sera were assayed for the specific antibodies without any selection.

In the group treated with intact DIIIa, although the titer levels of both specific antibodies differed

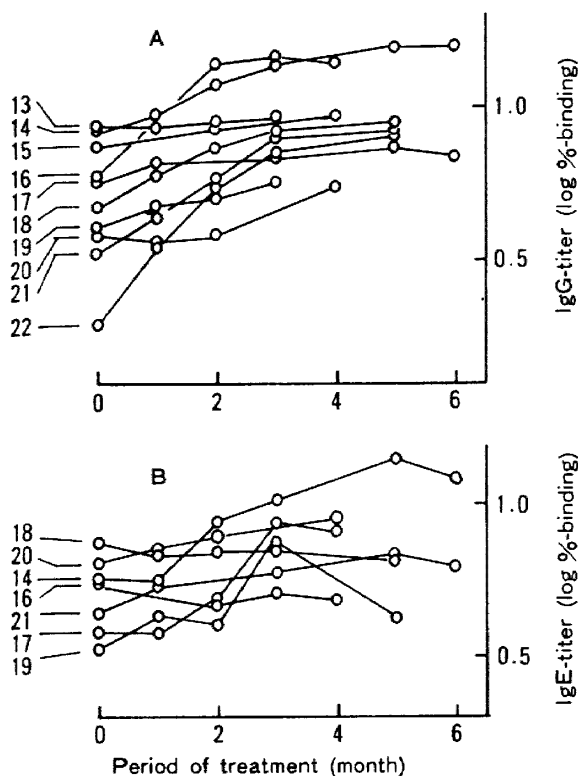


Fig. 5. Influence of hyposensitization therapy with poly-DIIIa-G on titers of Ei-M-specific IgG (A) and IgE (B) in sera of allergic patients. The numbers on the left indicate individual patients who were successfully hyposensitized with poly-DIIIa-G treatment; the therapy was markedly effective in patients Nos. 13, 14, 15, 17 and 18, and effective in patients Nos. 16, 19, 20, 21 and 22.

substantially in individual patients, no significant changes were detected in either the specific IgG or IgE titers for any of the unsuccessfully treated patients, with values of  $<0.041$  and  $0.11$  SD, respectively, in terms of the logarithm of percent binding of radioactivity (Figs. 3A and 3B); in this group, no serum was available from the successfully treated patients as the minority.

In the group successfully treated with poly-DIIIa-E, the specific IgG titer increased significantly in 3 of 6 patients (Nos. 7, 8, and 10) as judged from the SD value in Fig. 3 (Fig. 4A). However, the specific IgE titer appeared to be independent of the therapy in 3 patients examined (Fig. 4B).

In the group treated with poly-DIIIa-G, the hyposensitization showed either marked or normal efficacy, and the specific IgG titer increased significantly in all of the subjects except patient

No. 13 (Fig. 5A). Again, however, no significant changes in the specific IgE titer were detected in any of the subjects except patients Nos. 14 and 17 (Fig. 5B).

These observations suggested that polymerization of DIIIa significantly improved the low immunogenicity of the antigen to induce the specific IgG antibody capable of acting as a blocking antibody. As suggested in the previous study<sup>6)</sup>, the specific IgG increased by adding the therapy-induced IgG could overcome the specific IgE in competition for an asthma-inducing antigen, like DIIIa, resulting in the therapeutic effect, even when the induction of the IgG was not always detectable by the present assay method<sup>6)</sup>.

## DISCUSSION

As summarized in Table 3, a polymerized DIIIa preparation, poly-DIIIa-G, comparable to Gi-rep (MW 106,000) in MW-distribution showed high therapeutic efficacy comparable to that observed in Ei-M, which was the most effective of the three sea squirt antigens, Gi-rep, Ei-M, and DIIIa<sup>6)</sup> in the hyposensitization of sea-squirt allergy. Another product, poly-DIIIa-E, comparable to Ei-M (MW 22,800) in MW also showed high but slightly lower therapeutic efficacy relative to poly-DIIIa-G. However, in accordance with the previous observation<sup>6)</sup>, intact DIIIa was again apparently less effective in therapy. Since the allergy-specific carbohydrate epitope<sup>4)5)</sup> of DIIIa was considered to be unchanged in amount and quality before and after the polymerization treatment as examined by skin test (Table 1), the increased therapeutic efficacy of the polymerized antigens could be ascribed to their increased MW.

In the radioimmunometric analysis for the allergy-specific IgG and IgE antibodies using Ei-M as a target antigen, a significant increase in the specific IgG titer was observed in most patients successfully treated with poly-DIIIa-G (Fig. 5A), and an apparent increase in the specific IgG titer was also observed in some patients successfully treated with poly-DIIIa-E (Fig. 4A). However, similarly to the previous study<sup>6)</sup>, no significant change in the specific IgG titer was detected in patients unsuccessfully treated with intact DIIIa

(Fig. 3A). On the other hand, no significant correlation was detected again between the change in the allergy-specific IgE titer and the therapeutic effect in most patients observed in the previous study<sup>6)</sup>, although the IgE titer appeared to increase in a few cases (No. 14 and No. 17) (Figs. 3B, 4B, and 5B).

The apparent correlation between the increase in the specific IgG titer and the enhancement of the therapeutic effect suggested that polymerization enhanced the immunogenicity of DIIIa and resulted in a significant improvement in the therapeutic efficacy through an additional induction of the specific IgG antibody capable of competing, as a blocking antibody, with the specific IgE antibody for an asthma-inducing allergen, like DIIIa, in the treated patients<sup>6)</sup>. Enhanced immunogenicity in the polymerized antigens was also observed in mice (Fig. 2).

Although no significant increase in the specific IgG titer was detected in several cases of successfully treated patients (Nos. 12, 13, 17-20 in Fig. 3), it might be presumed, similarly to the previous study<sup>6)</sup>, that, in these patients, the IgG level could be increased over the unchanged specific IgE level by adding the newly induced IgG to express the therapeutic effect, even though the therapy-dependent increase in the specific IgG was almost undetectable by the present method.

Although Gi-rep of a substantially high MW seemed to be rather low both in the induction of the specific IgG and in the therapeutic efficacy, the lower efficacy compared to Ei-M and the polymerized antigens of Gi-rep could be ascribed again to its significantly lower content of the allergy-specific epitope compared to those of Ei-M and DIIIa<sup>4)6)</sup>.

Accordingly, it was considered that the efficacy of a sea squirt antigen in hyposensitization of sea squirt allergy was determined not only by its content of the allergy-specific carbohydrate epitope but also by the MW of its protein moiety as the carrier of the epitope; the higher MW was considered to enhance the immunogenicity to induce the specific IgG acting as a blocking antibody.

In the RIA experiments, the in vitro antigenicity depending on type  $\alpha$  epitope as a marker of the allergy-specific epitope<sup>1)-5)</sup> increased parallel with

the increase in MW of the DIIIa derivatives (Table 2). In this connection, it has been observed that the in vitro antigenicity of Gi-rep was significantly reduced by its Pronase digestion, while the allergenicity in skin was mostly conserved in the residual glycopeptides<sup>7)8)</sup>. These observations suggested that the protein moiety of a substantially high MW in sea squirt antigens contributed to expression of both the antigenicity depending on type  $\alpha$  epitope and the immunogenicity depending on the specific carbohydrate epitope responsible for skin reaction.

On the other hand, no mucosal allergenicity was detected in the polymerized DIIIa preparations (Table 1), and the in vitro antigenicity depending on type  $\gamma$  epitope as a marker of DIIIa<sup>6)</sup> apparently decreased with the increase in the degree of polymerization (Table 2). These observations suggested that the efficacy of hyposensitization was independent of the mucosal allergenicity-supporting factor and type  $\gamma$  epitope residing in the protein moiety of DIIIa.

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## 重合による喘息誘発性ホヤ抗原, DIIIa の ホヤ喘息減感作における治療効率の改善

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喘息誘発性ホヤ抗原, DIIIa (MW 9,980) のホヤ喘息に対する極めて低い減感作治療効率を, グルタルアルデヒド重合によって顕著に改善した. Gi-rep (MW 106,000) と同程度の分子量の重合物 (poly-DIIIa-G) は, 最も有効な Ei-M に匹敵する高い治療効率を示すと同時に, ほとんどの有効症例で, 最も有効な治療抗原 Ei-M と反応する特異 IgG 抗体価に顕著な増加を認めた. 分子量が Ei-M (MW 22,800) と同程度の重合物 (poly-DIIIa-E) も, poly-DIIIa-G より僅かに低い, 高い治療効率を示し, 有効症例の約半数に特異 IgG 抗体価の増加傾向を認めた. しかし, DIIIa 治療の無効症例のほとんどについて, このような特異 IgG の有意な増加は認められなかった. 一方, 特異 IgE 抗体価に関しては, 治療効果の有無にかかわらず, ほとんどの患者について有意な変動は認められなかった. 特異 IgG 抗体価の増加と治療効果の増強との間のこのような相関は, この治療効率の改善が, 重合による DIIIa の免疫原性の顕著な増強によるものであることを示唆する. 重合 DIIIa により誘導される優勢な特異 IgG は, DIIIa のような喘息誘発性抗原に対する特異 IgE の結合を, 拮抗的に阻害する遮断抗体として機能するものと思われる.