[原著] Specific immunosuppression without genetic restriction in vivo due to spleen extract and diluted antiserum

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Specific immunosuppression without genetic restriction in vivo due to spleen extract and diluted antiserum

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The effect of spleen extract and diluted antiserum to antibody production was studied in order to clarify the role of spleen extract in regulation of antibody production. Extracts of normal or pertussis-vaccine-stimulated mouse spleen did not by themselves suppress the primary immune response of mice to sheep or horse red blood cells. Similarly, anti-sheep-red-blood-cell mouse serum did not suppress if administered after 20- to 50-fold dilution. However, when the spleen extract and the diluted antiserum were administered either together with, or a day before the antigen, a very strong suppression of the primary response was observed. Antibody production was not suppressed by a combination of kidney or liver extracts and diluted mouse antisera.

The suppressive action observed was specific, and the specificity was determined solely by the specificity of the diluted antiserum used. On the other hand, the stimulatory action of the spleen extract appeared non-specific. Indeed not only mouse spleen extracts but also rat spleen extracts or concentrated bovine spleen extracts exerted a suppressive action on antibody production in mice. The secondary response was suppressed very effectively by the administration of spleen extracts alone.

From the above-mentioned results, it was clarified that a specific immunosuppression without genetic restriction was due to spleen extract and diluted antiserum in vivo.

INTRODUCTION

There are many reports that primed spleen extracts or primed thymus extracts exhibit specific immunosuppression in vivo (6–8, 10–18). For example, Takemori et al. (12, 13) found that primed spleen or thymus extract specifically suppressed IgG antibody production and that this phenomenon occurred only when the donor and the recipient contained the same I region of H–2 locus.

In contrast, comparatively few reports exist on the suppression of antibody production by normal spleen extracts. Gevorkyan et al. (3) observed that suppression by normal spleen extracts was nonspecific when the extracts had a high concentration of protein, and that at low concentrations, they enhanced, rather than suppressed, antibody production. Reinertsen and Steinberg (9) and Egan et al. (1) reported that supernatants from concanavalin A-activated spleen cells had a nonspecific immunosuppressive effect. From the above-mentioned literatures, it was clarified that the active

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Abbreviations used in this paper : HRC, horse red blood cell ; PFC, plaque forming cells ; PV, pertussis vaccine ; SRC, sheep red blood cells.

spleen cell and extract played an important role in regulation of antibody production. However, until present time it was not clarified whether the normal spleen extract suppressed the antibody production in vivo or did not.

In the present paper, the role of normal spleen extract was studied in detail concerning with the regulation of antibody production in vivo.

That is to say, in the present study, extracts from normal spleen or pertussis vaccine (PV) enlarged spleen were shown to act as an immunosuppressant, but in a manner very different from the studies described above. Thus in our system, the spleen extracts by themselves were inactive. However, when the extracts were combined with diluted antisera, which again by themselves exerted little suppressive action, and administered to mice either together with or just before the antigen, a remarkable suppression of the primary response was observed. Under certain conditions the number of plaque forming cells (PFC) in the experimental mice could be made as small as those in normal, unimmunized mice. This suppressive action was specific to antigen to which the diluted antiserum was directed. The effect of the spleen extracts, on the other hand, was not limited by the histocompatibility barrier.

MATERIALS AND METHODS

Animals : Outbred female ICR mice (6–12 wks old) were purchased from Nihon Clea Co. and Oriental Co., and maintained on pellets and water ad a lib. Wistar rats and outbred dogs were purchased from Fujii Co., and bovine spleen was obtained from the Kobe Food Center.

Antigens : Sheep red blood cells (SRC) and horse red blood cells (HRC) purchased from the Japan Blood Co. were washed twice with saline. An immunizing dose of 10^9 erythrocytes was used.

Antisera : ICR mice were immunized with a single intraperitoneal (i.p.) injection of 10^9 SRC or HRC and bled 8 days later. The antisera were inactivated at 56°C for 30 min and diluted 20–, 25–, or 50–fold (anti–SRC sera) or 12.5–fold (anti–HRC sera) with saline and stored in small aliquots at −20° C until required. The hemolysin titers of the anti–SRC sera before dilution were 320–, 640–, or 1024–fold, and that of the anti–HRC serum was 80–fold according to the conventional antiserum dilution method. Preparation of spleen extracts : PV spleens were obtained from mice injected i.p. with a dose of PV containing 10^{10} cells 8 days before. Normal or PV spleens (10.0 g) were added to 11.0 ml of cold saline. The mixture was homogenized for 2 min with an Ultra Turrax Homogenizer and then disrupted with a Sonifier Cell Disruptor for 10–20 sec. All these procedures were carried out at 0° C. The spleen suspensions were centrifuged at 15,000 rpm for 60 or 30 min at 0°C and the supernatant solutions were used as the spleen extracts. These were either used immediately or stored at −80°C until required.

Preparation of kidney and liver extracts : Kidney and liver of PV–stimulated mice were extracted by the same procedure as that used for the preparation of spleen extracts.

Preparation of concentrated bovine spleen extracts : Fresh bovine spleen (10.0g) was added to 6.0 ml of cold saline. Subsequent procedures were the same as those used for the preparation of mouse spleen extracts.

Immunization : For the study of primary response, mice were injected i. p. with 0.5 ml of SRC (10^9 cells), 0.5 ml of normal or PV spleen extracts and 0.5 ml of diluted anti–SRC serum (1 : 20, 1 : 25 or 1 : 50 or anti–HRC serum (1:12.5). For the study of secondary response, mice were injected i. p. with

0.5 ml of SRC(10^9 cells), and after 20 days 0.5 ml of SRC (10^9 cells) was administered together with spleen extracts and diluted antisera. The number of mice per group was 4 to 9.

Hemolytic plaque technique : PFC to SRC or HRC were assayed by a modification of the Jerne plaque assay (16). The mice were sacrificed by anestesia. Spleen cell suspensions were prepared by dissociating the spleens with forceps in cold saline, and agitating gently with a pipette. Large cell clumps sedimented after standing for 10 seconds, and they were removed from the cell suspensions. Appropriately diluted cell suspension (0.1 ml) was added to 1.0 ml of 0.85% solution of Difco agarose containing 199 culture medium and 4×10^8 SRC or HRC at 50°C. The mixture was immediately poured onto a supporting bottom layer containing 1.4 % Difco agar-agar in a petridish. After incubation at 37°C for 30 min (or as soon as the agarose solidified), this layer was covered with 1.0ml of guinea pig complement (1 : 2.5 dilution), and the numbers of direct PFC (IgM PFC) were determined after a further incubation at 37°C for 2 hrs. The numbers of indirect PFC (IgG PFC) were determined by adding 0.1 ml of 1 : 40 dilution of goat antiserum mouse 7S-globulins (Hyland Co.) to the agarose solutions. The numbers of IgG PFC were obtained from the difference in values between the assays performed in the presence and absence of this anti-mouse IgG serum. The PFC assay were carried out 4 days after antigen injection, unless indicated otherwise.

Statistical analysis : Arithmetic means and standard errors (S. E.) were calculated, and the P value was determined by Student's t-test.

RESULTS

Suppression of the primary response by the simultaneous administration of spleen extracts and diluted mouse antisera.

In confirmation of earlier results (2, 4, 19, 20), we could also show that the primary immune response was suppressed by the presence of high concentrations of antibody. Thus when undiluted mouse anti-SRC sera (0.5 ml) were administered to mice together with the immunizing antigen, SRC, the primary response measured by the increase of PFC in spleen was moderately depressed (data not shown). However, when the anti-SRC sera were diluted 20-to 50-fold before administration, significant suppression was not observed (compare Group 2 with 1 in Table 1). In the study, we discovered that very strong suppression occurred when extracts of normal mouse spleen were administered together with the diluted antiserum (Group 5 in Table 1). It should be emphasized that the administration of spleen extracts alone (Groups 3) did not produce a statistically significant suppression; thus the suppression apparently requires the presence of both spleen extracts and antisera.

Extracts of spleen enlarged by the pretreatment of mice with pertussis vaccine (PV spleen extracts) showed a behavior identical to the extracts of normal spleen (cf. Groups 4 and 6 in Table 1). Since the treatment significantly improved the yield of the spleen extracts, they were always prepared from PV spleen in subsequent experiments.

The amounts of antisera and spleen extracts required for suppression were apparently rather critical. Thus in the experiment of Table 1, no or little suppression was observed if the anti-SRC sera were diluted 100-fold or if the dosage of the spleen extracts was reduced to one-half (data not shown).

Experiments were performed to determine whether extracts of organs other than spleen were

Table 1. Suppression of IgM PFC formation by mouse spleen extracts and diluted mouse anti-SRC sera assayed on day 4 after antigen challenge. a : SRC(10^9) given intraperitoneally. b : Spleen extracts from PV-stimulated mice. C : Arithmetic mean ± S.E.; 4 mice per group. The hemolysin titer to anti-SRC serum was 1024-fold. P values for groups 1 vs. 2, N.S.; 1 vs. 3, N.S.; 1 vs. 4, N.S.; 1 vs. 5, < 0.001 ; 1 vs. 6, <0.001.

Group No.	Antigen[a] Challenge	Extract Inj.(i.p.)	Diluted Mouse Antiserum Inj. (i.p.)	Anti-SRC Response on Day 4 IgM PFC/Spleen Mean(±S.E.)[c]
1	SRC	———	———	183,200 (±27,800)
2	SRC	———	Anti-SRC Serum (1 : 50)	114,400 (±15,400)
3	SRC	Normal Mouse Spleen	———	152,600 (±70,800)
4	SRC	PV Mouse Spleen[b]	———	122,600 (±31,800)
5	SRC	Normal Mouse Spleen	Anti-SRC Serum (1 : 50)	4,400 (±3,200)
6	SRC	PV Mouse Spleen	Anti-SRC Serum (1 : 50)	5,100 (±1,500)

Table 2. Suppression of IgM PFC formation by extracts of various organs and diluted mouse anti-SRC sera assayed on day 4 after antigen challenge. a : SRC (10^9) given intraperitoneally. b : Various organ extracts from PV-stimulated mice. c : Arithmetic mean ± S.E.; 4 to 8 mice per group. The hemolysin titer to anti-SRC serum was 320-fold. P values for groups 1 vs. 6, N.S.; 1 vs. 7, N.S.; 1 vs. 8, <0.01.

Group No.	Antigen[a] Challenge	Extract[b] Inj.(i.p.)	Diluted Mouse Antiserum Inj.(i.p.)	Anti-SRC Response on Day 4 IgM PFC/Spleen Mean (±S.E.)[c]
1	SRC	——	——	238,600 (±73,100)
2	SRC	——	Anti-SRC Serum (1 : 25)	184,600 (±37,300)
3	SRC	Kidney	———	388,000 (±80,900)
4	SRC	Liver	———	153,000 (±29,100)
5	SRC	Spleen	———	136,000 (±26,900)
6	SRC	Kidney	Anti-SRC Serum (1 : 25)	172,000 (±66,100)
7	SRC	Liver	Anti-SRC Serum (1 : 25)	81,000 (±42,800)
8	SRC	Spleen	Anti-SRC Serum (1 : 25)	17,600 (±16,200)

Table 3. Antigen-specific suppression by mouse PV spleen extracts and diluted mouse antisera. a : SRC (10^9) and HRC (10^9) given intraperitoneally. b : Spleen extracts from PV-stimulated mice. c : Arithmetic mean ± S.E.; 4 to 9 mice per group. The hemolysin titer to anti-SRC serum was 640-fold; that of anti-HRC serum was 80-fold. P values for groups — IgM PFC to SRC : groups 1 vs. 6, N.S.; 1 vs 7, N.S.; 1 vs. 8, < 0.001 — IgM PFC to HRC : group 1 vs. 4, N.S.; 1 vs. 6, <0.02; 1 vs. 7, < 0.001; 1 vs. 8, N.S.

Group No.	Antigen[a] Challenge	Extract Inj.(i.p.)	Diluted Mouse Antiserum Inj.(i.p.)	Anti-SRC Response on Day 4 IgM PFC/Spleen Mean(±S.E.)[c]	Anti-HRC Response on Day 4 IgM PFC/Spleen Mean(±S.E.)[c]
1	SRC+HRC	—	—	246,600 (±75,200)	31,400 (±8,000)
2	SRC	—	—	255,600 (±22,600)	650 (±330)
3	HRC	—	—	1,000 (±600)	30,200 (±7,900)
4	SRC+HRC	—	Anti-HRC Serum (1 :12.5)	274,600 (±56,600)	14,400 (±6,600)
5	SRC+HRC	—	Anti-SRC Serum (1 :20)	270,600 (±82,700)	29,500 (±3,900)
6	SRC+HRC	PV Spleen[b]	—	129,600 (±34,000)	8,900 (±1,200)
7	SRC+HRC	PV Spleen	Anti-HRC Serum (1 :12.5)	95,800 (±42,000)	630 (±510)
8	SRC+HRC	PV Spleen	Anti-SRC Serum (1 :20)	24,000 (±14,900)	15,900 (±8,100)

active (Table 2). No immunosuppressive action was observed in the groups (6 and 7) in which a combination of kidney or liver extracts and diluted anti-SRC sera was administered. In some experiments, suppression by lung extracts and diluted anti-SRC sera was seen, but the effect was not reproducible (data not shown).

Specificity of the immunosuppressive action

The immunosuppression in our system was apparently antigen specific, and the specificity was determined by the antigen specificity of the diluted antisera administered. Thus in an experiment in which SRC and HRC were used as antigens (Table 3), the mounting of the primary response to SRC and HRC was strongly suppressed only when spleen extracts plus diluted anti-SRC and anti-HRC sera, respectively, were administered (Group 7 and 8). Neither cross reaction nor antigen competition was observed. These results indicated that suppression of antibody production by spleen extracts and diluted antisera is specific to the antigen, to which the diluted antiserum was directed.

The activity of the spleen extracts was not hindered by the histocompatibility or even the species barrier. This is illustrated in Table 4, in which spleen extracts of xenogenetic animals were tested.

Table 4. Suppression of IgM PFC formation by spleen extracts from various animals and diluted anti-SRC sera assayed on day 4 after antigen challenge. a : SRC (10^9) given intraperitoneally. b : Spleen extracts from normal animals. c : Spleen extracts from PV-stimulated mice. d : Arithmetic mean ± S.E.; 4 to 8 mice per group. The hemolysin titer to anti-SRC serum was 320-fold. P value for groups 1 vs. 7, < 0.02; 1 vs. 8, N.S.; 1 vs. 9 < 0.005; 1 vs. 10, < 0.001.

Group No.	Antigen[a] Challenge	Source of the Spleen Extract Inj.(i.p.)	Diluted Mouse Antiserum Inj.(i.p.)	Anti-SRC Response on Day 4 IgM PFC/Spleen Mean(±S.E.)[d]
1	SRC	——	——	230,600 (±37,400)
2	SRC	——	Anti-SRC Serum (1 : 25)	187,800 (±45,300)
3	SRC	Dog Normal[b]	——	227,600 (±40,100)
4	SRC	Bovine Normal	——	150,000 (±42,300)
5	SRC	Rat Normal	——	216,600 (±31,200)
6	SRC	Mouse PV-stimulated[c]	——	122,200 (±67,500)
7	SRC	Dog Normal	Anti-SRC Serum (1 : 25)	102,600 (±13,400)
8	SRC	Bovine Normal	Anti-SRC Serum (1 : 25)	129,600 (±21,500)
9	SRC	Rat Normal	Anti-SRC Serum (1 : 25)	52,000 (±23,100)
10	SRC	Mouse PV-stimulated	Anti-SRC Serum (1 : 25)	14,600 (±6,800)

Thus immune response in mice was suppressed quite effectively by a rat spleen extract and diluted mouse anti-SRC serum (Group 9). A bovine spleen extract and diluted mouse anti-SRC serum did not display statistically significant suppressive action (Group 8). However, when a concentrated bovine spleen extract was administered with diluted mouse anti-SRC sera, the suppressive action was as strong as in the case where a combination of mouse spleen extract and diluted mouse anti-SRC serum was administered (Table 5).

Time course of IgM PFC production

Fig. 1 shows the time course of the suppression of antibody production by combined spleen extracts and diluted anti-SRC sera. Statistically significant suppression was observed on the 3rd, 4th and 5th day after immunization, but on the 6th day the difference between the experimental and the control groups became rather small.

Effect of the administration scheme

Table 5. Suppression of IgM PFC formation by conc. bovine spleen extracts and diluted mouse anti-SRC sera measured on day 4 after antigen challenge. a : SRC (10^9) given intraperitoneally. b : Concentrated bovine spleen extract (1.0ml) given intraperitoneally. c : Arithmetic mean ± S.E.; 4 mice per group. The hemolysin titer to anti-SRC serum was 320-fold. P values for groups 1 vs. 2, N.S.; 1 vs. 3, N.S.; 1 vs. 4 < 0.005.

Group No.	Antigen[a] Challenge	Nature of the Extract Inj.(i.p.)	Diluted Mouse Antiserum Inj.(i.p.)	Anti-SRC Response on Day 4 IgM PFC/Spleen Mean(±S.E.)[c]
1	SRC	——	——	308,000 (±110,000)
2	SRC	——	Anti-SRC Serum (1 : 25)	284,000 (± 35,200)
3	SRC	Bovine Conc.Spleen[b]	——	221,000 (± 48,300)
4	SRC	Bovine Conc.Spleen	Anti-SRC Serum (1 : 25)	14,600 (±6,500)

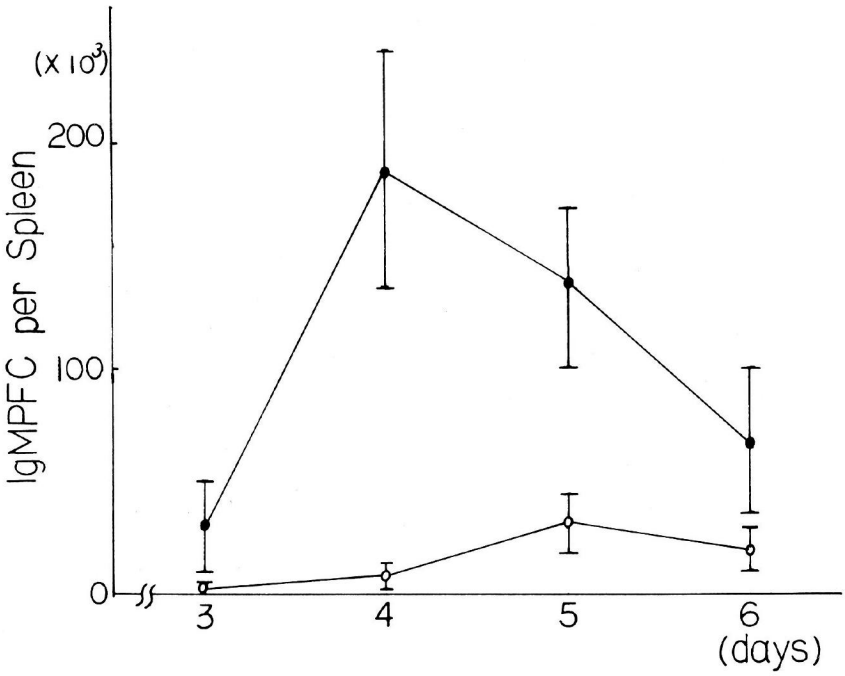

Fig. 1. Numbers of IgM PFC to SRC in spleen of mice at various times after intraperitoneal injection of 10^9 SRC or 10^9 SRC, PV mouse spleen extracts and diluted mouse anti-SRC sera (1 : 25). The hemolysin titer of the anti-SRC serum was 320-fold. Arithmetic means of results obtained from 4 mice are shown : the vertical bars represent the S.E. •—• mice administered SRC on day 0. ○—○ mice administered SRC, mouse spleen extracts and diluted mouse anti-SRC sera on day 0.

Table 6. The day of administration of mouse spleen extracts and diluted mouse anti-SRC sera and the suppression of IgM primary response. a : SRC (10^9) given intraperitoneally. b : Spleen extracts PV-stimulated mice. c : Arithmetic mean ± S.E.; 4 mice per group. The hemolysin titer to anti-SRC serum was 320-fold. P values for groups 1 vs. 2, < 0.05 ; 1 vs. 3, < 0.002, 1 vs. 4, < 0.001, 1 vs. 5, < 0.001; 1 vs. 6, N.S.

Group No.	Injection					Anti-SRC Response on Day 4 IgM PFC/Spleen Mean (±S.E.)[c]
	Day-3	Day-2	Day-1	Day 0	Day 1	
1	—	—	—	SRC	—	273,600 (±38,400)
2	Spl-Ext.[b] Anti-SRC Serum (1:25)	—	—	SRC	—	116,600 (±35,100)
3	—	Spl-Ext. Anti-SRC Serum (1:25)	—	SRC	—	48,400 (±37,600)
4	—	—	Spl-Ext. Anti-SRC Serum (1:25)	SRC	—	680 (±310)
5	—	—	—	SRC Spl-Ext. Anti-SRC Serum (1:25)	—	21,700 (±8,800)
6	—	—	—	SRC	Spl-Ext. Anti-SRC Serum (1:25)	172,400 (±50,900)

Table 6 shows the effect of the spleen extracts plus antisera administered at various times. The immunosuppressive action of spleen extracts and diluted anti-SRC sera was greater in Group 4 (administered on day −1 of antigen administration) than in Group 5 (administered on day 0 of antigen administration) and Groups 5 (administered on day −2 of antigen administration) and was not observed in Group 6 (administered on day 1 of antigen administration). We emphasize the observation that the suppression in Group 4 was so complete that the number of PFC was very close to that seen in untreated control (usually about 200 per spleen). The suppression was also as strong or even stronger when the spleen extracts plus diluted antisera were given twice, on day −1 as well as on day 0. In two such experiments, the number of PFC per spleen was 150 (±80) and 280 (±50) whereas the number of PFC per spleen of untreated mice was 200 (±40) and 200 (±50).

Immunosuppressive action of PV spleen extract and diluted mouse anti-SRC sera on the primary response of IgG PFC

The above experiments revealed that spleen extract and diluted anti-SRC sera exerted an immunosuppressive action on the primary response of IgM PFC

Fig. 2 illustrates the immunosuppressive action on IgG antibody production. In the groups in which a combination of spleen extracts and diluted anti-SRC sera was administered twice a day before and at the same time as the administration of antigen, significant suppression was observed throughout the periods of measurement (6, 8, and 11 days).

Suppression of the secondary response

Spleen extracts and diluted antisera were administered with SRC to mice previously immunized with SRC (Table 7). A very strong suppression was observed. In contrast to the suppression of the primary response, spleen extracts by themselves exerted a similarly strong suppression (cf. Group 3 in Table 7).

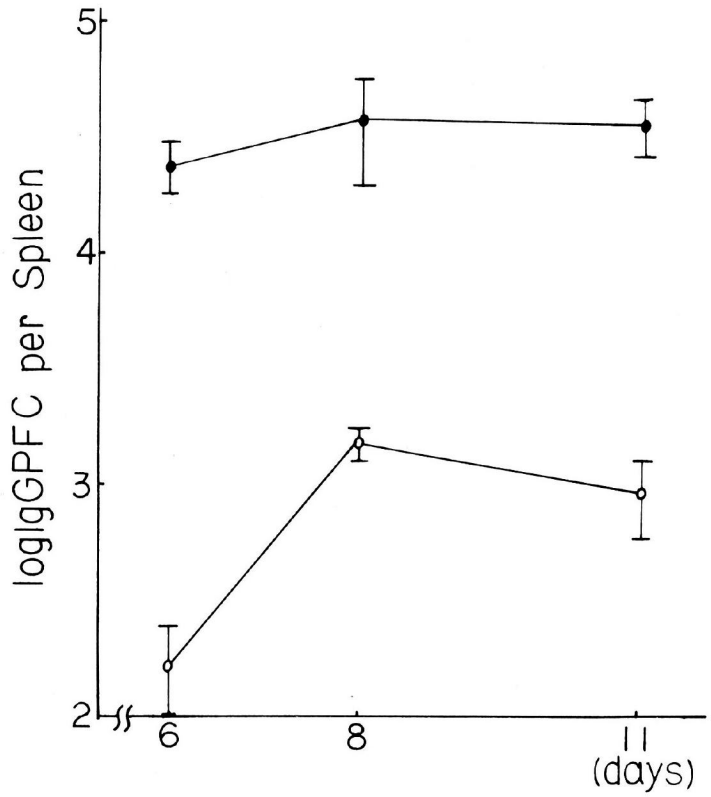

Fig. 2. Numbers of IgG PFC to SRC in spleen of mice at various times after intraperitoneal injection of 10^9 SRC or 10^9 SRC, PV mouse spleen extracts and diluted mouse anti-SRC sera (1: 25). The hemolysin titer of the anti-SRC serum was 320-fold. Arithmetic means of results obtained from 4 mice are shown : the vertical bars represent the S.E. •—• mice administered SRC on day 0, ○—○ mice administered SRC on day 0, mouse spleen extracts and diluted mouse anti-SRC sera both on day–1 and 0.

Table 7. The effect of PV spleen extracts on the secondary immune response a : SRC (10^9) given intraperitoneally. b: Spleen extracts from PV-stimulated mice. c : Arithmetic mean ± S. E.; 4 mice per group. The hemolysin titer to anti-SRC serum was 320-fold. P values for groups —— IgM PFC : 1 vs. 2, N.S.; 1 vs. 3, < 0.05; 1 vs. 4, < 0.05. P values for groups —— IgG PFC : 1 vs. 2, N.S.; 1 vs. 3 < 0.05, 1 vs. 4, < 0.05.

Group No.	Secondary Antigen Challenge[a]	Extract Inj.(i.p.)	Diluted Mouse Antiserum Inj.(i.p.)	Anti-SRC Response on Day 4 IgM PFC/Spleen Mean (±S.E.)[c]	Anti-SRC Response on Day 4 IgG PFC/Spleen Mean (±S.E.)[c]
1	SRC	——	——	60,000 (±19,300)	346,000 (±111,200)
2	SRC	——	Anti-SRC Serum(1:25)	165,600 (±71,800)	509,600 (±156,800)
3	SRC	PV Spleen[b]	——	5,500 (± 1,250)	17,800 (±3,100)
4	SRC	PV Spleen	Anti-SRC Serum(1:25)	5,300 (± 1,700)	24,000 (±7,450)

DISCUSSION

There are many reports on the immunosuppressive action of antibody (2, 4, 19, 20). In this study, we have also confirmed that the administration of undiluted mouse anti-SRC sera resulted in a moderate inhibition of the primary response to SRC. However, administration of 20-to 50-fold diluted antisera did not significantly affect the primary response (Table 1-3), and under these conditions we found that the simultaneous administration of spleen extracts had dramatic suppressive effects on the multiplication of PFC cells.

Although the mechanism of the suppressive effect observed is not totally clear, it seems reasonable to assume that the spleen extract non-specifically stimulates or augments the specific suppression mediated by the administered antibody, on the basis of the following observations. (i) The suppression was antigen specific and the specificity was determined entirely by the antisera administered. (ii) The combined use of normal mouse sera (undiluted) and spleen extracts caused no immunosuppression (data not shown). (iii) The secondary immune response was suppressed by the spleen extracts alone (Table 7), presumably because the animals already possessed low levels of circulating antibody. (iv) The action of the spleen extracts was not limited by histocompatibility or species barriers (Table 4 and 5). (v) Extracts of spleen from untreated mice and those from PV-stimulated mice were equally active. These results then suggest that factor(s) present in the spleen of normal animals strongly and non-specifically augment the antibody-mediated suppression of the primary immune response.

There are many reports on the suppressor factors involved in the regulation of antibody production (1, 3, 6-9, 10-18). The factor (s) present in our spleen extract, however, appear to be quite different from those previously reported. Firstly, most of those already reported, including the T-cell produced factors (7, 12, 13), are antigen-specific (15, 16), whereas ours are apparently non-specific and the specificity of our system is determined by the added antisera. Secondary, many of those factors previously reported were produced only by animals stimulated either non-specifically (for example, ref. 1, 9) or specifically with antigen (for example 11-13), whereas our factor(s) were present apparently in similar concentrations in both normal and PV-stimulated animals. Thirdly the non-specific factor described by Reinertsen and Steinberg (9) was active by itself, whereas ours required the simultaneous administration of diluted antisera.

It is important to ask whether the observed effect of spleen extracts are physiologically significant in the regulation of immune response, or are simply artefacts of our conditions of assay. We believe that they are physiologically relevant on the basis of the following considerations. (i) Although the doses of spleen extracts were rather large, they were still in the "physiological" range (up to two spleen equivalents from untreated mouse, one spleen equivalent from PV injected mouse). (ii) We had to use rather high doses of spleen extracts, because exceedingly low doses of antisera were used to minimize the "background" suppression due to the antibodies alone. It seems highly probable that much smaller amounts of spleen factor(s) would be effective in the presence of higher (i.e. more "physiological") concentrations of the antibody. (iii) The suppression by spleen extracts plus diluted antisera can be quite exhaustive, and absolutely no increase in PFC was observed if the injection was repeated on day -1 and 0 (see Results). In contrast, even repeated injections of undiluted antisera, without spleen extracts, could not eliminate the residual primary response to SRC, producing an increase in PFC from 230 ($\pm$50) in untreated mice

to 21,000 (±11,900) per mice (data not shown).

Our data thus indicate that the activity of the spleen extracts is certainly worthy of further investigation. Preliminary experiments on the chemical properties of spleen extracts revealed that the suppressive activity, which was unstable to heat, became weak within a few hours at 37°C. The suppressor factor(s) was not dialyzable, and may thus have a high molecular weight (unpublished data).

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