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B Cell Mitogenic Activity of Sea Squirt Antigen

Abstract

The activity of sea squirt antigen, one of the allergy-inducing substances for humans, on murine and human lymphocytes was studied in vitro. Sea squirt antigen stimulated normal mouse spleen cells to proliferate, as detected by [³H]-TdR incorporation, in a dose-dependent manner. The responder cells are B cells because the response was reduced by the treatment of spleen cells with anti-immunoglobulin antibody and complement and passing through a nylon wool column, but not with anti-Thy-1 antibody and complement. Spleen cells of C3H/HeJ mice, which are lipopolysaccharide low responders, were also stimulated as well as spleen cells of C3H/HeN mice, suggesting that this response is not due to lipopolysaccharide in the antigen fraction. Sea squirt antigen stimulated not only proliferative response of B cells, but also polyclonal immunoglobulin production. Furthermore, sea squirt antigen also stimulated human lymphocytes to proliferate and to produce immunoglobulin. All these results suggest that sea squirt antigen has mitogenic activity on B cells, and this ability is concerned with the induction of allergic reaction.

Key Words

B cell mitogenic activity
 Immunoglobulin production
 Sea squirt antigen

Introduction

Sea squirt allergy, Hoya asthma, observed among oyster culture workers is a typical type I allergy, and the patients with asthmatic attacks have high titers of immunoglobulin E antibody in their sera [1, 2]. This asthmatic attack is caused by an inhalation of the body fluid of sea squirt (*Protochorda*) grown on the oyster shells during oyster shucking work. Oka et al. [3–5] and Suzuki et al. [6] purified the antigen from sea squirt body fluid and fractionated it into three fractions termed Gi-rep, Ei-M, and DIIIa on the basis of the capacity to induce a skin reaction in the patients. Among these three fractions, DIIIa is most active in the induction of skin test, while Gi-rep and Ei-M induce IgG class antibody which can be used for hyposensitization. Oka et al. [7] also found that the antigenic determinant reacting with antibody

resides in carbohydrate chains of a sea squirt antigen. However, the biological activity of the sea squirt antigen remains to be elucidated. In this paper, we studied the effects of sea squirt antigen on murine and human lymphocytes in vitro.

Materials and Methods

Mice

C3H/HeN and C3H/HeJ mice were obtained from Seiwa Experimental Animal (Yoshitomi, Ohita, Japan) and maintained on Purina rodent chow and water ad libitum. Both male and female mice were used at 6–8 weeks of age.

Sea Squirt Antigen

Crude antigen was prepared from sea squirt (*Styela plicata*) body fluid and was dialyzed against phosphate-buffered saline (pH 7.2) and lyophilized. Three fractions, termed Gi-rep, Ei-M, and DIIIa (molec-

ular weights 106,000, 22,800, and 9,980), were separated by gel chromatography on the basis of the capacity to induce a skin reaction in asthmatic patients with sea squirt allergy as previously described [6].

Assay of Proliferative Response

A spleen cell suspension was prepared from normal mice and was treated with 0.83% NH_4Cl in 0.017 M Tris buffer (pH 7.2) to lyse red blood cells. Spleen cells (2×10^5) were cultured with several concentrations of sea squirt antigens, 10 $\mu\text{g}/\text{ml}$ concanavalin A (ConA; EY Laboratories, San Mateo, Calif., USA), 10 $\mu\text{g}/\text{ml}$ lipopolysaccharide (LPS) from *Escherichia coli* (Sigma Chemical, St. Louis, Mo., USA), or 0.01% *Staphylococcus aureus* cowan I (SAC; Funakoshi Yakuhin, Tokyo, Japan) in 0.2 ml of the Eagle-Hanks amino acid medium described by Corradin et al. [8] containing 10% fetal calf serum (Grand Island Biological, Grand Island, N.Y., USA) in wells of flat-bottomed microtiter culture plates (No. 3072; Falcon Plastics, Oxnard, Calif., USA) at 37°C for 3 days in 5% CO_2 and 95% air. The cells were labeled for the last 18 h with 0.5 μCi of tritiated thymidine (^3H]-TdR, specific activity 6.0 Ci/mmol; Amersham, Buckinghamshire, UK) and were harvested with the aid of a semiautomated cell harvester (Abe Kagaku, Chiba, Japan) [9]. The amount of radioactivity incorporated into DNA in the cells was measured with a liquid scintillation spectrophotometer. The results are expressed as the mean counts per minute (cpm) of triplicate cultures with standard error.

Fractionation of Spleen Cells

Spleen cells were treated with 100 times diluted anti-mouse Thy 1.2 monoclonal antibody (Olac 1976; Blackthorn Biester, Oxon, UK) or 20 times diluted rabbit anti-mouse immunoglobulin sera (Cedarlane Laboratories, Hornby, Canada) and six times diluted rabbit complement (Cedarlane Laboratories) at 37°C for 1 h and B cell and T cell fractions were obtained. Purified T cells were also prepared by passing spleen cells through a nylon wool column as previously described [10]. After treatment of spleen cells with anti-Thy 1 antibody or anti-immunoglobulin sera and complement Thy-1-positive and immunoglobulin-positive cells decreased to less than 1 and 5%, respectively, as detected by flowcytometry using fluoresceinated antibody, while the untreated spleen cells contained about 40% Thy-1-positive cells and 40% immunoglobulin-positive cells. After the nylon wool column passing, immunoglobulin-positive cells decreased to less than 1%.

Assay of IgG and IgE Production by Spleen Cells

The production of IgG and IgE by spleen cells was detected by sandwich enzyme-linked immunosorbent assay (ELISA). Spleen cells ($1 \times 10^6/\text{ml}$) were cultured with sea squirt antigen or LPS in the presence or absence of 50 units/ml recombinant interleukin 4 (IL-4) [11] (kindly donated by Dr. K. Hama, Ono Pharmaceutical, Osaka, Japan) for 7 days. Then the culture supernatant was harvested by centrifugation. For the assay of total IgG or IgE, flat-bottomed microtiter plates (Sumitomo Bakelite, Tokyo, Japan) were coated with sheep antimouse IgG (5 $\mu\text{g}/\text{ml}$; Binding Site, Birmingham, UK) or anti-IgE monoclonal antibody [12] (6HD5, 5 $\mu\text{g}/\text{ml}$; kindly donated by Dr. N. Watanabe, Jikei University School of Medicine, Tokyo, Japan) at 37°C for 2 h and at 4°C for overnight. After blocking with 1% bovine serum albumin solution in phosphate-buffered saline, the culture supernatant was added to the plate and incubated at 37°C for 1 h. After washing with phosphate-buffered saline, peroxidase-conjugated sheep antimouse IgG or IgE antibody diluted 800 or 200 times (Binding Site) was added to the plate and incubated at 37°C for 1 h. After washing with phosphate-buffered saline, *o*-phenylenediamine dihy-

drochloride (5.5×10^{-4} M; Wako Junyaku, Kyoto, Japan) in 0.01% H_2O_2 solution was added as a substrate and incubated at room temperature for 30 min. The reaction was stopped with 8 M H_2SO_4 , and the optical density (OD) at 490 nm was measured (Immuno Reader NJ-2000; Japan Intermed, Tokyo, Japan). The results are expressed as mean values $\mu\text{g}/\text{ml}$ or optical density of triplicate cultures with standard error.

Assay of Anti Sea Squirt Antibody Production

C3H/HeN mice were immunized with crude sea squirt antigen (100 μg) with aluminum hydroxide (2 mg) intraperitoneally. Four weeks after immunization, anti-sea squirt antibodies in their sera were detected by ELISA as described above using crude, Gi-rep, Ei-M, and DIIIa as antigens instead of antimouse IgG or IgE antibody.

Culture of Human Peripheral Blood Lymphocytes

Peripheral blood lymphocytes were prepared from heparinized peripheral blood of normal volunteers by centrifugation on a lymphocyte separation medium (Organon Teknica, Durham, N.C., USA). For preparation of T cell and B cell fractions, peripheral blood lymphocytes were treated with anti-CD3 or anti-CD20 antibodies (Seikagaku Kogyo, Tokyo) and rabbit complement (Cedarlane Laboratories) at 37°C for 1 h. After this treatment, CD3-positive cells and CD20-positive cells decreased to less than 2%, as detected by flowcytometry using fluoresceinated antibody, while CD3-positive and CD20-positive cells of the untreated peripheral blood lymphocytes amounted to about 70 and 20%, respectively. The assay system for proliferative response and immunoglobulin production was the same as that used in mouse experiments described above.

Statistics

All experiments were repeated at least three times. Statistical analysis was performed by Student's *t* test. A confidence level of $p < 0.05$ was considered significant [13].

Results

Mitogenic Activity of Sea Squirt Antigen on Murine Spleen Cells

At first, we studied the effect of sea squirt antigen and its fractions on the proliferative response of mouse spleen cells. As shown in table 1, crude sea squirt antigen markedly stimulated the proliferative response of normal spleen cells at concentrations of 1–100 $\mu\text{g}/\text{ml}$. Gi-rep also stimulated spleen cells comparable to crude antigen. Ei-M and DIIIa significantly stimulated spleen cells, although their activity was rather weak. In the following experiments, we mainly used 100 $\mu\text{g}/\text{ml}$ of crude antigen. Figure 1 shows the time course of proliferative responses of spleen cells stimulated with crude antigen. The peak response was observed after 2 days, and then the response gradually decreased. Therefore, in the following experiments we assayed on day 3.

Table 1. Proliferative response of spleen cells stimulated with sea squirt antigen

Source of antigen	[³ H]-TdR incorporation (cpm) of spleen cells stimulated with sea squirt antigens ^a			
	100 µg/ml	10 µg/ml	1 µg/ml	0 µg/ml
Crude	30,035±1,027 ^b	21,124±2,109 ^b	10,062±634 ^b	2,381±186
Gi-rep	24,751±1,251 ^b	8,691±286 ^b	6,045±250 ^b	
Ei-M	6,959±400 ^b	4,566±363 ^b	3,440±261	
DIIIa	6,980±590 ^b	5,169±537 ^b	3,911±261	
ConA		56,179±11,719 ^b		
LPS		27,086±1,341 ^b		

^a 2×10^5 spleen cells were cultured in vitro with several antigens or mitogens as indicated for 3 days, pulsed with 0.5 µCi/well of [³H]-TdR for the last 18 h, and harvested. [³H]-TdR incorporated was counted by a liquid scintillation spectrophotometer. The results are expressed as mean values ± SE of triplicate cultures.

^b Significantly stimulated.

Table 2. Responder cells are B cells for the stimulation with sea squirt antigen

Spleen cells treated with	[³ H]-TdR incorporation of spleen cells (cpm) stimulated with ^a			
	(control)	ConA	LPS	sea squirt
Complement	824±160	15,922±208	8,375±463	10,173±22
Anti-Thy 1 + complement	1,006±203	879±86 ^b	10,933±118 ^c	13,376±249 ^c
Anti-immunoglobulin plus complement	982±12	17,725±385 ^c	3,357±492 ^b	1,965±313 ^b

^a 2×10^5 spleen cells treated as indicated were cultured with 10 µg/ml ConA, 10 µg/ml LPS, or 100 µg/ml sea squirt antigen.

^b Significantly decreased as compared with the control group.

^c Significantly increased as compared with the control group.

Responder Cells Are B Cells

In order to determine the responder cells to sea squirt antigen, we fractionated spleen cells using anti-Thy-1 or anti-immunoglobulin antibody and complement. Control cells were treated with complement alone. As shown in table 2, control cells responded well to ConA, T cell mitogen, LPS, B cell mitogen, and sea squirt antigen. Anti-Thy-1 antibody and complement treatment completely abrogated the ConA response, but the responses of LPS and sea squirt antigen were better than that of the control cells. On the other hand, anti-immunoglobulin antibody and complement treatments depleted the response of LPS and sea squirt antigen, but the ConA response was enhanced. Since the B cell depletion from spleen cells with anti-immunoglobulin antibody and complement treatment was not complete, we next depleted B cells by using a nylon wool column. As shown in table 3, nylon-purified T cells alone weakly responded to ConA, LPS, and sea squirt antigen. The addition of mitomycin C (50 µg/ml; Kyowahakko Kogyo, Yokohama, Japan) treated spleen adherent cells as accessory cells could restore the

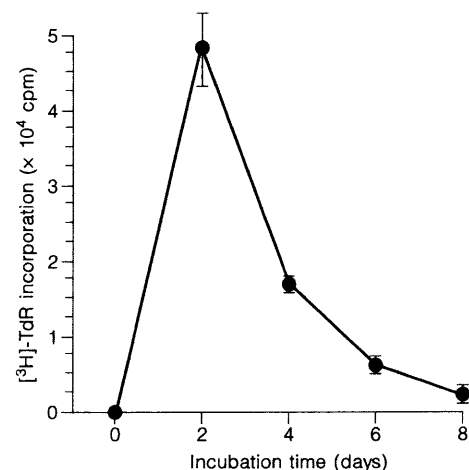


Fig. 1. Time course of proliferative response of spleen cells. 2×10^5 spleen cells were stimulated with 100 µg/ml sea squirt antigen and assayed on the days indicated. The cells were pulsed with [³H]-TdR for the last 18 h and harvested. Results expressed as mean ± SE of triplicate cultures.

Table 3. Nylon-purified T cells do not respond to sea squirt antigen

Responder cells	Addition of macrophages	[³ H]-TdR incorporation of spleen cells (cpm) stimulated with ^a		
		(control)	ConA	sea squirt
Spleen cells	—	5,677±547	86,331±2,514	49,731±3,943
T cells	—	515±49 ^b	7,013±560 ^b	987±165 ^b
T cells	+	2,837±262 ^b	59,991±2,569 ^{b,c}	2,903±146 ^b

^a 2×10^5 spleen cells or nylon column purified T cells were cultured with 10 µg/ml ConA or 100 µg/ml sea squirt antigen in the absence or presence of 1×10^5 mitomycin C treated spleen macrophages.

^b Significantly decreased as compared with the untreated spleen cells.

^c Significantly enhanced in the presence of macrophages.

Table 4. C3H/HeJ spleen cells can respond to sea squirt antigen

Source of spleen cells	[³ H]-TdR incorporation of spleen cells (cpm) stimulated with ^a			
	(control)	ConA	LPS	sea squirt
C3H/HeN	1,205±33	44,398±1,649	20,686±1,914	29,037±536
C3H/HeJ	3,312±180	31,478±2,165 ^b	5,555±442 ^b	51,613±3,941 ^c

^a 2×10^5 spleen cells were cultured with 10 µg/ml ConA, 10 µg/ml LPS, or 100 µg/ml sea squirt antigen.

^b Significantly suppressed as compared with C3H/HeN mice.

^c Significantly enhanced as compared with C3H/HeN mice.

ConA response, but not sea squirt antigen. These data suggest that the responder cells to sea squirt antigen are B cells but not T cells.

Mitogenic Activity Is Not Due to LPS

A representative B cell mitogen is LPS. Thus, a possibility is that the mitogenic activity of sea squirt antigen may be LPS contaminated in the antigen preparation. Then, we compared the responsiveness of spleen cells from C3H/HeN and C3H/HeJ mice which are low responders to LPS. As shown in table 4, C3H/HeN spleen cells responded to ConA, LPS, and sea squirt antigen, while C3H/HeJ spleen cells responded to ConA and sea squirt antigen, but not to LPS. In some experiments the responsiveness of C3H/HeJ to sea squirt antigen was better than that of C3H/HeN. Furthermore, polymyxin B, an inhibitor of LPS response, did not reduce the response of sea squirt antigen (data not shown). These results suggest that the mitogenic activity of sea squirt antigen is not due to LPS.

Sea Squirt Antigen Stimulates Immunoglobulin

Next, we studied the effect of sea squirt antigen on the production of immunoglobulin by B cells. As shown in table 5, sea squirt antigen enhanced the polyclonal IgG pro-

duction detected by sandwich ELISA as well as LPS. The addition of IL-4 further enhanced the IgG production. The production of IgE also had a tendency to be enhanced, but this was not statistically significant. The treatment of spleen cells with anti-Thy-1 antibody and complement had no effect on IgG production stimulated with sea squirt antigen. These results suggest that sea squirt antigen stimulates not only a proliferative response of B cells, but also polyclonal immunoglobulin production which is T cell independent.

Immunization of Mice with Sea Squirt Antigen Induces Both IgG and IgE Production

Then, we studied the antibody production in mice immunized with sea squirt antigen in aluminum hydroxide. As shown in table 6, the immunized mice markedly produced IgG and moderately IgE antibodies specific to sea squirt antigen, especially to crude and Gi-rep. The immunized spleen cells also showed the enhanced proliferative response upon the stimulation with sea squirt antigens.

Sea Squirt Antigen Also Stimulates Human B Cells

Finally we studied the effect of sea squirt antigen on human lymphocytes. As shown in table 7, sea squirt antigen stimulated human lymphocytes to proliferate. This re-

Table 5. Sea squirt antigen stimulates B cells to produce immunoglobulin

Spleen cells ^a		IgG (μg/ml) IL-4 ^b		IgE (OD at 490 nm × 10 ⁻³) IL-4 ^c	
treated with	cultured with	–	+	–	+
No treatment	control	0.45±0.02	0.50±0.01	14±1	7±1
	sea squirt	1.20±0.03 ^d	1.45±0.09 ^d	12±1	9±1
	LPS	1.18±0.03 ^d	2.17±0.39 ^d	13±1	21±2 ^d
Anti-Thy1 + complement	control	0.64±0.02	0.62±0.02	11±5	2±1
	sea squirt	1.15±0.03 ^d	1.71±0.25 ^d	11±1	6±1
	LPS	1.29±0.02 ^d	2.81±0.05 ^d	7±1	13±4 ^d

^a Spleen cells (10⁶/ml) were cultured with sea squirt antigen in the presence or absence of IL-4, and total IgG and IgE production was detected by ELISA.

^b IgG production expressed as micrograms per milliliter.

^c IgE production expressed as OD at 490 nm.

^d Significantly enhanced.

Table 6. Anti-sea-squirt-antibody production in immunized mice^a

Antigen	Antibody titer (OD×10 ³) ^b				Proliferative responses of spleen cells (cpm) ^a	
	IgG		IgE		normal	immune
	normal	immune	normal	immune		
Crude	102±7	595±43 ^d	66±3	149±16 ^d	12,891±1,077	33,926±664 ^d
Gi-rep	98±2	1,116±110 ^d	56±1	180±30 ^d	4,943±150	7,938±536 ^d
Ei-M	75±2	125±7 ^d	64±3	64±11	2,070±291	2,269±49
DIIIa	83±2	128±2 ^d	65±3	70±7	2,868±247	3,478±327

^a C3H/HeN mice were immunized with crude sea squirt antigen (100 μg) with aluminum hydroxide (2 mg) intraperitoneally.

^b 4 weeks after immunization, anti-sea-squirt antibodies in their sera were detected by ELISA using crude antigen and Gi-rep, Ei-M, and DIIIa antigens. The antibody titer was expressed as OD at 490 nm by using × 40 diluted sera.

^c Spleen cells were cultured with crude antigens and Gi-rep, Ei-M, and DIIIa antigens for 3 days, and the proliferative responses were detected by [³H]-TdR incorporation. The results are expressed as mean cpm ± SE of triplicate cultures.

^d Significantly enhanced by the immunization.

Table 7. Sea squirt antigen stimulates human B cells to proliferate

Peripheral blood lymphocytes treated with	[³ H]-TdR incorporation of PBL (cpm) stimulated with ^a			
	(control)	ConA	SAC	sea squirt
Complement	1,866±93	10,846±200	4,077±266	7,577±482
Anti-CD 3 + complement	3,762±292 ^c	5,181±554 ^b	5,385±350 ^c	12,011±1,203 ^c
Anti-CD 20 + complement	1,600±112	10,243±916	2,610±245 ^b	5,198±438 ^b

^a 2 × 10⁵ PBL treated as indicated were cultured with 10 μg/ml ConA, 10 μg/ml SAC, or 100 μg/ml sea squirt antigen.

^b Significantly decreased as compared with the control group.

^c Significantly enhanced as compared with the control group.

Table 8. Sea squirt antigen stimulates B cells to produce immunoglobulin

Peripheral blood lymphocytes cultured with ^a	IgG (ng/ml) IL-4 ^b		IgE (OD at 490 nm $\times 10^{-3}$) IL-4 ^c	
	-	+	-	+
Control	10 $\pm$ 1	8 $\pm$ 2	10 $\pm$ 1	11 $\pm$ 3
Sea squirt	15 $\pm$ 3	22 $\pm$ 3 ^d	16 $\pm$ 2 ^d	56 $\pm$ 5 ^d
SAC	9 $\pm$ 1	9 $\pm$ 1	55 $\pm$ 6 ^d	59 $\pm$ 11 ^d

^a Peripheral blood lymphocytes (10⁶/ml) were cultured with sea squirt antigen for 7 days in the presence or absence of IL-4, and the total IgG and IgE production was detected by ELISA.

^b IgG production expressed as nanograms per milliliter.

^c IgE production expressed as OD at 490 nm.

^d Significantly enhanced.

response was partially depleted after anti-CD20 and complement treatment of peripheral blood lymphocytes, but enhanced with anti-CD3 and complement-treatment, suggesting that the responder cells to sea squirt antigen in humans are B cells as is the case with murine lymphocytes. Furthermore, sea squirt antigen also stimulated the production of polyclonal IgG and IgE (table 8), although the response was weaker than that of murine B cells. These results suggest that sea squirt antigen activates human as well as murine B cells.

Discussion

Sea squirt allergy is induced by inhalation of a body fluid of sea squirt [1, 2]. Specific and nonspecific IgG antibody levels are elevated in patients with sea squirt allergy, especially during the season for harvesting oyster (December to March). Oka et al. [3–5] and Suzuki et al. [6] extensively studied the immunological nature of the sea squirt antigen and suggested that it is a glycoprotein. However, the biological activity of sea squirt antigen is not fully understood. In this paper, we studied the mechanism of lymphocyte activation by sea squirt antigen using murine and human lymphocytes in vitro.

Sea squirt antigen markedly stimulated normal murine lymphocytes to proliferate dose dependently. The peak response was observed on day 2. The mitogenic activity of sea squirt antigen is very strong, and the response is sometimes comparable to that of ConA and LPS as representative mitogens. Fractionated sea squirt antigens, Gi-rep, Ei-M and DIIIa, also stimulated lymphocytes. However, the activity

of Ei-M and DIIIa is weak as compared with that of Gi-rep. This is considered to be due to the size of the molecule. Ei-M and DIIIa have molecular weights of 22,800 and 9,980, respectively, much smaller than that of Gi-rep (106,000). Thus, the epitope of Ei-M and DIIIa recognized by lymphocytes is much smaller than that of Gi-rep. The responder cells to sea squirt antigen are B cells because the responsiveness is reduced by the treatment of lymphocytes with anti-immunoglobulin antibody and complement and nylon wool column passing, but not with anti-Thy-1 antibody and complement. The representative B cell mitogen reported until now is LPS. However, the response to sea squirt antigen is not due to LPS in the antigen fraction, because C3H/HeJ mice, which are low responders to LPS, responded as well as C3H/HeN mice, and polymyxin B which suppresses the LPS response did not suppress the response to sea squirt antigen. Oka et al. [7] and Shigeta et al. [14, 15] reported that the antigenic activity of sea squirt antigen to react with specific antibody is not reduced after heat and pronase treatment, but after periodate treatment. These authors also suggested that the epitope of sea squirt antigen resides on carbohydrate molecules, but not on protein molecules, especially N-acetyl galactosamine and N-acetyl glucosamine. In fact the B cell mitogenic activity is heat stable and resistant to protease treatment. Thus, B cell stimulating activity can be considered to reside on carbohydrate molecules of sea squirt antigen different from LPS. Sea squirt antigen also stimulated lymphocytes to produce immunoglobulin, especially IgG. This immunoglobulin production is independent of T cells, because the immunoglobulin production was not affected by the treatment of lymphocytes with anti-Thy-1 antibody and complement. This is consistent with the phenomenon that sea squirt antigen stimulates B cells. Sea squirt antigen also stimulated human B cells to proliferate and to produce immunoglobulin. However, the response of human B cells is weaker than that of murine B cells. The reason to cause this difference can be considered to be due to the source of B cells. Murine B cells are from the spleen, and human B cells are from the peripheral blood. In fact, the response of murine lymph node lymphocytes was weaker than that of spleen cells.

In patients with sea squirt allergy, specific IgG and IgE and nonspecific IgE antibodies were elevated in their sera [2]. How can the relation of B cell mitogenic activity of sea squirt antigen and the elevation of specific antibody be explained? We showed that IL-4 enhanced total IgG and IgE production by spleen cells or peripheral blood lymphocytes stimulated with sea squirt antigen. When administered to mice, sea squirt antigen induces specific IgG and IgE production. Recently, the presence of two functionally different

helper T cell subsets, Th1 and Th2, has been reported [16]. Th1 cells produce IL-2 and interferon- γ and regulate the production of IgG antibody, while Th2 cells produce IL-4, IL-5, and IL-6 and participate in the regulation of IgE production. Thus, sea squirt antigen seems to stimulate both Th1 and Th2 cells. All these results suggest that sea squirt antigen has two activities: nonspecific B cell mitogenic activity and specific T cell priming activity. In a preliminary

experiment we observed that another representative allergy-inducing antigen, *Dermatophagoides farinae* antigen, also has murine and human B cell stimulating activity in vitro [in preparation]. Thus, it can be considered that allergy-inducing antigens are intrinsically B cell mitogens. The role of nonspecific B cell mitogenic activity of allergy-inducing antigens for the specific B and T cell activation is under investigation.

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