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Effect of an acidic polysaccharide produced by *Serratia piscatorum* on immune responses in mice

II. STIMULATORY EFFECTS IN NORMAL AND IMMUNOLOGICALLY IMPAIRED ANIMALS

T. MATSUMOTO, NORIKO NONOYAMA, K. OOTSU & T. HOKAN *Biological Research Laboratories, Central Research Division, Takeda Chemical Industries Ltd, 17-85, Jusohonmachi 2-chome, Osaka, Japan*

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Summary. An acidic polysaccharide (PS) of *Serratia piscatorum* enhances the IgM PFC responses against heterologous erythrocytes in mice. Early and late IgM responses were increased significantly by increasing the number of immunizing erythrocytes and the dose of PS, whereas the IgM PFC response was suppressed by higher doses of PS and antigen. A stimulatory dose of PS significantly increased the secondary IgM and IgG responses against sheep erythrocytes. PS restored the reduced PFC response against sheep erythrocytes in adult-thymectomized, ^{60}Co-irradiated and bone marrow-transferred mice (ATXBM) and nude mice (nu/nu), and thus the stimulatory effect of PS appeared greater in immunologically impaired mice than in normal ones.

Spleen cells taken at the time of the peak PFC response from mice treated with higher doses of sheep erythrocytes and PS, suppressed the primary IgM production of normal syngeneic spleen cells against sheep erythrocytes *in vitro*. The suppressing activity of the spleen cells was increased by prior treatment with anti-theta serum and complement, while it was reduced by treatment with anti-mouse Ig serum and complement. These results suggested that immunoglobulin-bearing cells may have a role on the suppressing activity of spleen cells.

Correspondence: Dr T. Matsumoto, Biological Research Laboratories, Central Research Division, Takeda Chemical Industries Ltd, 17–85 Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan.

INTRODUCTION

An acidic polysaccharide isolated from the culture filtrate of *Serratia piscatorum* consists of L-rhamnose, D-galactose and partially acetylated D-galacturonic acid (Kita, Igarasi & Isono, 1974). This polysaccharide (PS) has been found to have very similar biologic activities *in vitro* to those reported for bacterial lipopolysaccharides (LPS) (Matsumoto, Shimonishi & Ootsu, 1975). In mouse-cell cultures PS has been shown to exert at least four effects: (1) it induces DNA synthesis in B lymphocytes selectively; (2) mitogenic concentrations of PS suppress the peak haemolytic plaque-forming cell (PFC) response to SRBC; (3) low concentrations of PS stimulate the PFC response and (4) higher concentrations of PS increase background PFC response of spleen cells to sheep erythrocytes (SRBC) in the early phase of culture. However, PS differs from LPS in that it does not contain detectable amounts of lipid A compo-

nents (Kita, Igarasi, Nakanishi & Isono, 1974), and it is not immunogenic in mice in dose ranges that stimulate specific antibody production to SRBC.

This paper reports further on the effects of PS *in vivo* on normal and impaired immune responses against heterologous erythrocytes in mice. It is shown that PS stimulates or suppresses the PFC responses against erythrocyte antigens, depending on the dose of immunizing antigen, and that PS restores the impaired immune responses in B (ATXBM and nude) mice. The significance of these results is discussed in relation to suppressor-cell functions.

MATERIALS AND METHODS

Mice

Specific pathogen-free DBA/2, C3H/HeMs, and C57BL/6 mice of 8–12 weeks old were obtained from inbred stock maintained on our farm. Congenitally athymic nu/nu mice, predominantly on BALB/c background, were obtained from a specific pathogen-free subunit of the Central Research Laboratories for Experimental Animals, Kanagawa, Japan, and bred in our laboratories in barrier-sustained sterilized conditions. They were fed on sterilized CA-1 mouse chow (Japan Clea Incorporated, Osaka) and drinking water *ab libitum*. The mice were used for experiments after a conditioning period of 5–10 weeks.

Polysaccharide

The acidic polysaccharide produced from *Serratia piscatorum* was prepared by the method of Kita *et al.* (1974) as described previously (Matsumoto 1975). To eliminate possible experimental fluctuations due to differences between lots, six PS preparations were mixed together after testing their analytical values and PFC-stimulating activities, and the activity of the mixture was confirmed before the experiments. This mixture is referred to as PS in the present paper. PS was dissolved in sterile pyrogen-free saline and diluted to the desired concentration with saline, and then volumes of 0·2 ml were injected into i.v. or i.p.

Antigens

SRBC in Alsever's solution were obtained from Shiihashi Company, Tokyo, Japan. Bovine (BRBC) and horse (HRBC) erythrocytes in Alsever's solution were purchased from Nikken-Kagaku Co., Kyoto, Japan. Hamster (HamRBC) and rabbit (RRBC) erythrocytes were obtained freshly from Syrian hamster and New-Zealand white rabbits, respectively. These erythrocytes were washed three times and resuspended in sterile saline at the desired concentration for injection. For immunization, 0·2-ml volumes of suspension containing the required numbers of erythrocytes were injected i.v.

Haemolytic plaque-forming cell (PFC) assay

Spleen cells releasing IgM haemolysin 2, 4 and 6 days after immunization of mice with heterologous erythrocytes were counted on agar by the method of Jerne & Nordin (1963). Spleens were removed asceptically from mice and teased apart in cold Eagle's minimal essential medium (MEM, GIBCO, Grand Island, New York). Each spleen was dispersed in 2 ml of MEM and diluted appropriately. Four to five mice were used for each experimental group, and duplicate plates were prepared for each spleen. Rabbit anti-mouse Ig serum (Miles Lab., Kankakee, Illinois) was used for IgG plaque-forming cell assay after absorption with mouse erythrocytes. Data in this paper are representative of at least three separate experiments, unless otherwise noted.

ATXBM mice

Eight- to 12-week-old DBA/2 and C57BL/6 female mice were thymectomized, irradiated (1,000 R from ^{60}Co source) and reconstituted with 3×10^7 syngeneic bone-marrow cells as described previously (Matsumoto *et al.*, 1975). These mice were examined under a dissecting microscope for the presence of thymic remnants before testing their spleens for PFC assays. Mice with thymic remnants were excluded from the study.

Mixed cell culture

The procedure described previously (Matsumoto *et al.*, 1975) was used. Briefly, 5×10^6 spleen cells taken from mice treated 4 days earlier with either 50 mg/kg of PS or 1×10^8 SRBC, or both simultaneously were added respectively to a 1-ml culture containing 1×10^7 normal spleen cells. Three cultures were prepared for each group. These cultures were kept for 5 days at 37° in a humid atmosphere of 7 per cent CO_2 in air in the presence or absence of 1×10^8 SRBC, and the number of cells making IgM to SRBC was measured with the method of Jerne & Nordin (1963).

Antisera and treatment of spleen cells

Anti-theta (Thy 1, 2) serum was prepared by immunizing AKR mice with C_3H thymocytes. This serum consistently kills 92–98·5 per cent of thymocytes and 30–50 per cent spleen cells with a titre of 1:10 in dye exclusion microcytotoxicity assays. To eliminate theta-bearing cells, spleen cells for addition to cultures were adjusted to 10×10^6 cells/ml, packed lightly by centrifugation, and incubated with 1 ml of anti-theta serum diluted ten-fold with Eagle's minimal essential medium (MEM, CTIBCO, New York). After 30 min at 37° 1 ml of fresh guinea-pig complement diluted 1:10 in PBS were added, and the cells were incubated at 37° for 30 min under a humid atmosphere of 7 per cent CO_2 in air. Under these experimental conditions, 75–86 per cent of spleen cells were left alive after treatment with antisera and complement, while 82–96 per cent cells remained alive in spleen cells treated with normal rabbit or mouse serum. The cells were then washed three times with MEM containing 1 per cent foetal calf serum (FCS, Flow Lab. Incorporated, Rockville, Maryland), suspended in 1 ml of MEM containing 10 per cent FCS, and 0·5 ml of the cell suspension were added to 0·5 ml of basal spleen cell culture which contain 20×10^6 normal spleen cells. Similarly, spleen cells for addition were treated with anti-mouse Ig serum (rabbit anti-mouse Ig, Miles Lab., Kankakee, Illinois, preabsorbed with mouse packed erythrocytes) and guinea-pig complement. For controls, either AKR normal serum or rabbit normal serum preabsorbed with mouse erythrocytes was substituted for the anti-theta or anti-Ig serum.

RESULTS

Effect of the dose of PS on the PFC response

PS at doses ranging from 0·001–100 mg/kg was injected i.v. into groups of C3H/HeMs mice within 30 min after administration of 1×10^8 SRBC. The peak of PFC response of mice against SRBC is known to be maximal 4 days after the immunization with a standard dose of antigen, so the direct PFC response was assessed 2 (early), 4 (peak) and 6 (late) days thereafter.

The early PFC response was generally stimulated in a dose-dependent manner by PS. The late PFC response was also stimulated but less markedly (Fig. 1). Doses of PS of about 1 mg/kg had weak stimulatory effect on the peak PFC response, and higher doses (10–100 mg/kg) suppressed it. The background PFC response against SRBC was increased by administration of higher doses of PS. Doses of PS of more than 1 mg/kg significantly increased the spleen indices of mice immunized with the standard dose (1×10^8) of SRBC (data not shown).

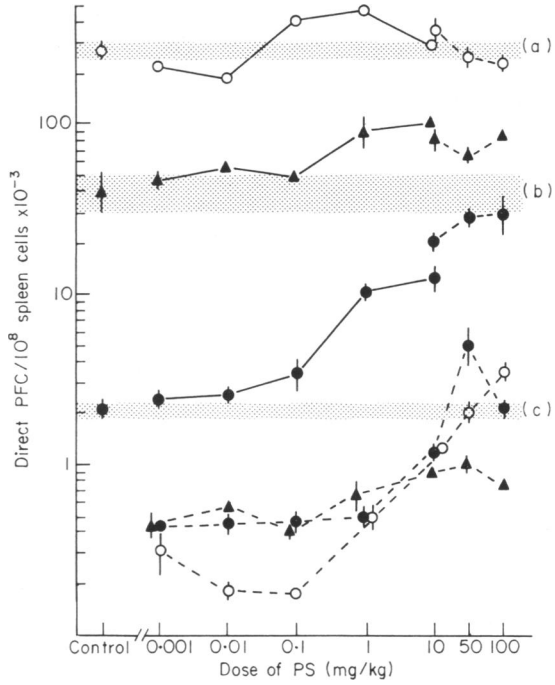

Figure 1. Effect of the dose of PS given i.v. at the time of antigen injection on the IgM PFC response in C3H/HeMs mice. SRBC (1×10^8) were injected i.v. and the PFC numbers of each group (five mice/group) were determined 2 (●), 4 (○) and 6 (▲) days later. Solid and broken lines show results of two similar experiments. Shaded areas represent control PFC values without PS (a) 4 days, (b) 6 days; (c) 2 days. Dashed lines indicate background PFC with PS. Vertical bars represent standard errors of the means.

Effect of PS on the PFC responses to various amounts of SRBC

A stimulatory dose of PS (10 mg/kg) was given to groups of four C3H/HeMs mice with antigen in amounts ranging from 10^3–10^8 SRBC and the direct PFC response was determined 2, 4 and 6 days after immunization. The results in Fig. 2 show a good correlation between the antigen dose and stimulation of the early PFC response by PS as well as a significant difference in the PFC values between PS-

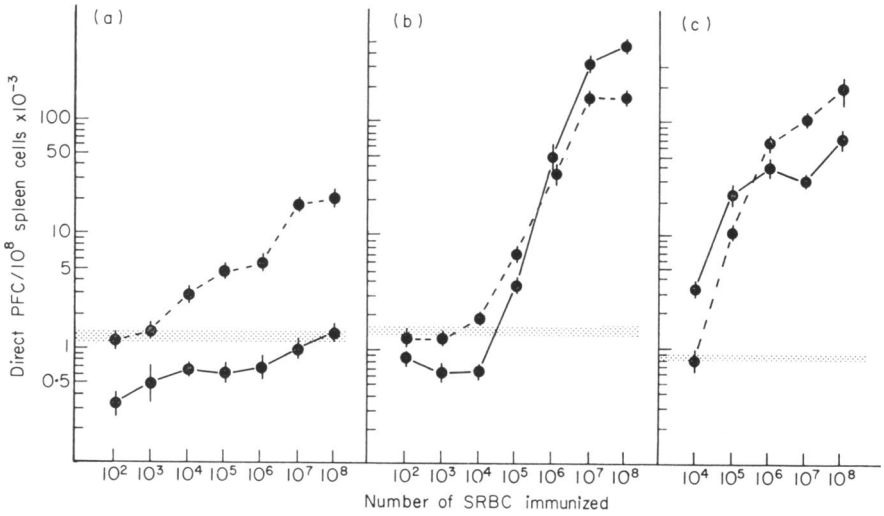

Figure 2. Effect of simultaneous administration of PS on the IgM PFC counts in C3H/HeMs mice immunized with various amounts of SRBC. Values are results of two to four separate experiments. Groups of mice (four mice/group) were assayed for IgM PFC production 2 (a); 4 (b) and 6 days (c) after antigenic stimulation. (———) SRBC alone; (– – – –) SRBC and PS (10 mg/kg, i.v.) simultaneously; shaded line, PS alone.

treated and untreated groups of mice. The PFC response on day 4 was stimulated using less than 10^5 SRBC for immunization, but it was depressed using more than 10^6 SRBC. The reverse was observed on day 6. On both days about 1×10^5 SRBC was found to be a critical dose of antigen for change in the effect of PS (Fig. 2).

Effect of the time of administration of PS on the PFC response

The results in Fig. 3 show that the PFC response varied depending on the time of administration of PS. Stimulation of the early PFC response was greatest when the mice were treated with antigen (1×10^8 SRBC) and PS (10 mg/kg) simultaneously. Treatment with PS 1 day before antigen stimulated the late (6 days) PFC response, whereas treatment with PS at times after the antigen generally had less effect on the PFC responses. The peak response (4 days) was depressed when PS was given 1 day before antigen, but it appeared greater than that of untreated controls when PS was given 3 days before or 2 days after antigen. The pattern of the effect of PS on the peak response was the reverse of that on the late response; PS depressed the latter when given on day 2 and stimulated it most when given one day before antigen.

Effects of PS on the PFC responses against various erythrocyte antigens

Results of a representative experiment are shown in

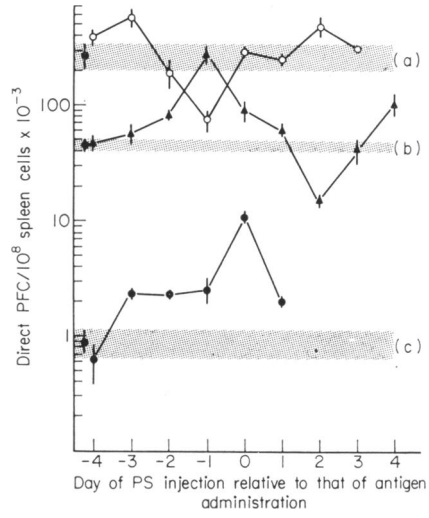

Figure 3. Effect of time of treatment with PS (10 mg/kg, i.v.) on the IgM PFC response. Day 0 is the day of SRBC (1×10^8) injection. IgM PFC was measured 2 (●), 4 (○) and 6 days (▲) after antigen injection. The shaded zones show the control values for 2 (a), 4 (b) and 6 days (c). Values are averages of those in 5 mice and vertical bars represent standard errors of the means.

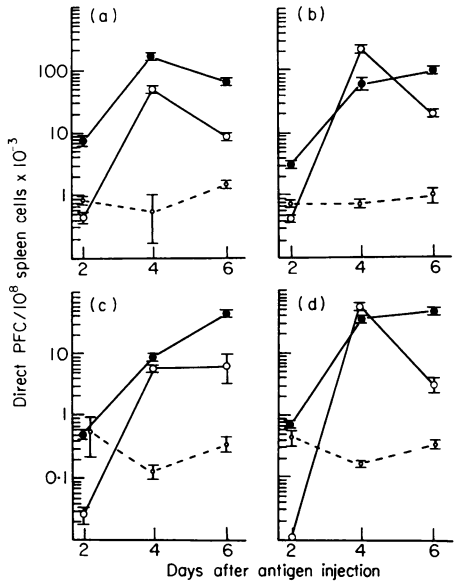

Figure 4. Effect of i.v. administration of PS (10 mg/kg) on IgM PFC counts in C3H/HeMs mice immunized against various kinds of heterologous erythrocytes. A standard dose (1×10^8 cells) of erythrocyte antigen (HRBC (a); BRBC (b); HamRBC (c) or RRBC (d)) was given i.v. a few hours before PS injection. (○), erythrocytes alone; (●), erythrocytes and PS. Dashed lines represent background PFC values for each erythrocyte antigen. Values are the mean of those in five mice ± s.e.

Fig. 4. Administration of PS significantly increased the number of early PFC over that seen after injection of erythrocyte antigen alone. It had no appreciable effect on the peak PFC responses against BRBC, RRBC and HamRBC, but caused approximately four-fold increase in that against HRBC (Fig. 4). PS stimulated the late PFC responses with all four types of erythrocytes.

Effects of PS on the secondary PFC response to SRBC

Adult C3H/HeMs mice were divided into four groups A, B, C and D, of twenty mice each (Table 1). In the first treatment, group A was kept not treated, and groups B, C and D, respectively were injected i.v. with 1×10^8 SRBC, 10 mg/kg of PS, and both simultaneously.

Three weeks later each group was divided into four subgroups of five mice each and these groups were treated like groups A–D. The mice were all sacrificed 4 days after this second treatment (25 days after the first treatment) to determine their direct and indirect PFC counts. Results of a representative experiment are shown in Table 1. Group A showed essentially the same pattern of response as that seen in the primary PFC response: PS significantly increased the number of early PFC, but had little effect on the peak response. Administration of PS at the time of the

Table 1. Effect of PS on the IgM and IgG PFC responses in primed and unprimed C3H mice

Group	First treatment* (mg/kg, i.v.)	Second treatment† (mg/kg, i.v.)	Plaque-forming cells‡/IgM	Spleen cells ± s.e., day-4 IgG
A	Normal	Normal	432 ± 24	0
		SRBC 1×10^8, i.v.	87,670 ± 14,999	0
		PS (10)	2323 ± 633	0
		SRBC + PS (10)	103,040 ± 10,593	0
B	SRBC 1×10^8, i.v.	Normal	648 ± 43	0
		SRBC	4830 ± 590	179,679 ± 20,229
		PS (10)	1422 ± 71	0
		SRBC + PS (10)	7068 ± 1473	244,469 ± 22,261
C	PS (10)	Normal	386 ± 60	0
		SRBC	169,246 ± 26,858	0
		PS (10)	583 ± 26	0
		SRBC + PS (10)	128,117 ± 17,170	0
D	SRBC + PS (10)	Normal	2182 ± 423	0
		SRBC	63,992 ± 14,730	222,768 ± 14,431
		PS (10)	2269 ± 303	0
		SRBC + PS (10)	128,995 ± 22,450	> 350,000§

* The first treatment was on Day 0.

† The second treatment was on Day 21.

‡ The plaque-forming cell numbers were determined 25 days after the first treatment. Averages from five mice per group ± s.e.

§ Haemolysed plates.

Table 2. IgM PFC responses in single- and mixed-cell cultures

Basic cells (No. of cells cultured)	Added cells (no. of cells cultured)	PFC*/10^6 spleen cells ± s.e.
Normal spleen (20×10^6/ml)	No	1930·5 ± 518·8
Normal spleen (20×10^6/ml)	Normal spleen (5×10^6/ml)	1402·2 ± 388·9
Normal spleen (20×10^6/ml)	SRBC spleen (5×10^6/ml)	1161·9 ± 505·4
Normal spleen (20×10^6/ml)	SRBC+PS spleen (5×10^6/ml)	679·4 ± 280·9
Normal spleen (20×10^6/ml)	PS spleen (5×10^6/ml)	1068·2 ± 405·6
No	SRBC spleen (5×10^6/ml)	828·5 ± 400·1
No	SRBC+PS spleen (5×10^6/ml)	9719·6 ± 5260·0
No	PS spleen (5×10^6/ml)	10·3 ± 6·7
No	Normal spleen (5×10^6/ml)	5·0 ± 5·0

* PFC was assessed 5 days after culture with 2×10^7 SRBC. Background PFC were subtracted. Summary of eight separate experiments.

second antigen injection significantly stimulated the secondary IgM and IgG PFC responses (Group B). Pretreatment with PS alone (Group C) increased the IgM PFC responses compared to those of group A, but had no significant effect on the IgG PFC responses. However, when PS was given at the time of the primary injection of antigen, the secondary IgG PFC responses were increased (group D). In addition, it was found that the sera of PS-pretreated mice (group C) did not elicit any antibodies to PS even after the second injection of PS so far as revealed by quantitative and qualitative precipitation reactions.

Suppression of PFC response by PS-treated spleen cells *in vitro*

The finding that higher doses of PS suppressed the peak PFC response suggested a role of PS on the elicitation of suppressor cells in the antigen-primed animals. To examine this possibility, groups of DBA/2 mice were injected with either PS (50 mg/kg) or SRBC (1×10^8 cells), or both simultaneously. Spleen cells were taken from these mice 4 days after injection and cultivated respectively with normal syngeneic spleen cells in the presence or absence of SRBC. Direct PFC numbers were assessed 5 days after culture.

The results summarized in Table 2 show that the PS- and SRBC-treated mouse spleen cells increased strikingly the secondary PFC response against SRBC *in vitro*, while the same number of the spleen cells suppressed the primary response of the normal spleen cells in mixed cell culture. However, the PFC response of normal spleen cell cultures was negligibly influenced by the addition of SRBC-treated mouse spleen cells that showed a less significant secondary response *in vitro*. The spleen cells from mice treated with PS alone were inactive to suppress or to stimulate the PFC response in single and mixed

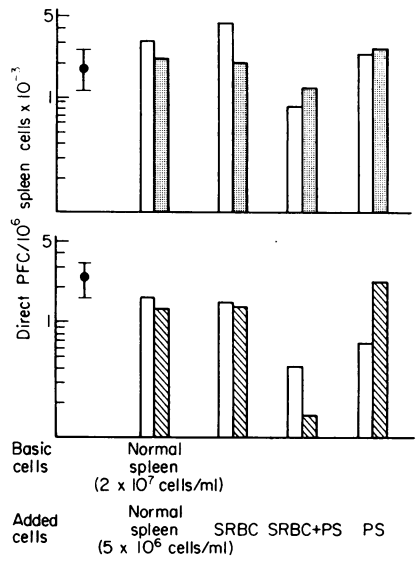

Figure 5. PFC responses of DBA spleen cells co-cultivated with syngeneic spleen cells taken from mice 4 days after injection of either SRBC (1×10^8 cells) or PS (50 mg/kg) or both simultaneously (SRBC+PS). Adding spleen cells were treated prior to culture with normal mouse or rabbit serum (open columns), anti-theta serum (hatched columns) or anti-mouse Ig serum and complement (stippled columns). PFC assay was done on pooled spleen cell cultures (three cultures per group) 5 days after culture. Figures from a representative experiment. Points (⊕) represent ranges of normal PFC response of spleen cells in single cultures.

Table 3. Effect of PS on the IgM responses in ATXBM and nude (nu/nu) mice

Mice	Treatment† (mg/kg, i.v.)	No. of animals Day-2	Day-4	Plaque-forming cells/10^8spleen cells ± s.e.‡ Day-2 (T/C index)§	Day-4 (T/C index)§
Normal C57BL/6	SRBC 1×10^8, i.v.	4	4	354±92	100,850±28,739
C57BL/6 ATXBM*	SRBC 1×10^8, i.v.	4	4	400±126	1182±110
	SRBC+PS (1)	4	4	337±68 (0·94)	6215±2723 (5·26)
	SRBC+PS (10)	4	4	2178±554 (5·45)	12,584±7754 (10·65)
Normal DBA/2	SRBC 1×10^8, i.v.	5	5	1074±119	180,632±21,461
DBA/2 ATXBM	SRBC 1×10^8, i.v.	4	4	252±21	874±41
	SRBC+PS (1)	4	3	378±118 (1·50)	3093±1401 (3·54)
	SRBC+PS (10)	3	4	498±230 (1·97)	3200±415 (3·67)
Nude (nu/nu)	SRBC 1×10^8, i.v.	4	4	1278±333	4056±1411
	SRBC+PS (1)	4	4	3808±959 (2·98)	9008±3677 (2·22)
	SRBC+PS (10)	4	4	4875±1891 (3·81)	7660±821 (1·89)

* Adult thymectomized C57BL/6 and DBA/2 mice were irradiated (1000 R) and bone-marrow cells (5×10^7 cells for C57BL/6, $2{\cdot}5\times10^7$ cells for DBA/2 mice) were transferred i.v., within 1 hr after irradiation.

† SRBC and PS were injected 2 weeks after bone-marrow-cell transfer.

‡ The number of plaque-forming cells in unimmunized B mice treated with 1 or 10 mg/kg of PS were 168–580/10^8 spleen cells on days 2 and 4 for C57BL/6, 256-521/10^8 spleen cells for DBA/2 and 320-695/10^8 spleen cells for nude mice.

§ The T/C index was calculated by dividing the average PFC number of the PS-treated group by that of the untreated immunized group.

cell cultures. The suppressing activity of the SRBC- and PS-treated mouse spleen cells was increased after treatment of the added spleen cells with anti-theta serum and complement prior to mixed-cell culture, while it was reduced by the similar treatment with anti-mouse Ig serum and complement (Fig. 5). The spleen cells thus treated significantly reduced the secondary PFC response against SRBC in single cell cultures.

Effects of PS on the PFC responses in B mice

To investigate how PS restored the impaired immune responses of B animals against T-dependent antigen, thymectomized, ^{60}Co-irradiated and bone marrow cell-transferred (ATXBM) DBA/2 and C57BL/6 mice, or athymic nude (nu/nu) mice were immunized i.v. with a standard dose of SRBC (1×10^8). The splenic direct PFC of these mice was determined 2 and 4 days after immunization (Table 3).

The PFC response of ATXBM DAB/2 and C57-BL/6 mice against SRBC was almost negligible on day 2, but became detectable on day 4. The number of PFC counted in ATXBM DBA mice 2 days after simultaneous i.v. injection of 1 mg/kg or 10 mg/kg of PS with antigen was almost equal to that seen with PS alone, whereas it was somewhat increased in ATXBM C57BL mice treated with 10 mg/kg of PS (Table 3). The number of PFC was remarkably increased in PS-treated DBA and C57BL mouse spleens on day 4.

Similar results were obtained on the PFC response of spleen cells from congenitally athymic nude mice. These mice were immunized with SRBC at 12–16 weeks of age under specific pathogen-free conditions, and no mortality or the usual wasting-like syndrome were seen throughout the experimental period. A highly significant increase in the number of PFC was observed both 2 days and 4 days after immunization in PS-treated nude mice (Table 3). PS did not afford any restoration of the impaired PFC responses in the mice after sublethal irradiation and transfer of syngeneic thymus cells (T-cell mice) (not shown).

DISCUSSION

The present experiments show that an acidic polysaccharide (PS) isolated from the culture filtrate of *S. piscatorum* enhances the early and late IgM PFC responses in mice against heterologous erythrocyte antigens *in vivo*. The early PFC response increased with the dose of PS and with a constant stimulatory dose of PS it increased linearly with the number of erythrocytes used for immunization. However, the peak response was suppressed by a stimulatory dose of PS when higher doses ($1–100\times10^6$ SRBC) of

antigen were used for immunization. An inverse relationship was observed between the effects of PS on the peak and late responses of SRBC-sensitized spleen cells on increase in the number of immunizing erythrocytes (Fig. 2). Injection of PS at different times relative to antigen also indicated an inverse relationship between peak and late PFC production.

These patterns of response in the PS-treated animals in response to SRBC coincided well with those obtained in *in vitro* experiments on PS activity (Matsumoto *et al.*, 1975). These results imply that the main cell populations responding to PS in the early PFC response may be different from those in the peak or late responses. Since PS has a mitogenic action on B cells (Matsumoto *et al.*, 1975), the dose-dependent stimulation of PS in the early PFC response may result from clonal proliferation of B lymphocytes in PS-treated animals. The early rise of the non-specific background PFC accompanied by increase in spleen weights also supported the notion that in the PS-treated spleens there may be an increase in the total number of B-cell clones that could develop into precursors of antibody forming cells.

The absence of stimulatory effect of PS on the peak PFC response could be due to a suppressor function of the spleen cells of PS-treated antigen-primed animals. Experiments *in vitro* on the effect of PS on mixed cultures of spleen cells and antigen-primed or unprimed spleen cells have suggested that PS may induce proliferation and/or maturation of suppressor cells which may be derived from either B or T cells responding to PS, and thus PS can regulate immune responses so that they do not exceed normal levels (Matsumoto *et al.*, 1975). The results presented in this paper also show that spleen cells taken from mice at the time of the peak PFC response following injection of higher doses of SRBC and PS *in vivo*, suppress IgM production of normal syngeneic spleen cells against SRBC *in vitro*. No such suppressing activity was observed with spleen cells taken similarly from mice treated with either PS or SRBC alone. Therefore, this suppression is not dependent merely on the physical interactions between the two cell populations in mixed cultures.

Furthermore, it is unlikely that T cells are responsible for the suppressing activity of PS- and SRBC-treated mouse spleen cells, because prior treatment with anti-theta serum and complement did not inhibit the suppressing activity of the spleen cells. The fact that treatment of the spleen cells with anti-Ig serum and complement reduced the activity of such cells suggests that an immunoglobulin-bearing cell may be the suppressor cell. There are some reports describing suppressive activities of bone marrow-derived lymphocytes to immune responses, though most of such activities are reported to be caused by those of thymus-derived cells (Gershon, Lance & Kondo, 1974). Neta & Salvin (1974) have suggested that a suppressor B cell may regulate the activity of a T cell in the expression of delayed skin hypersensitivity in guinea-pigs. Okumura & Kern (1975) reported that bone marrow cells inhibited the induction of immunoglobulin production by rabbit lymph node cells in tissue culture. This inhibitory activity of bone-marrow cells was reported to be reduced by prior treatment with anti-rabbit Ig antibody but was unaffected by treatment with antisera specific for thymus cells. Possibly antigen-primed B cells responding to PS may proliferate during mixed cell culture, and suppress the PFC response of normal spleen cells directly or indirectly through releasing immunoglobulins. However, glass-adherent cells in the spleen cannot be excluded as possible suppressor cells, since PS caused stimulation of adherent spleen cells *in vitro* (Matsumoto *et al.*, 1975).

On the basis of the above considerations PS should stimulate the PFC response of immunologically impaired animals more than that of normal animals. Thus, it was shown that in ATXBM and nude mice, treatment with SRBC and PS stimulated the PFC response more than SRBC alone. The restorative effect of PS in the immunosuppressed animals may be due largely to stimulation of B-cell clones, suggesting that PS may be able to replace the helper function of T cells in B mice. However, since T cells are present in murine bone marrow (Doenhoff, Davies, Leuchars & Wallis, 1970) and athymic animals possess small, but detectable levels of theta-bearing lymphocytes (Raff, 1973), stimulation of the PFC response in B mice by SRBC and PS may be due to the stimulatory effect of PS on the functions of these residual T cells. The observations that a low dose (1 mg/kg) of PS causes relatively great stimulation of the PFC response in SRBC-sensitized nude mice, and only slight stimulation of the PFC response in T-cell-transferred ATXBM mice (data not shown) suggests that the optimal dose of PS may stimulate the depressed immune response against T-dependent antigens in immunologically suppressed animals both by increasing the number of non-specific B-cell clones and by activating the helper functions of T cells.

The possibility that these activities of PS were related to contaminating endotoxin was thought to be unlikely, since no (or less than 0·02 per cent, as 100 mg PS) lipid could be detected in the PS preparation by gas-chromatography (Kita *et al.*, 1974a, b), and PS did not elicit any detectable antibody after two i.v. injections. However, a highly purified PS preparation had pyrogenic activity in rabbits at doses of more than 10 μg/kg, and gave a positive result in the *Limulus* lysate clotting assay (unpublished observations). Further analysis of the stereochemical properties of PS are required to explain the reason for these activities.

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