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Effect of stimulation and blockade of mononuclear phagocyte system on the induction of suppressor T cells of delayed footpad reaction to SRBC in mice

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Summary. The role of mononuclear phagocyte system (MPS) in the induction of suppressor T cells which depress the delayed footpad reaction to sheep erythrocytes (SRBC) was studied in mice in which MPS was blocked or stimulated. Colloidal carbon and diethylstilbestrol were used for blockade and stimulation respectively. Adoptive transfer of suppressor T cells was achieved by spleen cells of mice immunized intraperitoneally with varying doses of SRBC. In non-treated mice, 1×10^9 SRBC were required to induce suppressor T cells, while 6×10^8 could not induce the suppressor T cells. In MPS-blocked mice, however, even 6×10^8 SRBC could induce the suppressor T cells. On the other hand, 3×10^{10} SRBC were required for the induction of suppressor T cells in MPS-stimulated mice. These results suggest that the activity of macrophages as scavenger cells modulates the subsequent induction of suppressor T cells after immunization with high doses of SRBC.

INTRODUCTION

Macrophages were demonstrated to play important roles in the induction of immune responses as accessory cells for antigen presentation (Pierce, Kapp, Wood & Benacerraf, 1974) or antigen processing (Unanue, 1972). However, macrophages also function as scavenger cells in the elimination of antigen from the environment of immunocompetent cells (Metchnikoff, 1899: Perkins & Makinodan, 1965) and this function has been expected to influence the induction of immune responses against sheep erythrocytes (SRBC), (Yoshikai, Miake, Matsumoto, Nomoto & Takeya, 1979) or *Listeria monocytogenes* (Yoshikai, Miake, Matsumoto, Nomoto & Takeya, 1980).

The injection of an optimal dose of SRBC induces a high degree of delayed footpad reaction, while suppression of delayed footpad reaction occurs in mice when the immunizing dose of SRBC is increased (Lagrange, Mackaness & Miller, 1974). This suppression had been reported to be caused by B-cell response (Mackaness, Lagrange, Miller & Ishibashi, 1974) but more recent investigations have indicated that this suppression is mediated by suppressor cells which can be detected in the spleen of mice immunized with a high dose of SRBC and are θ-positive and antigen specific (Liew, 1977: Yamaguchi & Kishimoto, 1978).

In the present study, we examined the effects of blockade and stimulation of mononuclear phagocyte system (MPS) on the induction of the suppressor T cells in delayed footpad reaction to SRBC. It has been suggested that macrophages acting as scavenger cells modulate the induction of suppressor T cells.

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MATERIALS AND METHODS

Animals

Male mice of an inbred AKR strain were obtained from the Breeding Unit of Kyushu University. Ten-week-old mice were used for the experiments. Each experiment group consisted of five to seven mice.

Antigens

SRBC in Alsever's solution were obtained commercially and kept at 4° until use.

MPS blockade or stimulation

Pelikan carbon particles (C11/1431a, Günther Wagner, Hanover, Germany) were suspended at 25 mg/ml in phosphate-buffered saline (saline) containing 1% gelatin and 0·1 ml/10 g body weight was injected intravenously (i.v.) on day −1 and intraperitoneally (i.p.) on day 0 for MPS blockade. For MPS stimulation, diethylstilbestrol (DES, Iwai Kagaku Yakuhin, Japan) dissolved in peanut oil was administered subcutaneously (s.c.) as a single injection. Immunization was carried out on day 0.

Adoptive transfer of immune spleen cells

Four days after an intraperitoneal immunization with varying doses of SRBC, donor mice were killed, the spleens removed and single cell suspensions prepared. The cells were transferred i.v. into syngeneic recipients and 5×10^5 SRBC were injected i.v. to sensitize recipients. For elicitation of delayed footpad reaction, 1×10^8 SRBC in 50 μl of saline were injected into the left hind footpad 4 days after sensitization. The right hind footpad was injected with 50 μl of saline as control. Swelling of the footpad was measured 24 hr later with a dial-thickness gauge. The degree of reaction was expressed as the difference in thickness between the right and left hind footpad.

Separation of spleen lymphocyte subpopulation

T cell-enriched fraction. Spleen cells (5×10^8) were incubated at 37° on a nylon wool column (Wako Junyaku, Osaka, Japan) according to Julius, Simpson & Herzenberg (1973). Ten to fifteen percent of total applied cells were recovered in the non-adherent fraction and 93% were T cells reactive to anti-θ serum (Thyl.1) prepared by immunization of C3H/He mice with AKR thymus cells according to Reif & Allen (1964).

B cell-enriched fraction. One volume of 5×10^7 spleen cells were incubated at 37° for 45 min with an equal volume of six-fold diluted anti-θ serum and equal volume of ten-fold diluted guinea-pig complement (C). Forty to forty-five percent of total applied cells were viable and 94% of viable cells were reactive to rabbit anti-mouse immunoglobulin serum obtained commercially (Institute for Medical Biology, Nagoya, Japan).

Stimulation of spleen cells by mitogenic agents

Spleens were obtained from non-treated mice or from mice treated with colloidal carbon or DES. Cell suspensions (5×10^6/ml) were prepared in RPMI 1640 medium supplemented with 5% heat-inactivated normal AKR serum, 5×10^{-5} M 2-mercaptoethanol, 0·2% $NaHCO_3$, 20 mM HEPES, 100 μ/ml of penicillin and 100 μg/ml of streptomycin. The cell suspensions (200 μl) were added to wells of sterile tissue culture microplates (No. 3042 Falcon Plastics, Oxnard, Calif.) and an equal volume of medium alone or mitogen in medium was added. The mitogens used in this study were lipopolysaccharide (LPS, *E. coli* 0111; B4, Difco Lab.) and concanavalin A (Con A, Sigma Chemical Co., St Louis, Mo.). Microplates were cultured for 72 hr in a humidified atmosphere of 95% air–5% CO_2 at 37°. A 20 μl amount of medium containing 10 μCi/ml [^{3}H]-thymidine was added to each well 18 hr before harvesting cells on filter papers with a Labo Mash Semi Automatic Multiple Harvester (Labo Science, Co., Tokyo, Japan). The samples were counted in a liquid scintillation counter. Stimulation indices (SI) were calculated by dividing the mean count per minute (c.p.m.) by that of unstimulated control cultures.

Statistics

The statistical significance of the data was determined according to Student's t test. A P value of less than 0·05 was taken as significant.

RESULTS

Effect of DES or colloidal carbon on carbon clearance

To determine the effects of DES and colloidal carbon on MPS activity, a carbon clearance test was performed in mice injected with 1 mg of DES s.c. on day −3 or 25 mg/100 g of colloidal carbon i.v. on day −1 (Fig. 1). The phagocytic index (K value) was increased significantly by pre-treatment with DES ($P<0·001$) and decreased significantly by pre-treatment with colloidal carbon ($P<0·001$).

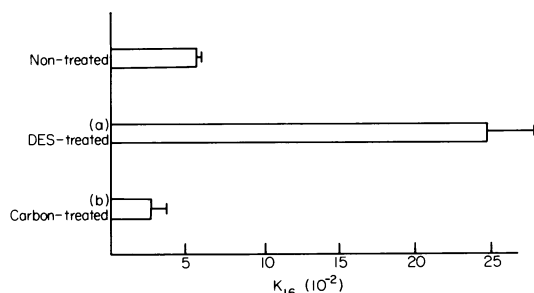

Figure 1. Effect of DES or colloidal carbon on the phagocytic activities of MPS. DES (1 mg) was given s.c. on day −3. Colloidal carbon (25 mg/100 g body weight) was given i.v. on day −1. Phagocytic indices (K_{16}) were measured by the carbon clearance. Horizontal bars represent standard deviations. Difference from non-treated mice was significant in (a) $P<0{\cdot}001$, (b) $P<0{\cdot}001$.

Effect of DES or colloidal carbon on the induction of suppressor T cells in spleens

In the spleen of mice immunized with a high dose of SRBC, suppressor cells have been reported to be generated which depress delayed footpad reactions to SRBC and these cells have been regarded as T cells but not B cells (Liew, 1977: Yamaguchi & Kishimoto, 1978). Our data also indicated that T cells, not B cells were responsible for the suppression of delayed footpad reactions (Table 1). In the present study, the adoptive transfer of suppressor cells of delayed footpad reactions was achieved by spleen cells from non-treated, DES-treated or carbon-treated mice immunized i.p. with varying doses of SRBC. When the immunizing dose of SRBC was 1×10^{10}, the T-cell-enriched fraction from non-treated mice or carbon-treated mice was able to suppress the delayed footpad reaction ($P<0{\cdot}005$) but that from DES-treated mice was not able to suppress the reaction ($P>0{\cdot}1$, Table 1). When the immunizing dose was increased to 3×10^{10} SRBC, however, such a cell-fraction from DES-treated mice was able to suppress the reaction ($P<0{\cdot}01$, Table 3). Even when the immunizing dose was reduced to 1×10^9 SRBC, such a cell-fraction from non-treated mice was able to suppress the reaction slightly ($P<0{\cdot}01$), but that from carbon-treated mice was more effective than that from non-treated mice ($P<0{\cdot}001$, Table 2). When the immunizing dose was 6×10^8 SRBC, the T-cell-enriched fraction from non-treated mice was no longer able to suppress the reaction ($P>0{\cdot}5$), whereas, that from carbon-treated mice was able to suppress the reaction ($P<0{\cdot}005$, Table 4). In these experiments, the suppressive activity appeared in the T-cell-enriched fraction (Tables 2, 3 and 4) but not in the B-cell-enriched fraction (Table 2). These results suggested that suppressor T cells were effectively generated in the spleen of carbon-treated mice, whereas the number of such T cells generated in the spleen of DES-treated mice was smaller than in the spleen of non-treated mice.

Table 1. T cell dependency of suppressive activity in spleen cells of mice immunized i.p. with 1×10^{10} SRBC

Pre-treatment of donors	Cell transferred*	Sensitization of recipients†	DFR‡ (0·1 mm)	*P*§
	—	—	0·9±0·3	
	—	5×10^5 SRBC	6·2±1·8	
Non-treated	Whole spleen cells	5×10^5 SRBC	3·1±1·8	<0·005
	Nylon wool non-adherent	5×10^5 SRBC	2·2±1·3	<0·001
	Nylon wool adherent	5×10^5 SRBC	6·1±1·1	>0·9
	C	5×10^5 SRBC	3·5±0·6	<0·005
	anti-θ+C	5×10^5 SRBC	7·0±2·0	>0·3

* Recipients were transferred i.v. with 5×10^7 spleen cells from donors immunized i.p. 1×10^{10} SRBC 4 days previously.

† Recipients were sensitized i.v. with 5×10^5 SRBC immediately after cell transfer.

‡ Footpad test was carried out 4 days after sensitization. Values are expressed as mean ± standard deviation.

§ Difference from non-transferred and sensitized control.

Table 2. Effect of colloidal carbon or DES on the induction of suppressor T cells in mice immunized i.p. with 1 × 10^9 or 1 × 10^{10} SRBC

Pre-treatment of donors	Immunization of donors	Cell transferred†	Sensitization of recipients‡	DFR (0·1 mm)	P§
		—	—	2·6±0·4	
		—	5 × 10^5 SRBC	8·0±1·1	
Non-treated	1 × 10^9 SRBC	Nylon wool non-adherent	5 × 10^5 SRBC	5·6±0·8	<0·01
		anti-θ+C	5 × 10^5 SRBC	7·0±1·8	>0·2
	1 × 10^{10} SRBC	Nylon wool non-adherent	5 × 10^5 SRBC	3·3±2·3	<0·005
		anti-θ+C	5 × 10^5 SRBC	6·8±2·3	>0·4
DES-treated	1 × 10^9 SRBC	Nylon wool non-adherent	5 × 10^5 SRBC	7·7±0·8	>0·2
		anti-θ+C	5 × 10^5 SRBC	7·6±1·5	>0·5
	1 × 10^{10} SRBC	Nylon wool non-adherent	5 × 10^5 SRBC	7·7±2·1	>0·1
		anti-θ+C	5 × 10^5 SRBC	6·7±1·1	>0·3
Carbon-treated	1 × 10^9 SRBC	Nylon wool non-adherent	5 × 10^5 SRBC	2·4±0·6	<0·001
		anti-θ+C	5 × 10^5 SRBC	8·5±0·5	>0·3
	1 × 10^{10} SRBC	Nylon woold non-adherent	5 × 10^5 SRBC	1·8±0·3	<0·001
		anti-θ+C	5 × 10^5 SRBC	6·1±1·0	>0·05

* Donors were pre-treated either 1 mg of DES s.c. on day −3 or 25 mg/100 g of colloidal carbon i.v. on day −1 and i.p. on day 0 and were immunized i.p. with 1 × 10^9 or 1 × 10^{10} SRBC.

† Recipients were transferred i.v. with 5 × 10^7 spleen cells from donors immunized 4 days previously.

‡ Values are expressed as mean ± standard deviation.

§ Difference from non-transferred and sensitized control.

Table 3. Effect of DES on the induction of suppressor T cells in mice immunized i.p. with 3 × 10^{10} SRBC

Pre-treatment of donors*	Cell transferred†	Sensitization of recipients	DFR‡ (0·1 mm)	P§
	—	—	1·7±0·3	
	—	5 × 10^5 SRBC	7·5±1·9	
Non-treated	Nylon wool non-adherent	5 × 10^5 SRBC	4·0±1·0	<0·005
DES-treated	Nylon wool non-adherent	5 × 10^5 SRBC	4·6±1·1	<0·01

* Donors were pre-treated with 1 mg of DES s.c. on day −3 and immunized i.p. 3 × 10^{10} SRBC on day 0.

† Recipients were transferred i.v. with 5 × 10^7 spleen cells from donors immunized 4 days previously.

‡ Values are expressed as mean ± standard deviation.

§ Difference from non-transferred and sensitized control.

Table 4. Effect of colloidal carbon on the induction of suppressor T cells in mice immunized i.p. with 6 × 10^8 SRBC

Pre-treatment of donors*	Cell transferred†	Sensitization of recipients	DFR (0·1 mm)	*P*§
	—	—	1·8±0·3	
	—	5 × 10^5 SRBC	6·5±1·1	
Non-treated	Nylon wool non-adherent	5 × 10^5 SRBC	6·0±2·6	>0·5
Carbon-treated	Nylon wool non-adherent	5 × 10^5 SRBC	2·4±0·4	<0·005

* Donors were pre-treated with 25 mg/100 g of colloidal carbon i.v. on day −1 and i.p. on day 0 and immunized i.p. with 6 × 10^8 SRBC on day 0.
† Recipients were transferred i.v. with 5 × 10^7 spleen cells from donors immunized 4 days previously.
‡ Values are expressed as mean ± standard deviation.
§ Difference from non-transferred and sensitized control.

Effect of colloidal carbon or DES on mitogen responsiveness of spleen cells

Since the purpose of this investigation was to determine the effect of MPS activity on induction of suppressor T cells, it was necessary to investigate whether DES or colloidal carbon altered lymphocyte functions. Therefore, groups of three mice were treated s.c. with 1 mg of DES on day −3 or i.v. with 25 mg/100 g of colloidal carbon on day −1 and the abilities of their spleen cells to proliferate in response to the T-cell mitogen Con A and in response to the B-cell mitogen LPS were measured (Table 5). Little difference was detected in the proliferative response of spleen cells to either Con A or LPS among DES-treated, carbon-treated and non-treated mice.

DISCUSSION

We have previously reported that the role of macrophages acting as scavenger cells in the elimination of antigen appears to modulate the subsequent immune responses against SRBC (Yoshikai, *et al.*, 1979) or *L. monocytogenes* (Yoshikai *et al.*, 1980). In the present paper, we have confirmed that macrophages play an important role in the modification of the induction of suppressor T cells. Our results showed that the suffi-

Table 5. Effect of DES or colloidal carbon on the response to T-cell or B-cell mitogen in mice

Pre-treatment*	Con A			LPS		
	μg/cc	c.p.m.†	SI‡	μg/cc	c.p.m.	SI
	0	4641±674		0	4641±674	
Non-treated	5·0	70980±5092	15·3	100	44533±2808	9·6
	10·0	74203±13791	15·9	250	51058±7007	11·0
	0	5001±309		0	5001±309	
DES-treated	5·0	73174±4924	14·6	100	43703±1787	8·7
	10·0	84501±5779	16·8	250	45605±1569	9·1
	0	3775±608		0	3775±608	
Carbon-treated	5·0	70120±2819	14·6	100	50070±2627	13·2
	10·0	63782±4832	16·8	250	37155±1919	9·8

* Groups of three mice were given either 1 mg of DES s.c. on day −3 or 25 mg/100 g of colloidal carbon i.v. on day −1.
† Values are expressed as mean count per minute ± standard deviation.
‡ S.I.: stimulation index calculated by dividing the mean c.p.m. of unstimulated control culture.

cient doses of SRBC needed for induction of suppressor T cells varied according to the phagocytic activity of MPS. In non-treated mice, 1×10^9 SRBC were required to induce suppressor T cells and 6×10^8 SRBC could not induce the suppressor T cells. In MPS-blocked mice, however, even 6×10^8 SRBC could induce the suppressor T cells sufficiently (Tables 2 and 4). On the other hand, 3×10^{10} SRBC were required for the induction of suppressor T cells in MPS-stimulated mice (Table 3). These results suggested that suppressor T cells were effectively generated in MPS-blocked mice, whereas the number of such T cells generated in MPS-stimulated mice was smaller than in non-treated mice.

There are several reports concerning the relationship between the activity of MPS and the induction of immunological tolerance involving suppressor T cells. Cruchard (1968) reported the effect of MPS blockade on immunological tolerance to bovine serum albumin and suggested that the induction of tolerance might be facilitated by MPS-blockade which would both delay antigen elimination from the tissue fluids and probably inhibit the antigen processing in the immune response. Chaouat & Howard (1976) studied the effect of MPS-blockade on the susceptibility to tolerance induction by a polysaccharide antigen in mice. They suggested that severe MPS-blockade could promote tolerance by suppressing the active roles of the macrophages in immune induction. Das & Leskowitz (1974) showed that the relatively high resistance of BALB/c mice to tolerization by bovine globulin (BGG) was associated with macrophage hyperactivity and they also studied the effect of stimulation or blockade of MPS on the tolerance induction to BGG in mice (Lukic, Cowing & Leskowitz, 1975). It was described that the stimulation of MPS with *Bacillus* Calmette Guérin (BCG) in DBA/2 mice decreased their susceptibility to tolerance, whereas MPS-blockade with carrageenan treatment of BALB/c mice made them more susceptible to tolerance induction. Although these observations are consistent with our results, the modification of tolerance induction was ascribed by Luckic *et al.* (1975) to the role of macrophages acting as antigen presenting cells in the induction of immune response. However, macrophages function not only as antigen presenting cells but also as scavenger cells in the antigen elimination of particulate substances. Our results in the present paper may be also ascribed to the different contribution of macrophages acting as scavenger cells in removing antigen from the environment of immunocompetent cells thus giving rise to a reduction of their ability to induce suppressor T cells due to antigen overload.

It is of great interest whether the scavenger function and antigen presenting function are mediated by the same cells or not. In recent reports (Yamashita & Shevach, 1977; Cowing, Schwartz & Dicker, 1979; Lee, Wilkinson & Wong, 1979), macrophages were separated into two sets based on the presence of detectable Ia antigen on their surface. Of these two groups of macrophages, the Ia-bearing set were responsible for antigen presentation (Schwartz, Yano & Paul, 1978; Beller & Unanue, 1980) and display limited phagocytic capacity (Beller, Kiely & Unanue, 1980). It has been shown that DES and colloidal carbon which affected the phagocytic activity of MPS (Fig. 1) did not appear to alter the lymphocyte functions directly (Table 5) but it is uncertain whether these agents can affect the macrophages acting as antigen presenting cells capable of inducing both effector and suppressor T cells of delayed footpad reaction to SRBC. We consider that these agents alter mainly the number and/or activity of macrophages acting as scavenger cells and have little influence on the antigen presenting cells. The relationship between these two functions of macrophages *in vivo*, however, is not yet clear. Further investigation should be carried out.

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