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A re-evaluation of the role of macrophages in carrageenan-induced immunosuppression

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Summary. Administration of a single dose of 1 mg carrageenan to mice caused a temporary blockade of hepatic phagocytosis of ^{51}Cr-labelled sheep erythrocytes (SRBC) and a prolonged reduction in the number of splenic plaque-forming cells (PFC) against SRBC. The *in vitro* responses to phytohaemagglutinin (PHA) and SRBC were also suppressed, whereas the response to the T cell-independent antigen DNP-Ficoll was not affected. Other *in vitro* experiments have shown that responses of normal cells can be actively suppressed by macrophages from carrageenan-treated mice and the possible mechanisms of this suppression are discussed.

INTRODUCTION

Carrageenan is a high molecular weight sulphated polygalactose extracted from the marine alga *Chondrus crispus* and its manifold biological properties have been reviewed elsewhere (Di Rosa, 1972). Among them is the capacity to induce an inflammatory process (Winter, Risley & Nuss, 1962) and the ability to interfere with both humoral (Aschheim & Raffel, 1972; Lukić, Cowing & Leskowitz, 1975; Thomson, Wilson, Cruickshank & Jeffries, 1976a; Thomson, Wilson, Cruickshank & Horne, 1976b) and cell-mediated (Schwartz & Leskowitz, 1969; Bice, Schwartz, Lake & Salvaggio, 1971; Rios & Simmons, 1972; Mizushima, Murata & Horiuchi, 1974; Schwartz & Catanzaro, 1973; Boros & Schwartz, 1975) immunological reactions.

When added to cells *in vitro* carrageenan is taken up by macrophages and causes the release of lysosomal enzymes (Allison, Harington & Birbeck, 1966) and, at higher concentrations, cell death (Catanzaro, Schwartz & Graham, 1971). The survival of lymphocytes (Catanzaro *et al.*, 1971) and their ability to respond to phytohaemagglutinin (PHA) (Lake, Bice, Schwartz & Salvaggio, 1971; Hanna & Leskowitz, 1973; Thomson *et al.*, 1976a) remain unimpaired. The observations made *in vitro* led to the suggestion that the *in vivo* suppressive effects of carrageenan were a reflection of macrophage killing and on that basis carrageenan has been used to establish the need for macrophage participation in certain reactions (Rios & Simmons, 1972; Lukić *et al.*, 1975; Lukić & Leskowitz, 1974; Hanna & Leskowitz, 1973; Lake *et al.*, 1971). Studies with carrageenan *in vivo* do not fully support the conclusions concerning its cytotoxicity to macrophages. Thus, macrophages in carrageenan-induced granulomas have a life-span of several weeks (Ryan &

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Spector, 1969) and carrageenan was demonstrated only in a proportion of phagocytic cells after doses which were immunosuppressive (Sawicki & Catanzaro, 1975). A number of authors have therefore concluded that the immunosuppressive properties of carrageenan are not related to its effects on macrophages (Aschheim & Raffel, 1972; Schwartz & Catanzaro, 1973; Boros & Schwartz, 1975; Sawicki & Catanzaro 1975).

The work reported here was undertaken in an attempt to clarify the role of macrophages in carrageenan-induced immunosuppression. Spleen cells were separated into adherent and non-adherent subpopulations so that the effect of carrageenan on their individual contribution to antibody formation and the response to mitogens *in vitro* could be ascertained. In addition, experiments on the effect of carrageenan on phagocytic activity and the antibody response were carried out *in vivo*. The results indicate that macrophages from carrageenan-treated mice exert an active suppressive effect upon lymphocytes.

MATERIAL AND METHODS

Mice

Inbred CBA female mice aged 3–4 months were used in all experiments.

Carrageenan

Sea Kem 9 carrageenan was a gift from Dr D. W. Renn, Marine Colloids Inc., Maine, U.S.A. This was dissolved in 0·9% sodium chloride at a concentration of 10 mg/ml just before use. Mice received 0·1 ml of the carrageenan solution intraperitoneally (i.p.) once or four times at 2-day intervals.

Phagocytic activity in vivo

This was assayed by measuring the organ uptake of ^{51}Cr-labelled sheep erythrocytes (SRBC), for this correlates with the rate of blood clearance (Warr & Šljivić, 1974). SRBC were labelled with ^{51}Cr (sodium chromate B.P., Radiochemical Centre, Amersham) as described elsewhere (Warr & Šljivić, 1974) and 10^8 cells were injected intravenously (i.v.) into mice. Mice were killed by cervical dislocation 30 min after injection and their organs were removed and weighed. The radioactivity of different organs was measured in a well-type scintillation counter and calculated as the percentage of injected radioactivity.

The antibody response in vivo

Mice were immunized i.v. with 5×10^6 or 10^9 SRBC. The number of direct plaque-forming cells was measured 4 days later as described by Cunningham & Szenberg (1968).

Preparation of cells for culture

The medium used throughout was RPMI 1640 with glutamine (Flow Laboratories Limited, Irvine, Scotland), containing heat-inactivated foetal calf serum (Flow), sodium bicarbonate, HEPES and antibiotics (Rumjanek, 1976; Watson & Šljivić, 1976). The spleens were teased apart and sieved through a fine metal sieve in the presence of medium. The cells were washed once (antibody response) or three times (mitogen response) and resuspended at the required concentration. Adherent cells were removed by incubating 15–20 ml of the suspension (20×10^6 cells/ml) in a 300 ml glass medical flask lying flat on its side. After 30 min at 37° the flask was turned over and the cells incubated for another 30 min. The non-adherent cells were then gently agitated and collected, after which the flask was vigorously rinsed with more medium, which was discarded. Fresh medium was added to the flask and the adherent cells were removed from glass by means of a rubber 'policeman'. The non-adherent cell fraction obtained in this way contained approximately 70% of the original cell number.

Peritoneal cells were obtained by washing the peritoneal cavity of untreated or carrageenan-treated mice with medium.

The antibody response in vitro

Spleen cells in RPMI 1640 medium containing 5% foetal calf serum (Watson & Šljivić, 1976) were cultured in Marbrook chambers (Marbrook, 1967) for 4 days at 37° in humidified air containing 5% CO_2. The cultures contained either unfractionated spleen cells or various combinations of non-adherent and adherent spleen cells and peritoneal cells. The details of fractionation procedures and concentration of cells have been described (Watson & Šljivić, 1976). The cells were cultured in the presence of either 2×10^6 SRBC or 0·1 μg dinitrophenylated Ficoll (DNP-Ficoll) as antigens.

At the end of the culture period the cells were harvested, washed once and resuspended in 1 ml medium. The antibody response to SRBC and DNP was measured in terms of direct plaque-forming cells (PFC) by the method of Cunningham & Szenberg (1968), using untreated SRBC and SRBC coated with DNP (Strausbach, Sulica & Givol, 1970), respectively. The results are given as the means ±s.e. PFC/culture of quadruplicate cultures of cells pooled from several mice.

In vitro *stimulation by mitogens*

Spleen cells in RPMI 1640 medium containing 10% foetal calf serum (Rumjanek, 1976) were cultured in the presence of mitogens PHA (phytohaemagglutinin, reagent grade, Wellcome Laboratories) or LPS (*E. coli* lipopolysaccharide 055:B5, Difco Laboratories). Aliquots (200 μl) of spleen cell suspensions (1×10^6/ml) were dispensed in quadruplicate into microtest plates (Microplates, flat bottomed wells, Nunc, Denmark). In some experiments peritoneal cells (2×10^4/ml) were added to non-adherent spleen cells (1×10^6/ml). The mitogens were added at a final concentration of 10 μg/ml (control cultures received medium instead) at the beginning of the culture period and the cells were incubated for 48 h at 37° in humidified air containing 5% CO_2. One microcurie of [^{3}H]thymidine ([methyl-^{3}H]thymidine, sp. act. 5 Ci/mM, Radiochemical Centre, Amersham) was added to each well 24 h before the end of the culture period. The cells were harvested on glass fibre filters using an automated sample harvester (Skatron, Lierbyen, Norway) and the radioactivity was measured in a Packard liquid scintillation spectrometer.

RESULTS

Effect of carrageenan on phagocytosis *in vivo*

Carrageenan given 24 h before ^{51}Cr-labelled SRBC caused a marked depression of hepatic uptake and an increase in the uptake by the spleen and lungs (Table 1). The organ uptake was little affected, however, in mice given carrageenan 7 days before or in repeated doses, and these animals showed hepatomegaly.

The effect of carrageenan on the antibody response *in vivo*

It has been shown that substances that increase splenic uptake of SRBC through blockade of hepatic phagocytosis also enhance the antibody response to

Table 2. Effect of carrageenan on the antibody response *in vivo*

Pre-treatment	Log_{10} PFC/spleen*	
	5×10^6 SRBC	10^9 SRBC
None	5·18±0·02 (150,891)	5·35±0·09 (226,116)
Carrageenan 1 day before	4·58±0·07 (37,914)	4·02±0·03 (10,362)
Carrageenan 7 days beore	3·62±0·54 (4,132)	4·60±0·07 (39,544)
Carrageenan on days −7, −5, −3, −1	4·16±0·20 (14,390)	4·40±0.15 (24,882)

* All values are means ±s.e. for 3–6 mice in each group. Geometric means are given in parentheses.

Table 1. Organ uptake of ^{51}Cr-labelled SRBC in carrageenan-treated mice

Treatment	Organ weight		Percentage injected dose		
	Liver (g/100 g body weight)	Spleen (mg)	Liver	Spleen	Lungs
None	5·29±0·10	75±4	72·5±4·5	10·2±1·9	0·78±0·39
Carrageenan 1 day before	4·97±0·20	83±7	6·4±1·7	22·7±4·7	8·38±0·45
Carrageenan 7 days before	6·18±0·12	71±1	71·4±1·3	13·8±0·9	0·92±0·13
Carrageenan on days −7, −5, −3, −1	6·54±0·13	78±2	78·4±2·4	10·1±1·6	0·93±0·21

All values are means ±s.e. for 4–5 mice in each group.

suboptimal doses of this antigen (Souhami, 1972; Bradfield, Souhami & Addison, 1974; Šljivić, Clarke & Warr, 1975). No such effect was, however, observed in mice immunized with 5×10^6 SRBC 1 day after administration of carrageenan (Table 2). On the contrary, the number of direct PFC elicited by the low as well as the high dose of SRBC was markedly reduced in all mice pretreated with carrageenan and ranged between 3 and 25% of the normal response (Table 2). This suggests a possible dysfunction of cells involved in the antibody response. In order to study the nature of the defect experiments were carried out on the antibody response of spleen cells cultured *in vitro*.

Effect of pretreatment with carrageenan on the antibody response *in vitro*

The results of a typical *in vitro* experiment are shown in Table 3, from which the following observations can be made. The response to SRBC was depressed in cultures containing spleen cells from mice given a single dose of carrageenan 1 day before, and depression was particularly strong after pretreatment with four doses (cultures 2 and 3 vs 1). In contrast, the response to DNP-Ficoll, a thymus- and macrophage-independent antigen *in vitro* (Mosier, Johnson, Paul & McMaster, 1974), was not affected. Non-adherent spleen cells cultured without adherent cells gave very low PFC response to SRBC, but the anti-DNP response was only slightly reduced (cultures 4–6). The addition of 10^6 peritoneal cells or adherent spleen cells to cultures of non-adherent cells largely restored the response to SRBC (cultures 10 and 7), but comparable cells from carrageenan-treated donors were far less effective (cultures 11, 12 and 8, 9). This defect was more marked after four doses of carrageenan than after a single dose given 1 day before. These results suggest that the depression of the antibody response to SRBC was associated with the adherent cell population.

The suppressive action of adherent spleen cells from carrageenan-treated mice was confirmed in another experiment in which cells from untreated and treated animals were co-cultured in various combinations (Table 4). Adherent spleen cells from treated animals failed to support an optimal response of normal non-adherent cells (cultures 6–8 vs 5), and again the failure to respond was greater when the cells were taken from donors treated 7 days before or in 4 doses (cultures 7 and 8 vs 6). Conversely, non-adherent cells from carrageenan-treated mice gave optimal responses to SRBC when they were co-cultured with adherent cells from untreated mice (cultures 9–11 vs 5),

Table 3. Effect of pre-treatment with carrageenan on the antibody response of spleen cells *in vitro* to SRBC and DNP-Ficoll

Culture no.	Cells in culture*				PFC/culture	
	Spleen			Peritoneal cells	anti-SRBC	anti-DNP
	Unfractionated	Non-adherent	Adherent			
1	Normal	—	—	—	2190±99	1700±78
2	C-1	—	—	—	1376±111	1610±31
3	C-X	—	—	—	666±79	1686±81
4	—	Normal	—	—	206±26	1470±99
5	—	C-1	—	—	256±62	
6	—	C-X	—	—	166±33	1566±128
7	—	Normal	Normal	—	1770±60	
8	—	Normal	C-1	—	990±99	
9	—	Normal	C-X	—	516±54	
10	—	Normal	—	Normal	1776±88	
11	—	Normal	—	C-1	1196±94	
12	—	Normal	—	C-X	496±75	

* Cell donors are designated as follows: normal—untreated; C-1—carrageenan 1 day before; C-X—carrageenan on days −7, −5, −3 and −1.

Table 4. Effect of pre-treatment with carrageenan on the antibody response of spleen cells *in vitro* to SRBC

Culture no.	Spleen cells in culture*			
	Unfractionated	Non-adherent	Adherent	PFC/culture
1	Normal	—	—	1966 ± 67
2	C-1	—	—	1130 ± 66
3	C-7	—	—	620 ± 68
4	C-X	—	—	696 ± 57
5	—	Normal	Normal	1746 ± 29
6	—	Normal	C-1	1216 ± 13
7	—	Normal	C-7	810 ± 42
8	—	Normal	C-X	490 ± 73
9	—	C-1	Normal	1800 ± 87
10	—	C-7	Normal	1746 ± 57
11	—	C-X	Normal	1710 ± 55
12	Normal	—	C-1	1626 ± 109
13	Normal	—	C-7	1166 ± 112
14	Normal	—	C-X	706 ± 107

* Cell donors are designated as follows: normal, untreated; C-1, carrageenan 1 day before; C-7, carrageenan 7 days before; C-X, carrageenan on days −7, −5, −3 and −1.

indicating a potentially normal lymphocyte function in treated animals. Finally, adherent spleen cells from carrageenan-treated mice suppressed the response of unfractionated normal spleen cells (cultures 12–14). In this respect carrageenan given 1 day before was least, and given in 4 doses most, effective.

The *in vitro* response of spleen cells from carrageenan-treated mice to mitogens PHA and LPS

When the *in vitro* stimulation by the T-cell mitogen PHA was studied, the response of spleen cells from carrageenan-treated mice was depressed when the cells were tested 7 days after a single dose or after 4 repeated doses (Table 5). No suppression occurred when spleen cells were tested 1 day after a single injection of carrageenan (Table 5); in fact, in 5 out of 6 experiments (data not shown) the response to PHA was slightly but significantly enhanced.

Table 5. The *in vitro* response of spleen cells from carrageenan-treated mice to PHA

Treatment of cell donors	Uptake of [^{3}H]thymidine*	
	Medium (control)	PHA
None	4835 ± 76	58020 ± 2356
Carrageenan 1 day before	5927 ± 316	88905 ± 266
Carrageenan 7 days before	4140 ± 150	20700 ± 2639
Carrageenan on days −7, −5, −3, −1	5137 ± 116	21548 ± 1561

* Mean ct/min ± s.e. of quadruplicate cultures.

The results of experiments with LPS, a B-cell mitogen, were inconsistent. Thus in 3 out of 6 experiments it paralleled the effect seen with PHA responses, whereas in 2 experiments the response to LPS was normal and in one experiment elevated.

The possibility that changes in responsiveness to PHA, too, were mediated by macrophages was next examined. The fact that PHA stimulation may proceed in the absence of accessory adherent cells (Table 6) allowed us to study the part played by these cells. Experiment 1 in Table 6 shows that non-adherent spleen cells from carrageenan-treated mice responded as well as cells from untreated animals, suggesting that T-cell function was not directly affected by carrageenan. Further evidence that the changes observed in PHA responsiveness were due to the participation of macrophages was obtained when normal non-adherent cells were cultured in the presence of peritoneal cells from normal or carrageenan-treated mice (Table 6, exp. 2 and 3). Peritoneal cells from mice pre-treated

Table 6. The response *in vitro* of non-adherent spleen cells to PHA in the presence of peritoneal cells from carrageenan-treated mice

	Cells in culture*		Uptake of [^{3}H]thymidine†	
Experiment	Non-adherent spleen cells	Peritoneal cells	Medium (control)	PHA
1	Normal	—	1565±59	11976±2189
	C-1	—	1742±345	13643±2158
	C-7	—	1783±219	13408±670
	C-X	—	1378±199	12410±527
2	Normal	—	7908±1132	21299±2269
	Normal	Normal	16281±2184	57634±6002
	Normal	C-1	18220±2052	75301±6716
	Normal	C-X	21740±1211	767±142
3	Normal	Normal	2898±606	12847±1001
	Normal	C-1	2336±275	15906±1420
	Normal	C-7	4526±420	3867±1090

* Cell donors are designated as follows: normal, untreated, C-1, carrageenan 1 day before; C-7, carrageenan 7 days before; C-X, carrageenan on days −7, −5, −3 and −1.
† Mean ct/min±s.e. of quadruplicate cultures.

with 4 doses of carrageenan, or with a single dose 7 days before, suppressed the response of normal non-adherent cells to PHA. Peritoneal cells from mice that had received carrageenan 1 day before slightly enhanced the response to PHA, a result similar to that obtained with unfractionated spleen cells.

DISCUSSION

The immunosuppressive properties of carrageenan are well documented. In the present study this suppression seemed to be mainly related to responses involving T lymphocytes, dependent on the time of carrageenan administration and associated with a regulatory role of macrophages.

One day after administration of carrageenan the hepatic uptake of ^{51}Cr-labelled SRBC was markedly reduced with a concomitant increase in splenic uptake. This resembles the temporary blockade of hepatic phagocytosis found after administration of colloidal carbon (Souhami, 1972) or dextran sulphate (Bradfield *et al.*, 1974), and it is followed within a few days by a recovery which is dependent on the influx of new cells from the circulation (Souhami & Bradfield, 1974). It has been shown that large molecular weight dextran sulphate forms complexes with fibrinogen and platelets which are removed from the circulation by Kupffer cells of the liver (Walton, 1954), and this seems to be responsible for the blockade of hepatic phagocytosis. Carrageenan, which is a large molecular weight polygalactose, probably acts in a similar way, for it has been reported to activate Hageman factor (Schwartz & Kellermeyer, 1969), induce intravascular coagulation (Thomson *et al.*, 1976b; Thomson & Horne, 1976) and cause thrombosis and infarction in the liver (Rumjanek, 1976; Thomson & Horne, 1976). The antibody response to SRBC was, however, markedly reduced in carrageenan-treated mice at a time corresponding to the blockade of hepatic phagocytosis, and this contrasts with the enhancement of the response to suboptimal doses of antigen found in similar circumstances after administration of colloidal carbon (Souhami, 1972; Šljivić *et al.*, 1975) and dextran sulphate (Bradfield *et al.*, 1974). This indicates that carrageenan interferes with the normal function of cells involved in the antibody response.

Evidence concerning the cell type affected by carrageenan was derived from experiments on the antibody response *in vitro*. An advantage of this approach was that the function of lymphocyte-rich (non-adherent) and macrophage-rich (peritoneal or adherent spleen cells) cell populations from carrageenan-treated and control animals could be assessed independently. The results showed that the *in vitro* response to DNP-Ficoll, a T lymphocyte and macrophage independent antigen (Mosier *et al.*, 1974) was not affected by administration of

carrageenan to cell donors, whereas the response to SRBC, which requires co-operation of these cells and B lymphocytes (Claman & Chaperon, 1969; Sjöberg, Anderson & Möller, 1972), was depressed. It was further found that lymphocytes from carrageenan-treated mice were fully capable of responding to SRBC in the presence of normal macrophages, but that peritoneal and splenic macrophages which had been exposed to carrageenan failed to support an optimal response of lymphocytes from untreated animals. Furthermore, macrophages from carrageenan-treated animals suppressed the response of unfractionated normal spleen cells These observations indicate that carrageenan suppresses humoral responses by interfering with macrophage function. This conclusion is also supported by the finding that spleen cells from carrageenan-treated mice responded normally to levan after transfer into irradiated recipients (Chaouat & Howard, 1976).

Carrageenan was shown to suppress, too, the *in vitro* incorporation of [^{3}H]thymidine into cells exposed to PHA, which is largely a T cell mitogen. This suppression was evident only with cells from mice which had received carrageenan in multiple doses or in a single dose 7 days, but not 1 day, before. The apparent delayed suppressive action of carrageenan under these conditions would explain the failure of several authors to affect the PHA response by adding carrageenan to cell cultures (Lake *et al.*, 1971; Bice *et al.*, 1971; Hanna & Leskowitz, 1973; Thomson *et al.*, 1976a). The suppressive effect of carrageenan on the response to PHA was mediated, as in the case of the humoral response, by macrophages. This was demonstrated by (a) the normal response of non-adherent cells from carrageenan-treated mice and (b) the suppressive effect which peritoneal cells from treated mice had on the response of non-adherent cells from control animals.

The way in which macrophages that have ingested carrageenan bring about suppression of immunological reactions remains unresolved. The depression of the antibody response to SRBC could be related to altered uptake and handling of a macrophage-dependent antigen (Unanue, 1972) but the available evidence does not support this possibility for it has been found that the uptake of SRBC by peritoneal macrophages from treated mice and their ability to elicit the antibody response to the intracellular antigen upon transfer into normal recipients were not diminished (Aschheim & Raffel, 1972). Several authors have reported that normal or activated macrophages and their soluble products can suppress [^{3}H]thymidine incorporation or, in some cases, proliferation of lymphocytes (Scott, 1972; Sjöberg, 1972; Nelson, 1973; Calderon, Williams & Unanue, 1974; Calderon & Unanue, 1975; Optiz, Neithamme, Lemke, Flad & Huget, 1975). Thymidine released through the breakdown of cells dying in culture is only one of these factors (Opitz, Neithammer, Jackson, Lemke, Huget & Flad, 1975).

The suppression of lymphocyte function in the presence of macrophages from carrageenan-treated animals may be related to some of the known effects of this agent on phagocytic cells. After uptake by macrophages, carrageenan is rapidly sequestered in the lysosomes (Allison *et al.*, 1966), thus causing their swelling (Catanzaro *et al.*, 1971), and it gives rise to a delayed and selective release of lysosomal enzymes (Allison *et al.*, 1966; Davies, Allison, Dym & Cardella, 1976), whilst the cells remain apparently viable (Allison & Davies, 1974; Davies *et al.*, 1976). As these enzymes are able to interact with a variety of substrates in the tissues (Allison & Davies, 1974) it is conceivable that they may interfere with lymphocyte function. Two observations appear to support this possibility. First, result of the present *in vitro* experiments indicate that responses of cells from mice receiving carrageenan 1 day before were less affected than when the intervals after carrageenan administration were longer. This correlates with the finding that the release of lysosomal enzymes by macrophages increases, after an initial delay, during the first few days after exposure to carrageenan (Allison *et al.*, 1966; Davies *et al.*, 1976). Second, it has been reported that poly-2-vinylpyridine N-oxide, which appears to stabilize lysosomes and protects against the toxic effects of silica (Holt, 1971), prevents or reduces immunosuppression caused by carrageenan (Rios & Simmons, 1972; Wilson, Cruickshank & Thomas, 1976).

If lysosomal enzymes are indeed responsible for the carrageenan-induced suppression of lymphocyte function, the following modes of action might be considered. (a) Lysosomal enzymes may interact directly with lymphocytes and alter their responsiveness. It has been reported, for example, that lysosomal neutral proteases can stimulate lymphocytes (Vischer, Bretz & Baggiolini, 1976) or enhance their

responses to PHA (Nakamura, Yoshinaga & Hayashi, 1976). We are not aware, however, of any reports on the possible effects on lymphocytes of acid hydrolases which are known to be released by macrophages exposed to carrageenan (Allison *et al.*, 1966; Davies *et al.*, 1976). (b) Activation of lysosomal enzymes, particularly phospholipase A_2, may increase the pool of fatty acids and thus contribute to the increased production of prostaglandins (reviewed by Lands & Rome, 1976). Increased levels of prostaglandin E have been found in carrageenan-induced exudates (e.g. Anderson, Brocklehurst & Willis, 1971; Willoughby, Giroud, Di Rosa & Velo, 1973; Velo, Dunn, Giroud, Timsit & Willoughby, 1973; Chang, Murota & Tsurufuji, 1975) and stimulated macrophages have been shown to produce PGE_2 *in vitro* (Gordon, Bray & Morley, 1976). E-type prostaglandins, and in particular PGE_2, have been reported to suppress a number of lymphocyte functions, including the blastogenic response to mitogens and antigens (Smith, Steiner & Parker, 1971; Stockman & Mumford, 1974), lymphokine production (Gordon *et al.*, 1976), antibody responses to SRBC *in vitro* (Plescia, Smith & Grinwich, 1975) and *in vivo* (Zurier & Quagliata, 1971), and antibody synthesis *in vitro* (Melmon, Bourne, Weinstein, Stearer, Kram & Bauminger, 1974), although these last two effects have been questioned (Quagliata, Lawrence & Phillips-Quagliata, 1973; Berenbaum, Purves & Addison, 1976). Using inhibitors of prostaglandin synthesis it was possible to enhance the antibody response to SRBC both *in vivo* and *in vitro* (Webb & Osheroff, 1976). These considerations suggest that prostaglandins may be involved as mediators of the macrophage-dependent carrageenan-induced suppression which we have described. It should be emphasized, however, that our experiments indicate that responses involving T lymphocytes are more readily suppressed than those of B lymphocytes. There is a paucity of reports on prostaglandin effects on lymphocyte classes, and those available appear to be contradictory (cf. Quagliata, *et al.* 1973; Stockman & Mumford, 1974). Further studies are obviously required in order to elucidate the basis of the carrageenan-induced immunosuppression and to substantiate the possible role of prostaglandins.

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