

Therapeutic effect and titers of the specific IgE and IgG antibodies in patients with sea squirt allergy (Hoya asthma) under a long-term hyposensitization with three sea squirt antigens

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Hyposensitization therapy with each of three sea squirt antigens, Gi-rep (molecular weight [MW] 106,000), Ei-M (MW 22,800), and DIIIa (MW 9980), have succeeded in 72%, 90%, and 36% of patients with sea squirt allergy, respectively, within the first year, and the effect has been maintained during subsequent 4-year maintenance therapy. All available sera from some of the hyposensitized patients were also examined for IgE and IgG titers against the most effective therapeutic antigen, Ei-M, and it was revealed that the Ei-M-specific IgG titer increased rapidly in the successfully hyposensitized patients, regardless of the therapeutic antigen used, except for a few patients. Furthermore, the high specific IgG titer, as well as the therapeutic effect, was maintained during the subsequent maintenance therapy. No such increase in the specific IgG titer was detected in all unsuccessfully hyposensitized patients. In contrast, the Ei-M-specific IgE titer was practically unchanged in all allergic patients, independent of the therapy. Therefore, the effect of the hyposensitization therapy was closely dependent on the induction of the specific IgG capable of competing with the specific IgE for a certain asthma-inducing antigen, like DIIIa. The apparently low therapeutic efficiency of DIIIa was attributed to its relatively low immunogenicity to induce the specific IgG. (J ALLERGY CLIN IMMUNOL 1989;83:386-93.)

The acidic glycoproteins termed Gi-rep (MW 106,000) and Ei-M (MW 22,800) are the antigen preparations that have been isolated from body fluid of a sea squirt, *Styela plicata*, based on the capability of inducing marked erythema at a concentration of $<10 \mu\text{g/ml}$ in skin of patients with asthma and with sea squirt allergy, Hoya asthma. However, neither has

Abbreviation used

MW: Molecular weight

antigen induced conjunctival nor bronchial reaction in the same patients.¹⁻⁴

It has also been observed that both antigens are markedly effective in hyposensitization therapy of the patients, although Gi-rep appears to be slightly less effective than Ei-M.⁵⁻⁹ The radioimmunometric analyses with Ei-M as a marker antigen have revealed that Ei-M-specific IgG titer was significantly higher in patients successfully hyposensitized with either Ei-M or Gi-rep, as compared with specific IgG titers of untreated allergic patients and healthy workers.⁷⁻⁹ In contrast, the Ei-M-specific IgE titer was significantly higher in all allergic subjects than in healthy subjects, being independent of the therapy.⁶⁻⁹

The correlation of the therapeutic effect with the significant increase of the specific IgG titer suggested

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that the hyposensitization therapy depended closely on the induction of the specific IgG capable of competing with the specific IgE for a certain asthma-inducing antigen in the patients. Thus, the specifically induced IgG was considered to act as a blocking antibody.⁸⁻¹¹

Recently, a weak acidic glycoprotein termed DIIIIa (MW 9980) was isolated as an asthma-inducing sea squirt antigen after monitoring the allergenic activity of the conjunctival reaction in the patients. DIIIIa was capable of inducing not only the conjunctival reaction and asthmatic attack but also the skin reaction similarly to the previous antigens.¹² However, DIIIIa was found to be significantly less effective in the hyposensitization therapy compared with the previous two antigens.¹³

In contrast, it has also been observed that DIIIIa, as well as Ei-M and Gi-rep, induced a marked histamine release from leukocytes in blood of the untreated allergic patients, whereas the release was apparently lower in the successfully treated patients who were hyposensitized with either Ei-M or Gi-rep.^{12, 14} The histamine release in blood of the untreated patients was inhibited dose dependently by addition of the plasma from the successfully hyposensitized patients.¹⁴

These in vivo and in vitro observations suggested that the specific IgG induced by either of the two previous antigens was cross-reactive to DIIIIa and that the allergenic epitope of DIIIIa was essentially the same in specificity as those of Gi-rep and Ei-M¹²⁻¹⁴; nevertheless, DIIIIa was apparently less effective in the hyposensitization therapy than the previous two antigens.⁹ In the present study, the therapeutic efficiency of the three sea squirt antigens was further examined in relation to the influence of the therapy on the specific IgE and IgG titers in the sera of the patients with allergy during long-term hyposensitization.

MATERIAL AND METHODS

Sea squirt antigens

Three antigen preparations, Gi-rep, Ei-M,^{3,4} and DIIIIa,¹³ were used in the present study. The molecular and immunologic properties of these preparations are summarized in Table I.

Subjects

All subjects in the present study were patients with asthma and with sea squirt allergy. They were highly sensitive to the three sea squirt antigens, as examined by skin test against the antigens at a concentration of <10 µg/ml. A typical asthmatic attack was induced in each patient only when the patient engaged in oyster-shucking work. The symptom was spontaneously improved after staying away from work for

TABLE I. Molecular and antigenic properties of sea squirt antigens

Property	Gi-rep ^{3,4}	Ei-M ^{3,4}	DIIIIa ¹³
Weight average MW	106,000	22,800	9,980
Hexosamine content (µmol/gm)	990	720	700
Skin reaction*	+	+	+
Conjunctival reaction†	—	—	+
Induction of asthmatic attack‡	—	—	+
Type of epitope detectable in vitro§	α	α and β	α and β
Affinity constant to rabbit anti-Gi-rep serum (ml/µg)	6.54	2.87	0.084

*Symbol + indicates that the antigen induces an erythema (>20 mm in diameter) in most patients with sea squirt allergy and with a skin test of a 20 µl injection of its solution at a concentration of 10 µg/ml.

†Symbol + indicates that the antigen induces conjunctival congestion in most patients with a challenge of a drop of its solution at a concentration of 100 µg/ml.

‡Symbol + indicates that the antigen induces asthmatic attack in most patients on provocation with the mist of 0.5 ml of its solution at a concentration of 100 µg/ml.

§Type α epitope is reactable to the two rabbit antisera prepared against Gi-rep and Ei-M in common, whereas type β epitope is reactable to the rabbit anti-Ei-M but unreactable to the anti-Gi-rep.^{3,4,13}

||The affinity constant was determined by the radioimmunometric experiments.^{3,4,13}

a few days. Thus, the causality is quite clear between the symptom and the presumed allergen.

Hyposensitization therapy

According to the previously described procedure,⁵⁻⁹ each of the patients received a subcutaneous injection of an antigen solution (100 µg/ml) once a week during the initial 2 months of therapy by increasing the dose from 10 µg up to 50 µg. The interval of the injection was gradually reduced to once a month at 50 µg dose to maintain the therapy.

When a patient required no symptomatic drugs or only a small amount to engage in oyster-shucking work, the hyposensitization was considered successful. Contrarily, when no significant reduction was observed in the drug requirement, the hyposensitization was considered unsuccessful.

Sera from the allergic patients

Sera was obtained each year in oyster-shucking season (January to March) and in the off season (August to October) from the allergic patients receiving hyposensitization. The sera were frozen and stored at -20°C until use. Since some patients dropped out of the long-term hyposensitization, available sera were limited; thus, without any selection, all

TABLE II. Therapeutic effect of hyposensitization therapy with the three sea squirt antigens

Period of therapy (yr)	Successful/unsuccessful cases in therapy with			
	Gi-rep	Ei-M	DIIIa	Symptomatic drug alone
1	34/13 (72%)	56/6 (90%)	5/9 (36%)	0/17 (0%)
2	23/8	50/4	3/6	0/7
3	16/7	42/3	2/6	0/6
4	16/2	31/1	2/5	—
5	13/1	27/1	2/5	—

Each of the successfully treated patients required only a small amount or no symptomatic drugs to engage in oyster-shucking work after therapy.

The number in parentheses indicates the percentage of successfully treated patients based on the total number of patients receiving each hyposensitization therapy.

The yearly decrease of patient number reflects the patients dropped from maintenance therapy but not the change in number from successfully to unsuccessfully treated nor from unsuccessfully to successfully treated patients.

TABLE III. Standard deviations for logarithms of the specific IgE and IgG titers in patients who received no hyposensitization therapy

Patient No.	IgE titer (log % binding)			IgG titer (log % binding)		
	Mean	n	SD	Mean	n	SD
1	1.305	5	0.189	0.402	5	0.045
2	1.320	5	0.149	0.162	5	0.048
3	0.424	4	0.058	0.079	4	0
4	0.418	4	0.075	0.069	4	0.018
5	0.495	5	0.088	0.605	5	0.057
6	1.313	5	0.084	0.317	5	0.009
7	1.386	3	0.070	0.238	3	0.029
Total		31	0.117		31	0.038

In each of the IgE and IgG titers, all the deviations of the logarithmic parameters from the corresponding mean values were squared and summed, and the total sum was divided by the degree of freedom (31 - 7 = 24) to obtain a mean value of the squared deviations. The square root of the mean value was obtained as SD for the logarithm of each titer. The values of 0.117 and 0.038 were obtained as the SDs for the IgE and IgG titers, respectively.

available sera were subjected to the examination of the specific antibodies.

Assay of the allergy-specific IgE and IgG antibodies

Ei-M was observed to demonstrate the highest efficiency in the hyposensitization therapy (Table II). The titers of IgE and IgG antibodies were assayed against Ei-M similarly to previous studies.^{5-11, 13}

Specific IgE. According to the same procedure as previously used,^{6, 9} the specific IgE antibody was assayed by RAST with paper disk-bound Ei-M as the allergosorbent. The titer of the specific IgE was expressed by percent binding of ¹²⁵I-labeled antihuman IgE antibody (Pharmacia Fine Chemicals, Uppsala, Sweden) to the serum IgE that was trapped on the allergosorbent.

Specific IgG. The specific IgG antibody was assayed by

the same method of radioimmunoassay as used in the previous studies.⁷⁻⁹ The titer of the specific IgG was expressed by percent binding of ¹²⁵I-labeled Ei-M to the serum IgG that was trapped on protein A-Sepharose CL-4B (Pharmacia Fine Chemicals).

RESULTS
Therapeutic effect of hyposensitization therapy with three sea squirt antigens

The results of long-term hyposensitization therapy of sea squirt allergy with the three sea squirt antigens are summarized in Table II. Therapy with Gi-rep, Ei-M, and DIIIa improved the symptoms in 72%, 90%, and 36% of the allergic subjects, respectively, within the first year, and no apparent improvement was observed in the control subjects treated with symptomatic drugs alone. It was again confirmed that

the hyposensitization therapy was most effective with Ei-M, slightly less with Gi-rep, but markedly less with DIIIa.

Although some patients dropped out of each group during the subsequent 4-year treatment, it was observed that the therapeutic effect was maintained in each patient who was successfully treated within the first year and received the subsequent maintenance therapy. Such clinical observation suggests that the hyposensitization with an antigen is mostly established within the first year of therapy for the patient for which the antigen is effective. However, for the subject receiving an ineffective antigen the first year, it is also suggested that no practical effect could be obtained by the same antigen even in a long-term therapy.

Seasonal change in the titers for specific IgE and IgG antibodies in patients who received no hyposensitization

Allergic patients continuously inhaled sea squirt antigens by way of the mist of body fluid of sea squirts throughout the working season in oyster farms. As a control, the sera from seven subjects treated with symptomatic drugs alone were examined each year in both in and out of season of oyster-shucking work for titers of IgE and IgG antibodies against Ei-M.

Although the titers of both specific antibodies were substantially different in levels in individual patients (Fig. 1), no significant seasonal change was detected in the IgE and IgG titers for each subject with values of <0.117 and 0.038 SD, respectively, in terms of logarithm of percent binding of radioactivity (Table III).

Influence of hyposensitization on titers of specific IgE and IgG antibodies

Specific IgE and IgG titers were assayed in the sera of the allergic patients with hyposensitization therapy with each of the three sea squirt antigens, and changes in the levels of the titers from the initial titer levels were examined for significance on the basis of the respective standard deviations calculated from the titers in the control group (Fig. 1).

In the group treated with Ei-M, successfully hyposensitized subjects were in the majority, and the specific IgG titer increased significantly in all subjects within the first year parallel with the therapeutic effect, as illustrated in Fig. 2, B, although the serum from the unsuccessfully treated patient was unavailable in the examination. However, the specific IgE titer appeared to be independent of the therapy in most subjects except for patients Nos. 18 and 25 (Fig. 2, A).

In the group hyposensitized with Gi-rep, as illus-

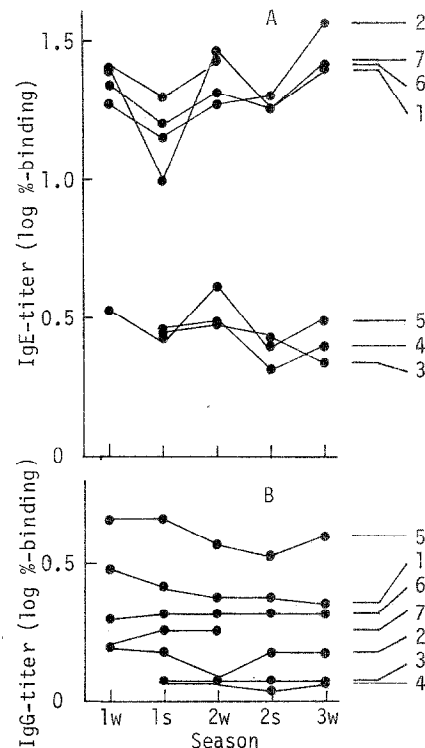


FIG. 1. Seasonal change in titers of Ei-M-specific IgE (A) and IgG (B) antibodies in sera of patients with sea squirt allergy with treatment with symptomatic drugs alone. Numbers on the right indicate the individual patients. No patient received hyposensitization treatment but did receive symptomatic drugs on occasion of asthmatic attack during the period of observation. No recognizable improvement was detected in all patients examined. Sera of the patients were examined each year in winter (January to March) and summer (August to October) for Ei-M-specific IgE and IgG. 1w and 1s indicate the first winter and the subsequent summer, respectively. Similarly, 2w, 2s, and 3w indicate the second winter, the second summer, and the third winter, respectively. Titrers of specific IgE and IgG are represented by % binding of ^{125} I-labeled antihuman IgE (RAST value) and that of ^{125} I-labeled Ei-M (radioimmunoassay value), respectively (see Material and Methods for the procedures).

trated in Fig. 3, B, Ei-M-specific IgG titer significantly increased in two successfully treated patients (Nos. 10 and 13) within the first year, similarly to the patients treated with Ei-M, whereas the increase of the IgG titer appeared to delay in phase from the appearance of the therapeutic effect in the other two patients (Nos. 12 and 14). No significant increase was observed in the specific IgG titer for the unsuccessfully treated patients (Nos. 8, 9, and 11) during the observation. In contrast, as illustrated in Fig. 3, A, the change in the Ei-M-specific IgE titer appeared again to be mostly independent of the therapy except for an unsuccessfully treated patient (No. 9).

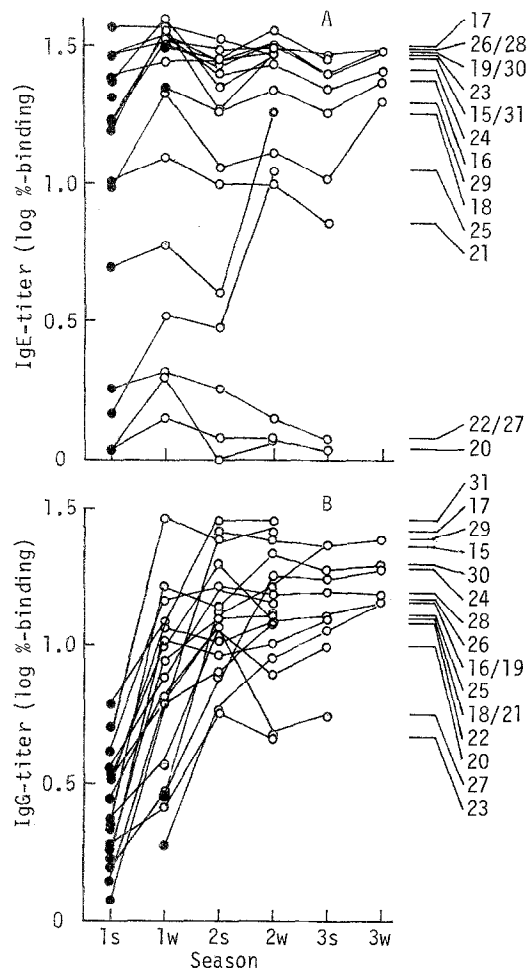


FIG. 2. Influence of hyposensitization therapy with Ei-M on the titers of Ei-M-specific IgE (**A**) and IgG (**B**) in sera of allergic patients. Numbers on the right indicate the individual patients who have succeeded in the hyposensitization with Ei-M treatment in the first year of therapy, and the therapeutic effect was maintained with the subsequent continuous therapy. Closed and open circles are the data for the patients just before the start of, and with the hyposensitization therapy, respectively. Terms for the titer and the seasons of examination are the same as in Fig. 1.

In the group hyposensitized with DIIIa, as illustrated in Fig. 4, unsuccessfully treated patients were in the majority, and no significant increase was observed in both the specific IgE and IgG titers for the unsuccessfully treated patients similar to the increase for the control group (Fig. 1) and for the unsuccessfully treated patients in the Gi-rep hyposensitized group (Fig. 3, Nos. 8, 9, and 11).

Two patients (Nos. 32 and 41) illustrated in Fig. 4, were successfully hyposensitized, even with DIIIa. In patient No. 41 who had dropped out of the therapy

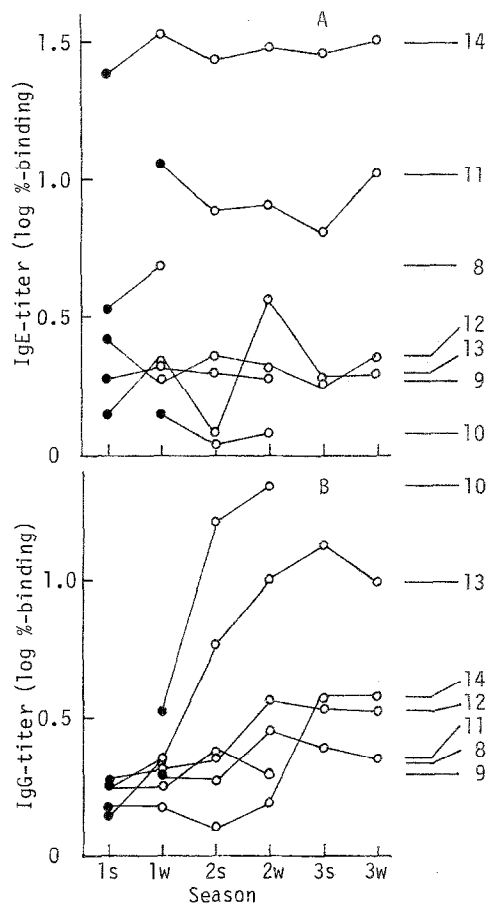


FIG. 3. Influence of hyposensitization therapy with Gi-rep on titers of Ei-M-specific IgE (**A**) and IgG (**B**) in sera of allergic patients. Numbers on the right indicate individual patients; patients Nos. 8, 9, and 11 were hardly improved in symptom, whereas the other patients were successfully hyposensitized within the first year of the treatment, and the improved symptom was maintained in each patient under continuous therapy. Terms for titers and seasons of examination are the same as in Figs. 1 and 2.

after 1 year, the specific IgG titer significantly increased, although the specific IgE titer also increased, but to a relatively less extent. In contrast, in patient No. 32, although both specific IgG and IgE titers appeared to be unchanged by the therapy, the level of the IgG titer was markedly higher when the level was compared to levels in the other patients from the start of the therapy.

These observations suggest a possibility that the therapeutic effect could be obtained when the level of specific IgG could be significantly increased over the level of specific IgE in competition for a presumed allergen, like DIIIa, by adding the therapy-induced IgG, even though the induction of IgG was not always detectable in the present assay method.

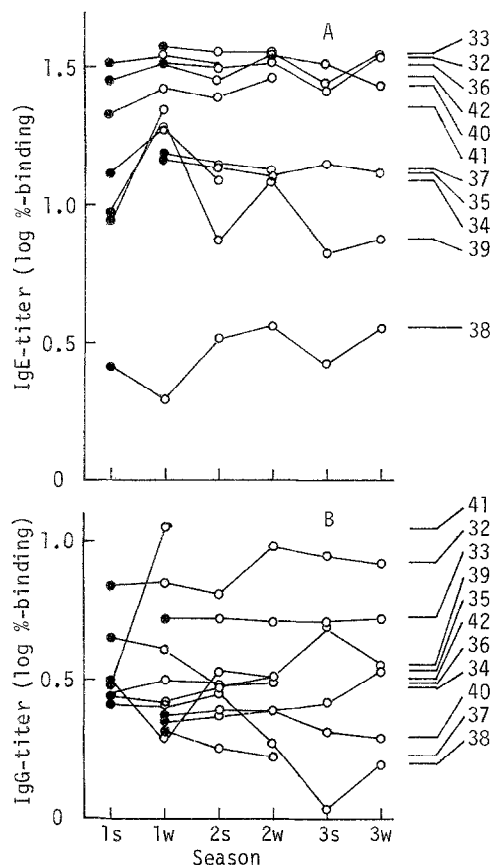


FIG. 4. Influence of hyposensitization therapy with DIIla on the titers of Ei-M-specific IgE (A) and IgG (B) in sera of allergic patients. Numbers on the right indicate the individual patients; a recognizable reduction of requirement for symptomatic drugs was observed in patients Nos. 32 and 41 within the first year, and the therapeutic effect was maintained under the subsequent continuous therapy, whereas the other patients were unsuccessful in the hyposensitization. Terms for the titers and the seasons of examination are the same as in Figs. 1, 2, and 3.

Influence of sequential treatments with DIIla and Ei-M on the symptom and the titers of specific IgE and IgG

An additional patient with sea squirt allergy was initially treated with DIIla, and no improvement was obtained in symptoms in the first working season. The therapeutic antigen was replaced with Ei-M in the next season, and therapy with Ei-M was continued for an additional 14 months, resulting in a significant improvement in symptoms as illustrated in Fig. 5.

As illustrated in Fig. 5, a significant increase in improvement was again observed in the specific IgG titer for the patient parallel with the improvement of the symptoms by the replacement of DIIla with Ei-M, although no significant change was detected in

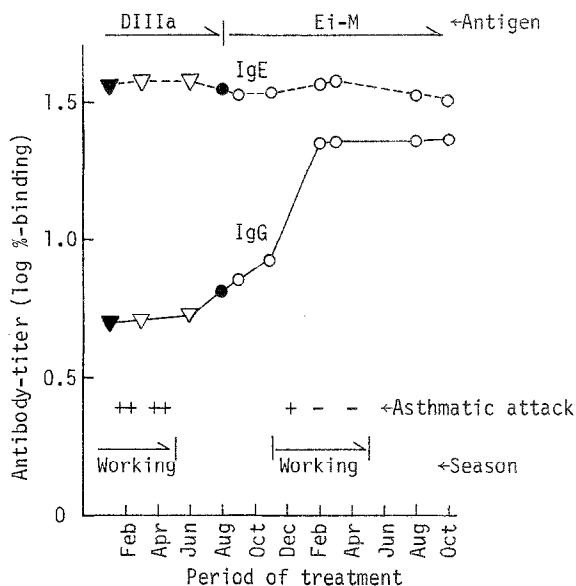


FIG. 5. Influence of sequential treatments with DIIla and Ei-M on symptom and titers of Ei-M-specific IgE and IgG in a typical patient with sea squirt allergy. No significant improvement was obtained in symptoms of a patient with sea squirt allergy by therapy with DIIla during the first working season. The therapeutic antigen was replaced with Ei-M at the treatment stage of August, and the therapy was continued for an additional 14 months, resulting in a significant improvement in symptoms in the next working season. In parallel with the therapy, titers of the specific IgE and IgG were also examined. Terms for the titers are the same as in Figs. 1 through 4.

the titers of the specific IgE and IgG as long as the treatment with DIIla was continued, as illustrated in Fig. 5. However, the IgE titer was practically independent of the therapeutic antigens. The influences of the therapy with Ei-M on the symptoms and the specific IgE and IgG titers were essentially the same as those observed in Fig. 3.

DISCUSSION

In the present study it has been observed that Ei-M-specific serum IgG titer significantly increased in most of Ei-M-hyposensitized patients within the first year parallel with the appearance of the therapeutic effect (Fig. 2, B). The specific IgG titer also increased in some patients hyposensitized with Gi-rep and DIIla within the first year parallel with the appearance of the therapeutic effect (Figs. 3, B, and 4, B). However, no such increase was detected in the IgG titer for unsuccessfully treated patients in the Gi-rep and DIIla hyposensitized groups. Furthermore, Ei-M-specific IgE titer appeared to be mostly independent of the therapy in all patients examined (Figs. 2, A, 3, A, and 4, A).

Such correlation between the significant increase in the specific IgG titer and the expression of the therapeutic effect supports the previous suggestion that the effect of hyposensitization closely depends on the IgG antibody that was specifically induced by the sea squirt antigens.⁸⁻¹¹

However, increase of the IgG titer apparently delayed in phase the appearance of the therapeutic effect in two successfully treated patients (Nos. 12 and 14) in the Gi-rep hyposensitized group. Furthermore, no significant increase was detected in the IgG titer in a successfully treated patient (No. 32) in the DIIIa hyposensitized group. Since the specific IgE level appeared to be unchanged during the therapy, it might be presumed that in these patients, although the increase of the specific IgG by therapy was almost undetectable by the present assay method, the IgG level could be increased over the unchanged IgE level by adding the newly induced IgG, resulting in the therapeutic effect.

In contrast, it was again recognized that the hyposensitization therapy was most effective with Ei-M and slightly less with Gi-rep, but markedly less effective with DIIIa (Table II). It has been considered that the allergy-specific epitope in DIIIa was essentially the same in specificity as those of Ei-M and Gi-rep because DIIIa induces the skin reaction in the allergic patients similar to reactions to the other two antigens.¹³ The similarity of the epitope was further supported by the *in vitro* observations that DIIIa as well as Gi-rep and Ei-M induced histamine release significantly from leukocytes in blood of the allergic patients.^{12, 14}

Nevertheless, only DIIIa was significantly less effective in the hyposensitization therapy as well as in the induction of the specific IgG (Figs. 4 and 5). The relatively low therapeutic efficiency of DIIIa could be attributed to its low immunogenicity. In fact, a low immunogenicity was recognized in immunization experiments with mice.¹⁵ It was also found that the immunogenicity of DIIIa significantly increased when it was polymerized by use of glutaraldehyde.¹⁵ In that case, the skin reactivity of DIIIa was mostly conserved in the polymerized DIIIa, whereas the conjunctival and bronchial provocation activities were eliminated.¹⁵ In the recent study it was also observed that the polymerized DIIIa (degree of polymerization, about 10) was as effective as Ei-M not only in the induction of the specific IgG but also in the hyposensitization therapy of allergic patients.¹⁶

These observations suggest that the therapeutic efficiency of an antigen is determined not only by the presence of the allergy-specific epitope but also by its immunogenicity depending on its molecular properties, such as MW.

It is also suggested that the conjunctival and bronchial provocation activities of DIIIa are attributable not only to the allergy-specific epitope but also to its permeability to mucosa, such as conjunctiva and bronchi, whereas no epitope other than the allergy-specific epitope is responsible to the mucosal reaction.^{15, 16} The results of polymerized DIIIa will be discussed elsewhere in detail.

The recent immunochemical study on Gi-rep suggested that an allergy-specific epitope responsible for the skin reaction was corresponded to a certain structural unit of a hexosamine-containing oligosaccharide residing in the carbohydrate chains of the antigens. The allergy-specific epitope was corresponded to an epitope that was detectable *in vitro* by the specific reactivity with a rabbit anti-Gi-rep serum and referred to as type α epitope.^{1-4, 13, 17} The content of type α epitope in Gi-rep has been estimated to be about 13% of those in Ei-M and DIIIa,¹³ and thus, the low content of type α epitope might reflect the slightly low efficiency of Gi-rep in the induction of the specific IgG and the therapeutic effect relative to that of Ei-M.

In the present study, both specific IgE and IgG titers were assayed against the most effective therapeutic antigen, Ei-M, containing not only type α epitope but also type β .^{1-4, 13} Since Gi-rep contains type α epitope alone, at least for the patients hyposensitized with Gi-rep, both titers are directed exclusively against type α epitope, and the induction of the specific IgG is also ascribed to type α epitope. However, for the patients hyposensitized with Ei-M and DIIIa containing the two types of epitopes, the specific titers might be directed against the epitopes of types α and β . Both specific titers should be further examined against Gi-rep to determine the specific role of type α epitope in the hyposensitization therapy.

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Hypodense eosinophilic granulocytes in normal individuals and patients with asthma: Generation of hypodense cell populations in vitro

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*Eosinophils from normal nonatopic healthy volunteers (195 ± 106 cells per microliter) were isolated by centrifugation over a discontinuous Percoll gradient, under isotonic conditions, with a recovery of $46.5 \pm 26.2\%$ from whole blood ($n = 21$; mean $\pm$ SD). More than 90% of the eosinophils (purity $>93\%$) with a density between 1.095 to 1.105 gm/ml were defined normodense. Less than 10% of the eosinophils had a density <1.095 gm/ml and were defined hypodense. Isolation of eosinophils of patients with atopic asthma revealed a cell population with 65% to 70% hypodense cells that was independent of the total eosinophilic cell count. In vitro activation of normodense eosinophils, measured by an increase in superoxide production, induced quantities of hypodense eosinophils in the range found in patients with asthma. The amount of hypodense eosinophils induced by different stimuli was in the same order as the increase in superoxide production (antibiotic calcium ionophore A23187 $>$ serum-treated zymosan $>$ platelet-activating factor $>$ N-formyl-methionyl-leucyl phenylalanine). During the stimulation of the normodense cells, no secretion of eosinophilic peroxidase or arylsulfatase B could be measured, even though hypodense eosinophils were produced. Enzymatic activity of arylsulfatase B within the eosinophils remained the same, before and after stimulation. The enzymatic activity of eosinophilic peroxidase in normodense eosinophils ($16.6 \pm 9.7 \mu\text{g}/10^6$ cells) did not change in the normodense fraction but was increased in the induced hypodense cells ($34.0 \pm 8.4 \mu\text{g}/10^6$ cells; $n = 7$; means $\pm$ SD; $p < 0.01$) after stimulation. These observations suggest that hypodense eosinophils, like those found in patients with atopic asthma, are not raised as a result of degranulation but are more likely generated by in vivo stimulation of the cells. (*J ALLERGY CLIN IMMUNOL* 1989;83:393-400.)*