EFFECT OF A B CELL MITOGEN EXTRACTED FROM A FUNGUS *PEZIZA VESICULOSA* ON ANTIBODY PRODUCTION IN MICE

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The effect of Vesiculogen (a hot-water extracted B cell mitogen from *Peziza vesiculosa*) on antibody production in mouse spleens was studied. The number of plaque-forming cells (PFC) elicited by injection with Vesiculogen and/or 2,4,6-trinitrophenyl substituted sheep red blood cells (TNP-SRBC) were comparatively assayed. In the mice injected with 100 μg of Vesiculogen (*i.p.*), an obvious increase in the numbers of anti-SRBC and anti-TNP SRBC PEC in spleens was observed after 2 d of injection, and the numbers of PFC reached a maximum on day 3. Injections to 1—1000 μg of Vesiculogen were effective. This effect was shown when Vesiculogen was administered by *i.p.* or *i.v.* injections. In some cases, the numbers of PFC in mice injected with Vesiculogen and antigen exceeded the sum of each number of PFC elicited by polyclonal B cell activation activity of Vesiculogen and by antigenic stimulation of TNP-SRBC. Pretreatment with Vesiculogen within 4 d before immunization markedly reduced the PFC response. These results suggest that the effect of Vesiculogen on antibody response in mice attributes to its activities as a polyclonal B cell activator and adjuvant.

Keywords — *Peziza vesiculosa*; fungal immunomodulator; polyclonal B cell activator; adjuvant; Vesiculogen

INTRODUCTION

Various kinds of substances are known to stimulate the deoxyribonucleic acid (DNA) synthesis of lymphocytes polyclonally (mitogenic effect) and to induce their effector cell functions. These substances are obtained from various microorganisms. Bacterial components such as lipopolysaccharide (LPS)^{1,2)} peptidoglycan,³⁻⁵⁾ lipoprotein^{6,7)} endotoxin protein,⁸⁾ pneumococcal capsular polysaccharide,⁹⁾ capsular polysaccharide of *Klebsiella pneumoniae*,¹⁰⁾ purified protein derivatives (PPD) from *Mycobacterium*,¹¹⁾ and components of *Nocardia*¹²⁾ and *Actinomyces*,¹³⁾ are extensively studied. The substances from mycoplasma¹⁴⁾ and virus^{15,16)} can also stimulate lymphocytes. These substances are different from each other on several points in the chemical and biological properties, and provide a powerful means for study of immune response.

We examined the immunomodulating activi-

ties of fungal products, because the existence of the active substance(s) in fungal as well as in other microorganisms could be expected, and if the active substance(s) exist, the property would be expected to differ from that of active substances originating from the other microorganisms. In the first report of this series, we showed that the water-soluble substances from the fruiting bodies of several species of fungi, which belong to the *Ascomycotina*, possessed mitogenic activity.¹⁷⁾ In addition, a substance (Vesiculogen) from *Peziza vesiculosa* was shown to be a B cell mitogen and to act as a polyclonal B cell activator (PBA).¹⁸⁾ We have further examined the immunomodulating activities of the substances from the fungi (8 species) mentioned above, and found that almost all of the substances tested showed *in vivo* and *in vitro* PBA activity and *in vivo* adjuvant activity, although only the substance from *P. vesiculosa*¹⁹⁾ showed

in vitro adjuvant activity. Some points remained to be examined, are as follows; 1) different results may be obtained if any other assay conditions including doses, timing and routes of administration of sample and antigen are used, and 2) optimal timing and antigen dose for simultaneous assay of both PBA and adjuvant activities ought to be selected, because these effects on antibody production may be different from each other.

In this paper, effects of Vesiculogen as both PBA and adjuvant on *in vivo* antibody production in mice under the several experimental conditions are described.

MATERIALS AND METHODS

Mice — Six to 8 week-old male ICR mice were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka. They were fed standard animal diet and tap water freely under conventional condition.

Preparation of Vesiculogen — Vesiculogen was prepared by the method previously described,⁸⁾ except for lyophilization. Briefly, the dried fruiting bodies were boiled in water. After filtration, the filtrate was dialyzed against distilled water and the non-dialysable fraction was recovered by lyophilization. This preparation is referred to as Vesiculogen in the present paper, and is composed of protein (*ca.* 60% as bovine serum albumin), carbohydrate (*ca.* 30% as glucose) and a small amount of amino sugar, uronic acid, and phosphate. Vesiculogen was dissolved in saline and diluted to the desired concentration with saline.

Estimation of the Spleen Weight and the Number of Nucleated Cells in Spleen — Mice were killed by cervical dislocation, and their spleens were removed and weighed. They were teased in ice-cold Eagle's minimum essential medium (MEM) (Nissui Seiyaku Co. Ltd., Tokyo) to prepare single cell suspensions. The cell suspensions to be tested were diluted with turk solution, and the cells were counted with a hemocytometer.

Antigen — Sheep red blood cells (SRBC)

were obtained from Nippon Biotest Co. Ltd., Tokyo. 2,4,6-Trinitrophenyl substituted SRBC (TNP-SRBC) were prepared by modification of Rittenberg and Pratt.²⁰⁾

Plaque-Forming Cell (PFC) Assay — The direct PFC response to TNP and SRBC was determined by a modification of the hemolytic assay of Jerne and Nordin.²¹⁾ The number of TNP-specific PFC was calculated by subtracting the response to SRBC from the TNP-SRBC response. Mice were killed by cervical dislocation and cell suspensions from the spleens were prepared by teasing the spleen in the cold MEM medium. Each cell suspension (0.1 ml) was mixed with 0.05 ml of a suspension of TNP-SRBC and 1.5 ml of soft agar (0.8% agar, 0.5% lactalbumin hydrolysate and 0.05% diethylaminoethyl (DEAE) dextran in Hank's solution) kept at 47 °C after boiling. Each mixture was then layered onto a plastic dish (7.5 cm diameter) which was previously layered with a base agar (1.4% agar, 0.5% lactalbumin hydrolysate and 0.05% DEAE dextran in Hank's solution). After incubation of dishes for 60 min at 37 °C, 1.0 ml of guinea pig serum, which had been absorbed with TNP-SRBC and diluted with MEM medium, were added as the complement source and the dishes were further incubated at 37 °C for 60 min. Results of the plaque assay are expressed as the mean number of PFC $\pm$ standard error (SE) per spleen.

RESULTS

Effect of Vesiculogen on the Weight and the Number of Nucleated Cells of Spleen

In the course of studies of Vesiculogen, we have found that the injection of Vesiculogen into mice induces significant splenomegaly. Then, the time course of changes in the weight and number of nucleated cell in spleens of mice injected with Vesiculogen, was examined. The results are shown in Fig. 1. Both the spleen weight and the number of cells in treated mice increased markedly at 2 d after the injection, and were about 1.6 times larger than those in control mice. The peak level was maintained until day 3,

and decreased gradually thereafter.

Kinetics of PFC Responses after Injection of Vesiculogen, Antigen or Both

In order to determine the kinetics of PFC response, mice were injected *i.p.* with 100 μg of Vesiculogen or *i.v.* with 1×10^8 TNP-SRBC or both. PFC responses were assessed over 2–6 d (successively) and at 14th day after the injection (Table I). The PFC number was observed after 2 d of injection, and the responses reached a maximum on 3 d. On the other hand, when the mice were injected with TNP-SRBC together with Vesiculogen, the numbers of anti-SRBC and TNP PFC reached maximum after 4 d of injection (70336 ± 14703 and 49456 ± 8165 per spleen, respectively).

Dose Effect of Vesiculogen on PFC Response in Mice

FIG. 1. Changes in the Weight and the Number of Nucleated Cells of Spleens in Mice Injected with Vesiculogen

Five hundred μg of Vesiculogen (● — ●) or saline (○ — ○) was injected *i.p.* Each point represents arithmetic mean $\pm$ SE for 3 mice.

To determine the effect of doses of Vesiculogen on PFC responses, mice were injected *i.p.* with 1, 10, 100, 250, or 1000 μg of Vesiculogen or *i.v.* with 1×10^8 TNP-SRBC or both. PFC responses were assessed at 3rd and 4th days after the injection (Table II). In the mice injected with Vesiculogen, the PFC responses on day 3 was stimulated strongly. When various doses of Vesiculogen were injected together with antigen, the anti-SRBC PFC responses observed in mice injected with Vesiculogen together with antigen were higher than the sum of each response induced by PBA activity and antigenic stimulation.

Relationship between Doses of TNP-SRBC and Effect of Vesiculogen on PFC Responses

To examine the relationship between doses of antigen and effect of Vesiculogen on PFC response, mice were injected *i.v.* with 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 or 1×10^9 TNP-SRBC with or without 500 μg of Vesiculogen (*i.p.*). PFC responses were assessed at 3rd and 4th days after injection (Table III). On day 3, marked enhancement on PFC response in antigen and Vesiculogen-injected mice was observed when 1×10^5 or 1×10^6 TNP-SRBC were used as antigen. Their stimulating indices against corresponding doses of antigen-injected group were 10.5 or 12.5 in anti-SRBC and 22.4 or 18.1 in anti-TNP SRBC, respectively. On day 4, the enhancing effects on PFC response in antigen and Vesiculogen injected groups of mice against corresponding dose of antigen injected groups were low in comparison with those on day 3. The peak enhancing effect was observed when 1×10^6 TNP-SRBC, which was not able to elicit strong antibody response, was used as antigen (Stimulating index; 3.6 in anti-SRBC, 11.2 in anti-TNP, respectively).

Relation between Injection Routes and Effect of Vesiculogen on PFC Response

To examine whether the effect of Vesiculogen is affected by the injection routes of its own and antigen, 500 μg of Vesiculogen and/or 1×10^8 TNP-SRBC were injected into mice, and PFC responses were assessed at 3rd and 4th days

TABLE I. Kinetics of PFC Responses in Mice after Injection with Vesiculogen, TNP-SRBC or Both

	Number of PFC/spleen ^{a)}			
	Nil	TNP-SRBC ^{b)}	TNP-SRBC + Vesiculogen ^{b)}	Vesiculogen ^{b)}
Anti-SRBC				
Day 2	64 ± 32	80 ± 42	472 ± 102	184 ± 93
Day 3	160 ± 46	4 688 ± 795	2 792 ± 650	368 ± 66
Day 4	200 ± 63	53 272 ± 15 486	70 336 ± 14 703	224 ± 60
Day 5	80 ± 33	33 240 ± 7 013	20 336 ± 2 721	72 ± 15
Day 6	24 ± 10	7 410 ± 616	10 032 ± 1 446	32 ± 15
Day 14	144 ± 47	600 ± 86	4 712 ± 362	304 ± 113
Anti-TNP				
Day 2	1 488 ± 655	1 992 ± 506	7 984 ± 1 408	5 760 ± 1 557
Day 3	320 ± 87	10 408 ± 1 284	10 312 ± 1 930	8 024 ± 1 451
Day 4	1 830 ± 517	43 536 ± 17 618	49 456 ± 8 165	4 400 ± 1 115
Day 5	1 280 ± 555	10 352 ± 2 545	8 984 ± 2 647	3 960 ± 635
Day 6	1 128 ± 216	4 010 ± 1 194	8 752 ± 2 136	2 064 ± 403
Day 14	920 ± 277	2 050 ± 746	2 384 ± 1 412	3 560 ± 527

a) Arithmetic mean number of PFC ± SE for 5 mice. b) One hundred µg of Vesiculogen was injected i.p., and 1×10^8 TNP-SRBC were injected i.v.

TABLE II. Dose effect of Vesiculogen on PFC response

Vesicu- logen ^{a)}	TNP- SRBC ^{b)}	Number of PFC/spleen ^{c)}			
		Day 3		Day 4	
		Anti-SRBC	Anti-TNP	Anti-SRBC	Anti-TNP
—	—	16 ± 10 (1.0) ^{d)}	3 128 ± 567 (1.0) ^{d)}	96 ± 27 (1.0) ^{d)}	2 688 ± 1 685 (1.0) ^{d)}
1	—	248 ± 89 (15.5)	8 024 ± 1 123 (2.6)	160 ± 13 (1.7)	2 856 ± 348 (1.1)
10	—	296 ± 39 (18.5)	11 040 ± 2 339 (3.5)	80 ± 22 (0.8)	2 816 ± 650 (1.0)
100	—	416 ± 96 (26.0)	12 360 ± 1 268 (4.0)	144 ± 45 (1.5)	7 464 ± 2 009 (2.8)
250	—	624 ± 79 (39.0)	13 064 ± 1 687 (4.2)	136 ± 27 (1.4)	4 872 ± 1 166 (1.8)
500	—	2 008 ± 408 (125.5)	24 328 ± 4 459 (7.8)	180 ± 10 (1.9)	14 880 ± 1 435 (5.5)
1 000	—	1 376 ± 340 (86.0)	27 720 ± 4 112 (8.9)	504 ± 129 (5.3)	26 976 ± 3 340 (10.0)
—	+	872 ± 328 (1.0) ^{e)}	4 072 ± 908 (1.0) ^{e)}	13 896 ± 3 826 (1.0) ^{e)}	50 496 ± 11 290 (1.0) ^{e)}
1	+	5 096 ± 783 (5.8)	12 152 ± 2 190 (3.0)	51 264 ± 4 679 (3.7)	48 256 ± 7 849 (1.0)
10	+	2 432 ± 597 (2.8)	12 160 ± 1 115 (3.0)	57 320 ± 21 691 (4.1)	49 624 ± 6 905 (1.0)
100	+	3 160 ± 448 (3.6)	16 744 ± 2 645 (4.1)	43 040 ± 13 729 (3.1)	47 384 ± 10 484 (0.9)
250	+	2 496 ± 455 (2.9)	17 576 ± 3 091 (4.3)	54 648 ± 11 787 (3.9)	61 640 ± 14 395 (1.2)
500	+	3 200 ± 572 (3.7)	16 728 ± 1 350 (4.1)	43 480 ± 11 884 (3.1)	67 656 ± 13 494 (1.3)
1 000	+	4 936 ± 863 (5.6)	29 296 ± 5 437 (7.2)	50 280 ± 7 295 (3.6)	74 296 ± 13 585 (1.5)

a) Vesiculogen was injected i.p. b) 1×10^8 TNP-SRBC were injected i.v. c) Arithmetic mean number of PFC ± SE for 5 mice. d) Mean number of PFC in Vesiculogen-injected group/ Mean number of PFC in saline-injected group. e) Mean number of PFC in both Vesiculogen and TNP-SRBC-injected group/ Mean number of PFC in TNP-SRBC-injected group.

after the injection (Table IV). In the case of injection of Vesiculogen, both *i.p.* and *i.v.* administrations increased the number of PFC, although *i.v.* administration exceeded *i.p.* on both 3rd and 4th days. On the other hand, the greatest enhancing administration route of antigen was ob-

served when both Vesiculogen and antigen were injected *i.p.*

Relationship between Timing of Vesiculogen Injection and Its Effect of PFC Response

To examine the relationship between timing of injection of Vesiculogen and that of antigen,

TABLE III. *Relationship between Doses of TNP-SRBC and Effect of Vesiculogen on PFC Response*

Vesicu- logen ^{a)}	TNP- SRBC ^{b)}	Number of PFC/spleen ^{c)}							
		Day 3				Day 4			
		Anti-SRBC		Anti-TNP		Anti-SRBC		Anti-TNP	
—	10 ⁵	88±	39 (1.0) ^{d)}	952±	173 (1.0) ^{d)}	296±	41 (1.0) ^{d)}	3 100±	839 (1.0) ^{d)}
+	10 ⁵	928±	309 (10.5)	21 320±	6 302 (22.4)	944±	255 (3.2)	8 088±	1 641 (2.6)
—	10 ⁶	120±	36 (1.0)	1 136±	478 (1.0)	1 184±	209 (1.0)	2 560±	863 (1.0)
+	10 ⁶	1 504±	170 (12.5)	20 560±	1 943 (18.1)	4 224±	949 (3.6)	28 680±	6 960 (11.2)
—	10 ⁷	776±	361 (1.0)	3 096±	279 (1.0)	10 152±	1 564 (1.0)	20 352±	4 797 (1.0)
+	10 ⁷	1 576±	320 (2.0)	22 304±	4 953 (7.2)	24 848±	7 011 (2.4)	20 900±	8 753 (1.0)
—	10 ⁸	1 984±	261 (1.0)	9 168±	3 751 (1.0)	34 496±	5 853 (1.0)	17 160±	8 873 (1.0)
+	10 ⁸	6 528±	1 817 (3.3)	51 256±	11 429 (5.6)	57 472±	5 446 (1.7)	45 600±	8 794 (2.7)
—	10 ⁹	3 104±	750 (1.0)	12 560±	2 211 (1.0)	68 032±	15 254 (1.0)	15 744±	2 898 (1.0)
+	10 ⁹	4 940±	1 442 (1.6)	41 140±	4 726 (3.3)	97 312±	19 420 (1.4)	21 200±	10 667 (1.3)
—	—	70±	34	1 350±	386	1 640±	93	1 330±	786
+	+	1 104±	364	16 984±	2 655	1 750±	407	6 560±	1 771

a) Five hundred μ g of Vesiculogen was injected *i.p.* b) TNP-SRBC were injected *i.v.* c) Arithmetic mean number of PFC $\pm$ SE for 5 mice. d) Mean number of PFC in both Vesiculogen and TNP-SRBC injected group/ Mean number of PFC in TNP-SRBC-injected group.

TABLE IV. *Relationship between Injection Routes and Effect of Vesiculogen on PFC Response*

Vesicu- logen ^{a)}	TNP- SRBC ^{b)}	Number of PFC/spleen ^{c)}							
		Day 3				Day 4			
		Anti-SRBC		Anti-TNP		Anti-SRBC		Anti-TNP	
—	<i>i.p.</i>	616±	236 (1.0) ^{d)}	2 472±	644 (1.0) ^{d)}	4 704±	2 304 (1.0) ^{d)}	7 304±	2 766 (1.0) ^{d)}
<i>i.p.</i>	<i>i.p.</i>	4 072±	894 (6.6)	31 272±	4 279 (12.7)	28 560±	8 026 (6.1)	26 920±	4 712 (3.7)
<i>i.v.</i>	<i>i.p.</i>	3 288±	1 284 (5.3)	16 552±	4 330 (6.7)	8 240±	3 186 (1.8)	11 224±	2 907 (1.5)
—	<i>i.v.</i>	7 896±	2 064 (1.0)	10 328±	1 593 (1.0)	42 384±	15 028 (1.0)	11 510±	4 204 (1.0)
<i>i.p.</i>	<i>i.v.</i>	7 912±	2 098 (1.0)	52 472±	3 037 (5.1)	60 864±	8 720 (1.4)	34 816±	11 370 (3.0)
<i>i.v.</i>	<i>i.v.</i>	5 808±	1 218 (0.7)	24 192±	2 504 (2.3)	32 640±	4 387 (0.8)	50 994±	11 113 (4.4)
<i>i.p.</i>	—	432±	114	17 328±	2 523	160±	55	5 960±	1 324
<i>i.v.</i>	—	1 040±	232	23 080±	3 118	168±	50	10 008±	822
—	—	144±	43	1 312±	270	32±	15	1 320±	510

a) Five hundred μ g of Vesiculogen was injected. b) 1×10^8 TNP-SRBC were injected. c) Arithmetic mean number of PFC $\pm$ SE for 5 mice. d) Mean number of PFC in both Vesiculogen and TNP-SRBC-injected group/ Mean number of PFC in TNP-SRBC-injected group.

mice were injected *i.p.* with 500 μg of Vesiculogen at varying times either before or after the immunization with 1×10^8 TNP-SRBC (*i.v.*). The PFC responses in these mice were assessed 4 d after the immunization (Table V). The greatest response was observed when mice were injected with Vesiculogen together with antigen. Pre-treatment with Vesiculogen within 4 d before immunization reduced the response. However, the responses obtained with 1 or 2 d after was almost equal to that observed in the control immunized mice.

DISCUSSION

As described by Dziarski,⁴⁾ B cell mitogens can be classified into three types according to their ability to stimulate polyclonal antibody secretion as follows; type 1 causes a strong DNA synthetic response and only a small increase in polyclonal antibody synthesis (*e.g.* dextran sulfate and lanatoside C), type 2 causes a high-rate antibody synthesis with a very limited increase in DNA synthesis (*e.g.* PPD of *Mycobacterium*), and type 3 causes both strong DNA synthesis and high-rate antibody synthesis (*e.g.* *Nocardia* water soluble mitogen and lipoprotein). In the previous paper,¹⁷⁻¹⁹⁾ we have demonstrated that

Vesiculogen was a potent B cell mitogen for murine lymphocytes, and Vesiculogen was also able to induce polyclonal antibody synthesis. Continuously, the characterization of PBA activity of Vesiculogen in mice was performed in this report. In the kinetics, the response became obvious after 2 d of Vesiculogen-injection, and reached a maximum on day 3 (Table I). The stimulating effect of Vesiculogen was dose-dependent at wide doses ranging from 1 μg to 1000 μg /mouse tested (Table II), and the stimulating indices of Vesiculogen-injected mice against control mice on day 3 were 125.5 or 86.0 in anti-SRBC and 7.8 or 8.9 in anti-TNP. The PBA activity of Vesiculogen was observed when Vesiculogen was administered by either *i.p.* or *i.v.* injection (Table IV). This potency of Vesiculogen in mice is comparable to that of LPS and lipoprotein.^{6,22)} Therefore, it is considered that Vesiculogen belongs to type 3.

The injection of Vesiculogen into mice induced significant changes in the weight and the number of nucleated cells of spleens (Fig. 1), and the pattern of time course was very similar to that of PFC response in spleens of Vesiculogen-injected mice (Table I). Similar results were obtained in the *in vitro* experiment (data not

TABLE V. Relationship between Timing of Vesiculogen Injection and Its Effect

	Number of PFC/spleen (day 4) ^{a)}	
	Anti-SRBC (significance $p < $) ^{d)}	Anti-TNP (significance $p < $) ^{d)}
Nil	288 $\pm$ 191	744 $\pm$ 130
TNP-SRBC ^{b)}	70 016 $\pm$ 10 968	34 272 $\pm$ 9 653
Day 0 ^{c)}	95 424 $\pm$ 20 409	93 770 $\pm$ 15 415
Day -1	68 360 $\pm$ 12 840 (ns)	18 928 $\pm$ 7 519 (0.01)
Day -2	37 760 $\pm$ 13 235 (0.05)	11 850 $\pm$ 5 635 (0.01)
Day -4	43 480 $\pm$ 21 935 (ns)	23 160 $\pm$ 12 174 (0.01)
Day -7	29 216 $\pm$ 7 860 (0.05)	19 544 $\pm$ 3 163 (0.01)
Day +1	76 240 $\pm$ 22 645 (ns)	32 328 $\pm$ 8 625 (0.01)
Day +2	49 288 $\pm$ 19 595 (ns)	66 000 $\pm$ 19 607 (ns)

a) Arithmetic mean number of PFC $\pm$ SE for 5 mice at 4th day from TNP-SRBC injection. b) 1×10^8 TNP-SRBC were injected *i.v.* (day 0). c) Five hundred μg of Vesiculogen was injected *i.p.* at indicated days. d) Variances were tested by Equal. T-test or Cochran-Cox test. $p < 0.05$ being taken as a significant difference. ns = not significant.

shown). These findings suggest that the PBA activity of Vesiculogen correlates to their ability to increase the number of spleen cells.

The polyclonal activation of B cells by PBA in mice is considered to be a result of a direct interaction of the activator with B cells.^{4,23)} However, the effect of PBA on antibody formation is not restricted only on B cells, *i.e.* PBA such as LPS administered with thymus-dependent antigen affect the antibody response by virtue of their effects on either helper T cells, macrophages or B cells, or on any conceivable combination of these cells.²⁴⁻²⁷⁾ Therefore, the antibody responses obtained by the administration of PBA with antigen were generally markedly higher than the sum of each response. The adjuvant effects of Vesiculogen were observed in some causes tested. These results suggest that the enhancing effect of Vesiculogen on antibody response in mice can be attributed to its activities as PBA and adjuvant though the significant adjuvant activity was not always observed in these assay systems.

Injection of Vesiculogen several days prior to immunization markedly suppressed the antibody response (Table V). The similar effects were observed in various microorganisms and their products.²⁸⁾ The mode of the suppressive effect particularly resemble to that of LPS and some other B cell mitogen.^{29,30)} The suppressive effect of Vesiculogen is considered to be due to the same mechanism as other B cell mitogens. Further studies are in progress to clarify this phenomenon.

Vesiculogen is heat-stable and is composed of large molecular weight molecules.^{18,31)} In addition, it was suggested that the molecule carrying mitogenicity in Vesiculogen may be a polypeptide containing anionic sites.³²⁾ This molecule also may carry the PBA activity of Vesiculogen since the substances such as LPS and peptidoglycan possess both mitogenic and PBA activities.¹⁻⁵⁾ On the other hand, we have shown the possibility of existence of similar PBAs in fungi belonging to *Pezizales* of the *Ascomycotina*.^{19,31)} The active materials in those fungi may be

resemblant to that in Vesiculogen.

In fungi, a group of materials such as glucan and mannan which have been found as antitumor polysaccharides were known as immunomodulator.³³⁻³⁵⁾ However, those materials do not possess potent B cell activating activity in mice. Additionally, Vesiculogen showed an antitumor activity against ascites form of Sarcoma 180,³⁶⁾ to which polysaccharides were generally ineffective. Therefore, it is possible to remark that the immunological and physicochemical properties of Vesiculogen is different from those of antitumor polysaccharides.

Various microorganisms and their products have been known to possess the ability to stimulate murine B cells polyclonally. As to bacteria, it was speculated that almost all of them possess the above ability, since their common cell surface components, peptidoglycan and/or LPS, generally can induce B cell mitogenic and polyclonal responses.^{1-5,12,22,37,38)} Johnson *et al.*⁶⁾ proposed an opinion about the biological significance of these materials, *i.e.* these bacterial components may influence the host-parasite relationship, particularly before establishment of specific humoral and cellular immune responses. Antitumor polysaccharides of resemblant structure have been found from many fungal cells.^{39,40)} Hence, we expect that some biological significance might exist in the fungal immunomodulators including Vesiculogen, Vesiculogen-like materials and antitumor polysaccharides.

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