

CONJUNCTIVAL ALLERGENICITY-SUPPORTING FACTOR (CASF) IN DIIIa AS THE SEA SQUIRT ANTIGEN CAPABLE OF INDUCING ASTHMATIC ATTACK AND CONJUNCTIVAL REACTION IN SEA SQUIRT ASTHMA

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Among three glycoproteins, DIIIa, Ei-M, and Gi-rep, isolated from sea squirt as antigens capable of eliciting skin reaction specific to sea squirt allergy, only DIIIa induces asthmatic attack and conjunctival reaction, whereas Ei-M and Gi-rep do not. Periodate oxidation eliminated not only the conjunctival allergenicity but also the skin allergenicity from DIIIa, suggesting that the conjunctival and skin allergenicities could both be ascribed to the epitope residing in the carbohydrate chains of DIIIa. On the other hand, proteolysis with Pronase E and chemical modifications of the carboxyl or amino groups by various methods eliminated the conjunctival allergenicity from DIIIa, whereas the skin allergenicity was still retained. Therefore, the expression of conjunctival reaction was expected to be supported by a relatively small structural factor residing in the protein moiety of DIIIa, which we have tentatively designated as conjunctival allergenicity-supporting factor (CASF). CASF might contribute to the permeability of the antigen into mucosa, enabling the carbohydrate epitope to interact with the allergy-specific IgE distributed in conjunctiva and bronchia. In specificity analysis of a rabbit anti-DIIIa serum, a periodate oxidation- and acid-stable but heat- and alkali-unstable epitope (type γ) in the protein moiety of DIIIa was also characterized as a useful marker of the antigen. However, the type γ epitope was apparently independent of CASF that was substantially stable to alkali and heat.

Key words: conjunctival reaction — epitope — mucosal permeability — periodate oxidation — skin reaction

INTRODUCTION

Recently, we have isolated and characterized a weakly acidic glycoprotein termed DIIIa (MW 9,980) from the body fluid of a sea squirt, *Styela plicata*, as the only antigen capable of inducing asthmatic attack and conjunctival reaction in addition to skin reaction in patients with sea squirt asthma¹⁾. Since the previously isolated two sea squirt antigens, Gi-rep (MW 106,000) and Ei-M

(MW 22,800), capable of inducing skin reaction have never induced allergic reactions in the bronchia or conjunctiva²⁾³⁾, DIIIa could apparently be distinguished from the two previous antigens¹⁾.

Radioimmunometric analyses carried out previously also revealed that DIIIa was apparently cross-reactive to rabbit anti-Gi-rep (containing type A antibody alone³⁾⁴⁾ and anti-Ei-M (containing type A and B antibodies³⁾⁴⁾) sera¹⁾, suggesting that DIIIa as well as Gi-rep and Ei-M carried a common epitope (type α ³⁾⁴⁾) specific to type A antibody, which was expected again to correspond to the epitope responsible for the common skin reaction¹⁾⁻⁴⁾.

Since the allergic reaction either in skin or in mucosa would be mediated by the cross-linking of the specific IgE antibodies through two or more epitopes on the allergen molecule, it was expected

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Abbreviations: CASF conjunctival allergenicity-supporting factor; 2Fuc 2-*O*-substituted fucose; GalNAc *N*-acetylgalactosamine; 3/4GalNAc 3- or 4-*O*-substituted *N*-acetylhexosamine; Hepes *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; MW molecular weight; Tris tris (hydroxymethyl) aminomethane

that the epitope responsible for the skin allergenicity should also elicit the allergic reaction even in the bronchia and conjunctiva. Nevertheless, the mucosal allergenicity was limited to DIIIa among the three sea squirt antigens. This fact suggests that only DIIIa contains a factor supporting the reaction of the antigen to the specific IgE antibodies located in mucosa.

In an attempt to elucidate the characteristics of the conjunctival allergenicity-supporting factor (CASF) as a representative of the supporting factor in mucosa, present investigations were made to clarify the effects of chemical, physical, and enzymatic treatments on the allergenicities in the conjunctiva and skin and on the in vitro antigenicities of DIIIa.

MATERIALS AND METHODS

Sea squirt antigens The purified antigens, DIIIa, Gi-rep and Ei-M, were prepared and characterized as reported in previous papers¹⁾⁻⁴⁾.

Anti-sea squirt sera Anti-DIIIa serum (L 708) was prepared by immunizing a rabbit with DIIIa with the aid of Freund's complete adjuvant according to the procedure used in previous studies²⁾⁻⁴⁾.

Antigenic activity Before and after various treatments, the antigenic activities of DIIIa were assayed against the rabbit anti-DIIIa serum (L 708) by radioimmunoassay (RIA) using ¹²⁵I-labeled DIIIa and Gi-rep according to the procedure used in previous studies²⁾⁻⁶⁾.

Skin test Skin tests were carried out according to the method reported in previous papers²⁾⁻⁶⁾. Briefly, 20 μ l of a sample solution (100 μ g/ml in 0.9% NaCl) was injected in patients with sea squirt asthma. Scores of 0, 0.5, 1, 2, and 3 were assigned for erythema of <10, 10 to 20, 20 to 30, 30 to 40, and > 40 mm in diameter at 15 min, respectively.

The allergenicity of a sample (DIIIa after a given treatment) was expressed by the ratio of the total scores obtained in 4~12 patients by the sample to that obtained by untreated DIIIa in the

same patients as (total score for the sample)/(total score for untreated DIIIa). For example, when scores of 0, 1, 0, and 1 were obtained by a sample, and scores of 2, 3, 2, and 3 were obtained by untreated 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 4 patients ($n=4$).

Conjunctival reaction test Conjunctival reaction tests were carried out in the same patients subjected to skin tests. In the same way as in the previous study¹⁾, a drop of a sample solution (1 mg/ml in 0.9% NaCl) was introduced as a challenge into a patient's eye, and the congestion of the conjunctiva was examined after 15 min. Simultaneously, an untreated DIIIa solution of the same concentration was also dropped into the other eye as a control challenge.

Scores of 0, 1, 2 and 3 were assigned for congestion observed as none, localized, intermediate, and strong, respectively. The allergenicity of the sample in the conjunctiva was then expressed by the ratio of the total score obtained by the sample to that obtained by the untreated DIIIa in the same patients, similarly to the method employed to express the allergenicity in the skin.

Treatment with acid, alkali and heat DIIIa (1 mg) was incubated in 200 μ l of 0.5 N HCl or 0.5 N NaOH at 22°C and neutralized at a given reaction time. The antigen (1 mg) in 200 μ l of 0.9% NaCl was also heated at 100°C for a given reaction time. The samples thus treated were dialyzed and freeze-dried.

Periodate oxidation In the same way as in the previous studies⁵⁾⁻⁷⁾, DIIIa (1 mg) was incubated with 4 mg of NaIO₄ in 2 ml of deionized water at 25°C for a given reaction time. After the addition of an excess amount of ethylenglycol, the reaction mixture was chromatographed on a Sephadex G-25 column (1.4 $\times$ 60 cm) with deionized water, and the periodate-oxidized DIIIa recovered in the effluent was freeze-dried.

Proteolysis According to the method employed in the previous studies⁵⁾⁶⁾⁸⁾, DIIIa (1 mg) was incubated with 0.4 mg of Pronase E (Kakenkagaku, Tokyo) at 37°C in 4 ml of 0.1 M Tris-HCl

buffer (pH 8.0) containing 20 mM CaCl_2 under a toluene layer. After 48 hr incubation, the reaction mixture was chromatographed on a Sephadex G-50 column (2.4×100 cm) with 1% NaCl, and the pronase-digest of DIIIa was recovered in the effluent, dialyzed and freeze-dried.

Composition analysis The methods used for composition analysis were the same as those used in the preceding studies¹¹⁻⁸⁾. Amino acids and hexosamines were determined on a KLA-5 amino acid analyzer (Hitachi, Tokyo) after hydrolysis of a sample in 6 N HCl at 110°C for 24 hr and in 4 N HCl at 100°C for 6 hr, respectively. Neutral sugars were determined on a 20 K gas-chromatograph analyzer (JEOL, Tokyo) after hydrolysis of a sample in 4 N trifluoroacetic acid at 100°C for 4 hr.

Modifications of ionic groups Amino and carboxyl groups in DIIIa were chemically modified as follows. The degree of *N*-substitution was measured as the reduction in reactivity of amino groups to *o*-phthalaldehyde; 20 μl of a sample solution (10 mg/ml) in deionized water was reacted with an equal volume of *o*-phthalaldehyde (80 mg/ml) in 0.35 M borate buffer, pH 10.4, at 55°C for 1 min, and the fluorescence from the reaction product was measured in a flow cell using emission λ at 434 nm with a FP-210 spectrofluorometer (JASCO, Tokyo) under excitation λ at 365 nm, with leucine as a standard.

Reductive *N*-methylation—According to the method of Jentoft and Dearborn^{9,10)}, 20 mg of DIIIa was reacted with 40 μmol of formaldehyde in 20 ml of 0.1 M Hepes buffer (pH 7.5) at 25°C in the presence of 0.4 mmol of NaBH_3CN for 4 hr. After dialysis and freeze-drying, the substitution of amino groups was 2.3 mol/mol in the product.

***N*-Acetylation**—According to the method of Riodan et al.¹¹⁾, 40 mg of DIIIa in 2 ml of 30% sodium acetate solution was reacted with five of 1.6 ml each of acetic anhydride at 25°C within 1 hr. After dialysis and freeze-drying, the substitution of amino groups was 3.2 mol/mol in the product.

***N*-Succinilation**—According to the method of Chu et al.¹²⁾, 20 mg of DIIIa was incubated with 30.2 mg of succinic anhydride in 2 ml of 5 M guanidine-HCl for 1 hr, keeping pH at 8.0 with 1 N NaOH. Then, 30 mg of hydroxylamine-HCl was added to the solution, and the reaction mixture was incubated for an additional 1 hr, keeping pH at 10 with 1 N NaOH. After dialysis and freeze-drying, the substitution of amino groups was 2.7 mol/mol in the product.

***N*-Maleylation**—According to the method of Butler et al.¹³⁾, 30 mg of DIIIa was reacted with 41.2 mg of maleyl anhydride in 3 ml of 0.1 M phosphate buffer at pH 8.5-9.0 for 1 hr, keeping the temperature at 0°C. After dialysis and freeze-drying, the substitution of amino groups was 2.8 mol/mol in the product.

De-maleylation of maleyl-DIIIa—According to the method of Glazer¹⁴⁾, 10 mg of maleyl-DIIIa was dialyzed against 10 mM of pyridine-acetic acid buffer, pH 3.5, at 25°C for 24 hr, and the product was dialyzed and freeze-dried.

***C*-Amidation**—According to the method of Carraway et al.¹⁵⁾, 20 mg of DIIIa and 890 mg of glycine methyl ester were dissolved in 10 ml of 5.0 M guanidine-HCl and adjusted to pH 4.75 with 2 N HCl and reacted with 30 mg of 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide at 25°C for 100 min, keeping pH at 4.75 with HCl. The reaction mixture was dialyzed and freeze-dried. The substitution of carboxyl groups was 2.4 mol/mol as measured from the increase of glycine in the amino acid composition.

Reagents All reagents used were of analytical grade.

RESULTS

Immunological reactivity of anti-DIIIa serum The immunological reactivities of a rabbit anti-DIIIa serum (L 708) were examined by RIA against the three sea squirt antigens, DIIIa, Gi-rep, and Ei-M. The binding of radiolabeled DIIIa to the rabbit anti-DIIIa serum was apparently inhibited

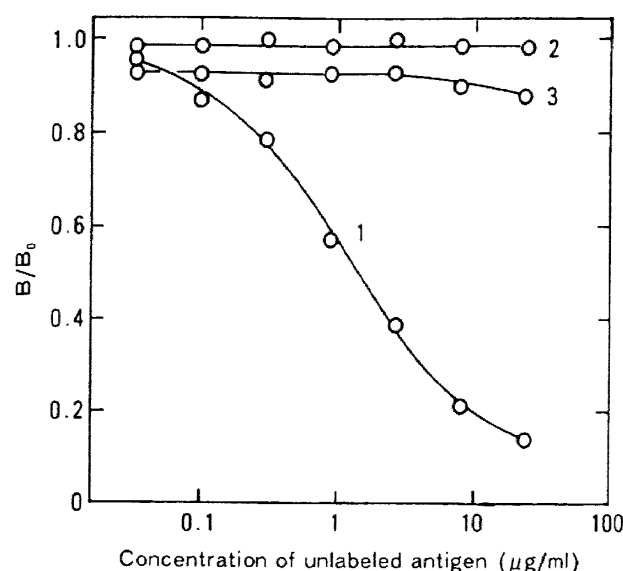


Fig. 1. Inhibition of binding reaction of radiolabeled DIIIa to a rabbit anti-DIIIa serum (L 708) by the three unlabeled antigens. The immunological reaction between ^{125}I -labeled DIIIa (ca. 10,000 cpm) and $1\ \mu\text{l}$ of a rabbit anti-DIIIa serum in $200\ \mu\text{l}$ of $0.05\ \text{M}$ PBS-1% NaCl-5% (v/v) normal rabbit serum was inhibited by unlabeled DIIIa (curve 1), Gi-rep (curve 2) and Ei-M (curve 3). B/B_0 is the ratio of antibody-bound radioactivity in the presence of an unlabeled antigen (B) to that in the control experiment with the radiolabeled DIIIa alone (B_0).

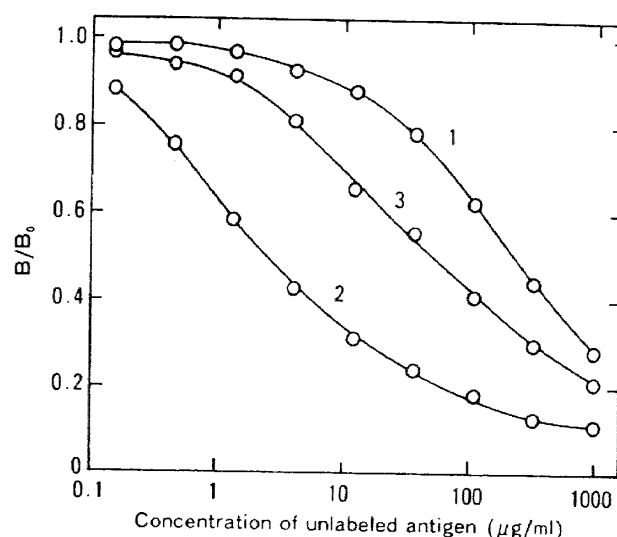


Fig. 2. Inhibition of binding reaction of radiolabeled Gi-rep to a rabbit anti-DIIIa serum (L 708) by the three unlabeled antigens. The immunological reaction between ^{125}I -labeled Gi-rep (ca. 8,000 cpm) and $0.17\ \mu\text{l}$ of a rabbit anti-DIIIa serum in $200\ \mu\text{l}$ of $0.05\ \text{M}$ PBS-1% NaCl-5% (v/v) normal rabbit serum was inhibited by unlabeled DIIIa (curve 1), Gi-rep (curve 2) and Ei-M (curve 3). B/B_0 is the same as in the legend to Fig. 1 except that radiolabeled Gi-rep was used.

with increasing concentrations of unlabeled DIIIa, whereas unlabeled Gi-rep and Ei-M hardly inhibited the binding (Fig. 1).

The limited inhibition by Gi-rep (containing type α epitope alone^{3,4)}) and Ei-M (containing types α and β epitope alone^{3,4)}) showed that the anti-DIIIa serum contained a large amount of the third type antibody (type C), in addition to the antibodies of types A and B characterized in the previous studies^{3,4)}. Since type C antibody was considered to be induced by and reacted to an additional epitope (type γ) specific to DIIIa, the in vitro antigenicity depending on type γ epitope as well as the in vivo allergenicity inducing asthmatic attack and conjunctival reaction were considered to be characteristic to DIIIa.

On the other hand, the binding reaction of radiolabeled Gi-rep to the same anti-DIIIa serum containing antibodies of types A, B, and C was inhibited not only by unlabeled Gi-rep but also by unlabeled DIIIa and Ei-M (Fig. 2). This observation supported the previous suggestion that DIIIa

as well as Gi-rep and Ei-M contained type α epitope, which might correspond to the epitope responsible for the skin allergenicity in common with the three antigens¹⁾. The antigenic activity depending on type α epitope in DIIIa was assayed to be $11\ \mu\text{g}/\text{ml}$ as Gi-rep from the distance between curve 1 (inhibition by unlabeled DIIIa) and curve 2 (inhibition by unlabeled Gi-rep) in Fig. 2.

In the following experiments, the antigenic activities depending on the epitopes of types γ and α were assayed by the RIA method using curve 1 in Fig. 1 and curve 2 in Fig. 2 for calibration, and referred to as γ - and α -activities, respectively.

Periodate oxidation The carbohydrate moiety of DIIIa was examined for its contribution to the in vivo and in vitro activities by periodate oxidation treatment. Periodate treatment simultaneously eliminated the conjunctival and skin allergenicities and α -activity from DIIIa, whereas γ -activity was mostly conserved even after treatment. (Table I-1).

This observation indicated, as with Gi-rep^{5,6)}, that both allergy-specific epitope and type α

Table I Influence of physical, chemical, and enzymatic treatments on antigenicities and allergenicities of DIIIa

Treatment	Antigenicity		Allergenicity		
	γ -activity as DIIIa ($\mu\text{g}/\text{mg}$)	α -activity as Gi-rep ($\mu\text{g}/\text{mg}$)	In con- junctiva	In skin	Number of patients (n)
0. Untreated	1000	11			
1. NaIO ₄ oxidation					
24 hr	880	0.4	5/16	3/9	(6)
48 hr	870	<0.1	1/12	0/11	(7)
2. 0.5 N HCl, 25°C					
1 hr	610	10	—	—	
4 hr	540	11	16/16	9/9	(6)
8 hr	560	11	—	—	
3. 0.5 N NaOH, 25°C					
1 hr	20	7.7	—	—	
4 hr	20	8.3	11/10	7/8	(5)
8 hr	<10	7.7	—	—	
4. Heat, 100°C					
1 hr	70	4.5	—	—	
4 hr	20	4.2	7/7	5/6	(4)
8 hr	<10	3.8	—	—	
5. Pronase digestion	<10	4.2	0/12	11/12	(7)

See MATERIALS AND METHODS for the methods of assay and expression of the antigenicity and allergenicity.

epitope resided in the carbohydrate moiety of DIIIa. Furthermore, it was also suggested that the periodate-unstable epitope responsible for the skin allergenicity was also essential for DIIIa to express the conjunctival allergenicity. After the periodate oxidation, significant amounts of sugars were eliminated from DIIIa, while most of amino acids seemed to be retained (Table II).

Treatment with acid, alkali and heat The protein moiety of DIIIa was also examined for its contribution to the allergenic and antigenic activities by treating the antigen with HCl, NaOH, and heat. The conjunctival and skin allergenicities and α -activity were all substantially stable to the three treatments. However, γ -activity was significantly unstable to alkali and heat, though it was stable to acid (Table I-2~4).

These results suggest that γ -activity depends partly on some conformational structure within the type γ epitope in the protein moiety of DIIIa. However, it was suggested that the conjunctival allergenicity as well as the skin allergenicity was independent of such a conformational factor.

Proteolysis The protein moiety of DIIIa was further examined for its contribution to the antigenic and allergenic activities by treating the antigen with Pronase E.

The exhaustive proteolysis eliminated not only γ -activity but also the conjunctival allergenicity. However, about 40% of α -activity and most of the skin allergenicity were still retained (Table I-5). The results suggested that the factor required for expression of the conjunctival allergenicity as well as type γ epitope resided in the protein moiety of DIIIa, though they were independent of each other. After the proteolysis, significant amounts of amino acids were eliminated from DIIIa, while most of sugars seemed to be retained (Table II).

Chemical modifications of ionic groups The ionic groups in DIIIa were further examined for their contribution to the antigenic and allergenic activities by various chemical modifications. All treatments for chemical modification of the amino or carboxyl groups in DIIIa resulted in the concomitant elimination of the conjunctival allergenicity and γ -activity, whereas the skin aller-

genicity and α -activity were mostly conserved even after the modifications (Table III-1~5).

These observations suggested that the con-

Table II Amino acid and sugar compositions of DIIIa, pronase-digest of DIIIa, and IO $\bar{I}$ -oxidized DIIIa

Component	DIIIa (μ mol/g)	IO $\bar{I}$ - oxidized DIIIa (μ mol/g)	Pronase- digest of DIIIa (μ mol/g)
Lysine	310	330	30
Histidine	42	44	18
Arginine	106	99	38
Aspartic acid	502	476	158
Threonine	428	439	208
Serine	366	351	144
Glutamic acid	540	450	106
Proline	208	203	80
Glycine	466	476	98
Alanine	416	419	100
Cystine	8	9	28
Valine	228	260	56
Methionine	72	60	24
Isoleucine	150	176	30
Leucine	224	227	40
Tyrosine	232	232	24
Phenylalanine	140	142	28
Glucosamine	370	370	580
Galactosamine	332	185	524
Fucose	150	45	281
Mannose	160	72	309
Glucose	61	37	198
Galactose	136	52	338

junctival allergenicity-supporting factor (CASF) and γ -activity both depended on a certain structure of a relatively small portion of the protein moiety of DIIIa. The assumption was supported by the finding that the maleylated DIIIa recovered the conjunctival allergenicity and γ -activity when the maleyl group was removed from the derivative (Table III-6).

DISCUSSION

The periodate oxidation treatment eliminated not only the skin allergenicity but also the conjunctival allergenicity from DIIIa, which was the only antigen capable of inducing asthmatic attack and conjunctival reaction in addition to skin reaction in patients with sea squirt asthma¹⁾ (Table I). Thus, the conjunctival and skin allergenicities were both ascribed to the epitope residing in the carbohydrate chains of DIIIa. It could be expected that the carbohydrate epitope of DIIIa was essentially the same as that responsible for the skin allergenicity of Gi-rep and Ei-M²⁾⁻⁶⁾.

In connection with this, radioimmunometric analysis revealed that a rabbit anti-DIIIa serum apparently cross-reacted with Gi-rep (containing type α epitope alone³⁾⁴⁾), and the result supported the previous suggestion that DIIIa also contained type α epitope in common with Gi-rep and Ei-M¹⁾ (Fig. 2). Furthermore, the antigenicity depending on type α epitope as well as the skin allergenicity

Table III Influence of chemical modifications of ionic groups on antigenic and allergenic activities of DIIIa

Treatment	Antigenicity		Allergenicity		
	γ -activity as DIIIa (μ g/mg)	α -activity as Gi-rep (μ g/mg)	In con- junctiva	In skin	Number of patients (n)
0. Untreated	1000	11			
1. C-Amidation	<10	7.1	1/17	16/21	(12)
2. Reductive N-Methylation	<10	12	4/12	13/13	(8)
3. N-Acetylation	<10	12	3/25	20/25	(14)
4. N-Succinylation	<10	13	4/24	21/22	(16)
5. N-Maleylation	<10	9.4	3/17	18/19	(11)
6. Demaleylation of N-maleyl-DIIIa	1110	10	14/13	16/17	(9)

See MATERIALS AND METHODS for the methods of assay and expression of the antigenicity and allergenicity.

of DIIIa was substantially unstable to periodate oxidation but stable to acid, alkali, heat, pronase digestion, and all the chemical modifications of the carboxyl and amino groups examined (Tables I and III). Therefore, type α epitope of DIIIa was considered to be a useful marker of the carbohydrate epitope responsible for the skin allergenicity in common with the three sea-squirt antigens, DIIIa, Gi-rep, and Ei-M⁽¹⁻⁶⁾.

On the other hand, the conjunctival allergenicity of DIIIa was eliminated by proteolysis with Pronase E and every chemical modification of the carboxyl or amino groups, whereas the skin allergenicity was still retained even after these treatments (Tables I and III). Therefore, the conjunctival allergenicity was considered to be supported by a relatively small structural factor residing in the protein moiety of DIIIa, which we have tentatively designated as conjunctival allergenicity-supporting factor (CASF). CASF might contribute to the permeability of the antigen into mucosa, enabling the carbohydrate epitope to interact with the allergy-specific IgE antibody distributed not only in conjunctiva but also bronchia.

Type γ epitope residing in the protein moiety of DIIIa was also characterized as a useful marker of the antigen by the specificity analysis of a rabbit anti-DIIIa serum (Fig. 1 and Tables I and III). However, type γ epitope was unstable to alkali and heat, and it was considered to be independent of CASF as this was substantially stable to alkali and heat.

In the recent study on Gi-rep⁵⁾, it was also observed that the skin allergenicity and the antigenicity depending on type α epitope were both apparently unstable to the periodate oxidation treatment, and it was suggested that both the skin allergenicity and the antigenicity closely depended on a certain oligosaccharide structure residing in both of the *O*- and *N*-glycosidically linked carbohydrate chains of the antigen. Subsequently, two disaccharide units composed of *N*-acetylgalactosamine (GalNAc) and 2-*O*-substituted fucose (2Fuc) or 3- or 4-*O*-substituted *N*-acetylhexosamine (3/4 HexNAc), GalNAc α 1 $\rightarrow$ 2Fuc1 $\rightarrow$ and GalNAc1 $\rightarrow$ 3/4 HexNAc1 $\rightarrow$, have been identified in the *N*-linked carbohydrate chain of Gi-rep as the possible major structural units in the carbohy-

drate epitope responsible for the skin allergenicity specific to sea squirt asthma⁶⁾.

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喘息並びに結膜反応誘発性ホヤ抗原, DIIIa 中の 粘膜アレルゲン性支援因子 (CASF)

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ホヤ喘息患者に対して特異的に皮内反応を与える 3 つの糖タンパク性ホヤ抗原, DIIIa, Ei-M, 及び Gi-rep の内, 喘息誘発能及び結膜反応性を合わせもつのは DIIIa だけで, Ei-M や Gi-rep はこれを欠く。この DIIIa を, 過ヨウ素酸で酸化すると, 皮内反応性と同時に, 結膜反応性も消失するので, この両アレルゲン活性は, いずれも, DIIIa の糖鎖上に存する同じエピトープに依存するものと考えられた。一方, プロナーゼ消化や, カルボキシル基及びアミノ基の化学修飾によっても DIIIa の結膜反応性は消失するが, 皮内反応性は保存される。したがって, DIIIa に特有な, 気管支や結膜などの粘膜におけるアレルギー反応の誘発は, DIIIa のタンパク部分に存在する比較的小さな構造的因子によって支援されている可能性が示唆される。この結膜アレルゲン性支援因子 (CASF) は, DIIIa に粘膜透過性を付与することにより, 粘膜中に分布するホヤアレルギー特異的 IgE に対して, 糖鎖性エピトープを介する DIIIa の免疫反応を可能にしているものと思われる。なお, ウサギ抗 DIIIa 血清の特異性を解析して, DIIIa マーカーとして有用な, 過ヨウ素酸酸化及び酸に安定で, 熱及びアルカリには不安定な, タンパク性のエピトープ (γ 型) の存在を検出した。しかし, γ 型エピトープは, この熱及びアルカリには安定な CASF とは一致しない。