

IOTA-CARRAGEENAN INDUCED REAGINIC ANTIBODY PRODUCTION IN THE RAT — I. CHARACTERISATION AND KINETICS OF THE RESPONSE

STEPHEN NICKLIN, HILARY ATKINSON and KLARA MILLER

Immunotoxicology Department, British Industrial Biological Research Association, Carshalton, Surrey, U.K.

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Abstract — The intraperitoneal injection of graded amounts of ovalbumin into inbred PVG strain rats was found to produce a dose dependent anti-ovalbumin antibody response without eliciting the production of ovalbumin-specific reagenic antibodies. The injection of ovalbumin admixed with iota-carrageenan enhanced the anti-ovalbumin response and simultaneously elicited the *de novo* production of reagenic anti-ovalbumin antibodies. Isotype analysis of the anti-sera revealed the presence of both IgG and IgE class anti-ovalbumin antibodies and demonstrated that non-reagenic IgE anti-ovalbumin antibodies were also a feature of this reaction. The reagenic antibody phase of the response, although transient in nature, was re-elicited by secondary antigen challenge. The administration of ovalbumin prior to the ovalbumin-carrageenan preparation was shown to abrogate the reagenic antibody response without significantly influencing the non-reagenic component of the response. Conversely the injection of iota-carrageenan prior to antigen, significantly suppressed the normal agglutinating anti-ovalbumin response but did not prevent the induction of reagenic antibodies. These observations demonstrate that iota-carrageenan can, under specific conditions, function as an efficient adjuvant, and is capable of by-passing the normal mechanisms responsible for controlling antigen specific reagenic antibody production.

Carrageenan is the generic term given to the high molecular weight (>100,000 daltons) fraction of sulphated polygalactan isolated from various species of marine algae. The numerous biological properties of various systemically administered carrageenans were reviewed by Di Rosa (1972); they include the ability to induce inflammatory/granulomatous reactions, to interfere with various components of the blood clotting process, to decrease the level of complement activity, and to affect macrophage activity in treated animals. It is also becoming increasingly apparent that the parenteral administration of carrageenan leads to altered immune reactivity in a number of animal systems. Results indicate that allograft survival time is increased (Rios & Simmons, 1972) the growth of allogeneic tumours is potentiated (Rumjanek & Brent, 1978; Sakemi, Kuroiwa & Nomoto, 1980) and type IV hypersensitivity reactions are suppressed (Bice, Schwartz, Lake & Salvaggio, 1971; Schwartz & Cantanzaro, 1973). Carrageenans have also been

demonstrated to depress both cytotoxic T cell responses (Yung & Cudkowicz, 1977) and antibody dependent cell mediated killing (Catalona, Ratliff & McCool, 1978).

Humoral responses are also modified by carrageenan administration; several authors have reported that carrageenan treated animals have a reduced capacity to mount primary antibody responses against T-cell dependent antigens (Aschheim & Raffel, 1972; Thomson, Wilson, Cruickshank & Horne, 1976; Ishizaka, Otani & Morisawa, 1977; Rumjanek, Watson & Sljivic, 1977). Antibody responses to T-cell independent antigens are either unaffected (Ishizaka, Otani & Morisawa, 1977; Wong & Herscowitz, 1979) or likewise suppressed (Chaouat & Howard, 1976).

Carrageenan induced suppression of the immune system is thus well documented, but, there are also reports that carrageenans can under certain circumstances enhance antibody responses (Richou, Lallouette & Legger 1968; Turner & Higginbotham,

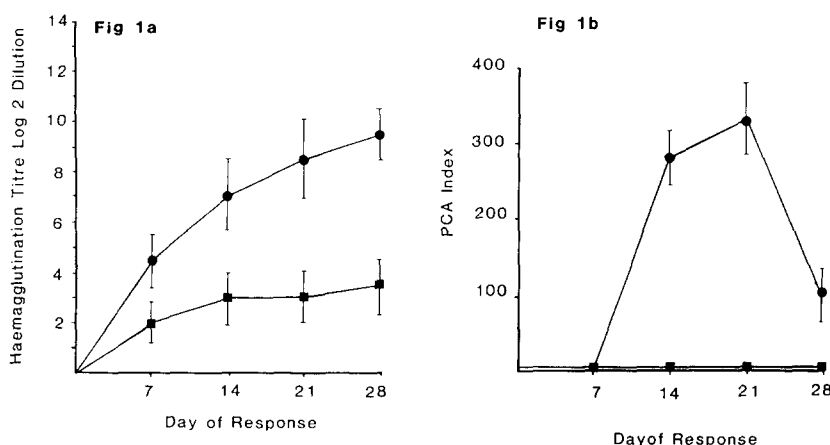


Fig. 1. Effect of systemically administered carrageenan on the agglutinating (a) and reaginic (b) anti-ovalbumin antibody response induced following the i.p. injection of 1 μ g ovalbumin on day 0 either alone (■—■) or admixed with 1000 μ g carrageenan (●—●). Results indicate mean response $\pm$ S.E., $n=6$. PCA index refers to the area of dye extravasation in mm^2 . Haemagglutination titre is given as the $\log_2$ dilution of the end point titre.

1979) and be as effective as Freund's complete adjuvant in potentiating the delayed hypersensitivity response to lysozyme (Mizushima, Murata & Horiuchi, 1974). In view of the paucity of data concerning the nature of this adjuvant effect we have examined the capacity of a carrageenan to modify the normal antibody response against a specific protein antigen. Carrageenan retains a net negative charge over a wide pH range, consequently a number of proteins can interact with it to form non-covalently associated complexes (Towle, 1973). In the present investigation we have restricted our attention to iota-carrageenan, this is the most sulphated form of carrageenan and hence carries the highest net negative charge. We therefore postulate that an antigen presented in association with iota-carrageenan could prove more immunogenic than antigen alone.

Our studies confirm and extend the earlier reports indicating that carrageenan can under certain conditions function as an adjuvant and in addition demonstrate the ability of carrageenan to initiate antigen specific reaginic antibody production against a protein antigen.

EXPERIMENTAL PROCEDURES

Animals

Specific pathogen-free (grade 3) PVG strain male rats weighing 200–250 g were purchased from Olac 1976 Ltd. (Bicester, U.K.). They were maintained under standard conditions of 12 h light, 12 h dark at

a temperature of $22 \pm 3^\circ\text{C}$ and with $60 \pm 15\%$ humidity. They were fed on Spratt's Laboratory Animal diet No. 1 (Spratt's Patent Ltd., Croydon, U.K.), and given water *ad libitum*.

Reagents and antigen preparation

Iota-carrageenan (food grade RE8809 batch No. 930402) was supplied by Marine Colloids Ltd., (F.M.C. Corporation, Springfield, NJ, U.S.A.).

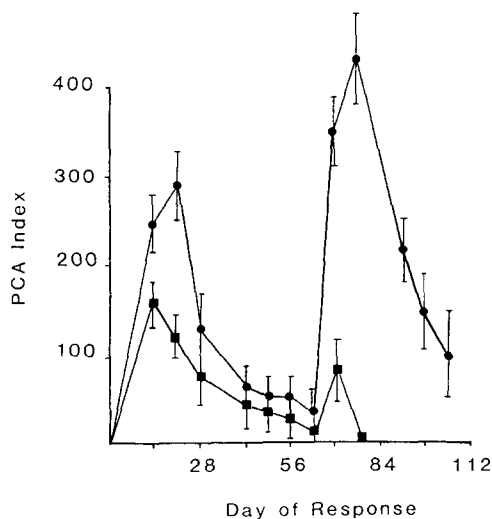


Fig. 2. Primary and secondary reaginic anti-ovalbumin antibody responses induced following the i.p. injection, on days 0 and 63, of 1 μ g ovalbumin admixed with 1000 μ g carrageenan (●—●) or precipitated with 1000 μ g alum (■—■). Results indicate mean response $\pm$ S.E., $n=6$. PCA index refers to the area of dye extravasation in mm^2 .

Prior to use the carrageenan was dissolved at 1 mg/ml in saline (0.15M NaCl) by heating to 60°C using a water bath. Stock solutions of ovalbumin (grade V, Sigma Chemical Company Ltd., Dorset, U.K.) were also prepared at 1 mg/ml in saline. The ovalbumin/carrageenan mixtures for injection were prepared by mixing the appropriate dilution of these solutions immediately prior to use. Alum precipitated ovalbumin was prepared according to the method of Hudson & Hay (1975), to give 1 µg precipitated protein/1000 µg alum.

Antibody determinations

Weekly blood samples (1 ml) for serum antibody analysis were taken from the retro-orbital sinus under ether anaesthesia. The total anti-ovalbumin antibody response was determined by passive haemagglutination using ovalbumin coated sheep red blood cells (SRBC's) (Oxoid Ltd., Basingstoke, U.K.). These cells were prepared according to the chromic chloride coupling technique described by Herbert (1967).

The agglutination assays were performed in round-bottom microtitre plates (Linbro/Titertek, Flow Laboratories Ltd., Strathclyde, U.K.), using 25 µl of serum, heat-inactivated at 56°C for 30 min and serially double-diluted in phosphate buffered saline pH 7.2 (PBS). After adding 25 µl of 1% ovalbumin coated SRBC's suspension to each well,

the plates were incubated at 4°C and scored for agglutination after 18 h. The end point titre was taken as the concentration at which agglutination was just detectable.

The level of circulating reaginic antibody in each serum sample was estimated by a passive cutaneous anaphylaxis (PCA) test (Ovary, 1964). Briefly, test and control serum were prepared at 1:2 dilution in saline and injected intradermally (0.1 ml/site) into the shaved flank of age and sex matched recipient rats. After 24 h the rats received by intravenous injection 0.6 ml ovalbumin (1 mg/ml) in saline together with 0.4 ml Evan's blue dye (2.0% in saline). The animals were killed after 20 min, their skin reflected and the area in mm² of dye extravasation around each injection site determined. An area of dye extravasation >5 mm was considered positive.

Isotype analysis of the anti-ovalbumin response

A solid-phase radio-immunoassay based on the method described by Liew, Hale & Howard (1982) was used to determine the IgE component of the anti-ovalbumin antibody response. Fifty microlitres of an ovalbumin solution (50 µg/ml) in absorption buffer (0.05 M sodium bicarbonate pH 9.6) were added to each well of a Dynatech U-bottom flexiwell microtitre plate (Dynatech Laboratories Ltd., Billingshurst, U.K.). After incubation at room temperature for 90 min, the wells were filled with a washing buffer (PBS, pH 7.2 containing 0.5 ml/l. Tween 40 and 2g/l. bovine serum albumin [BSA; Sigma grade V]) for 90 min and then washed thoroughly with distilled water. The plates were dried and stored at -20°C until used. Fifty microlitres of test serum serially diluted in Hanks' balanced salt solution (containing 10 mM Tris, pH 7.4 and 0.1% BSA) were added to each well and incubated at room temperature for 30 min. The plates were then washed, and 50 µl of 1/100 dilution (equivalent to 25 µg protein) of rabbit anti-rat IgE or IgG (Nordic Laboratory, Maidenhead, U.K.) were added. After 30 min incubation at room temperature the plates were washed as above, and 50 µl of ¹²⁵I-labelled goat anti-rabbit immunoglobulin antibody (1 µg protein) (Nordic Laboratories, IgG fraction) were added to each well. After a further 30 min the plates were washed again and each well counted in a gamma counter. Each serum titration was performed in triplicate and the end point titre was expressed as the highest serum dilution with counts greater than twice that of the corresponding dilution of normal PVG serum.

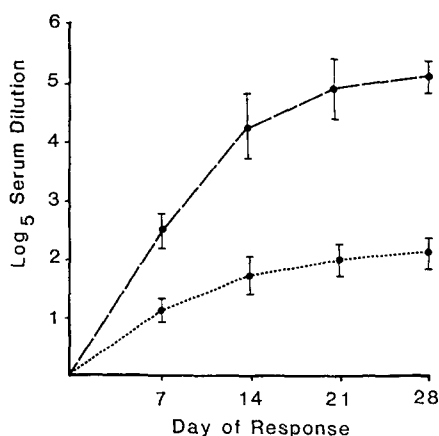


Fig. 3. Isotype analysis of the anti-ovalbumin response induced following the intraperitoneal injection of 1 µg ovalbumin admixed with 1000 µg carrageenan on day 0. IgE response (●---●), IgG response (●—●). Each serum titration was performed in triplicate with end point titres being expressed as the highest serum dilution with counts greater than twice the control. Results indicate mean ± S.E.

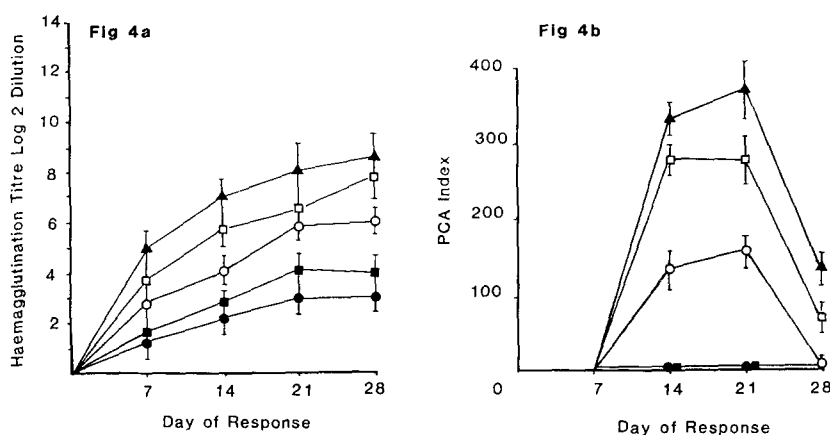


Fig. 4. Effect of varying the proportion of carrageenan to ovalbumin in the antigen preparation on the agglutinating (a) and reaginic (b) anti-ovalbumin antibody response. Rats received 1 µg ovalbumin i.p. on day 0 either alone (●—●) or admixed with 1 µg (■—■), 10 µg (○—○), 100 µg (□—□) or 1000 µg (▲—▲) of carrageenan. Results indicate the mean anti-ovalbumin response $\pm$ S.E., $n = 5$. PCA index refers to the area of dye extravasation in mm². Haemagglutination titre is given as the log₂ dilution of the end point titre.

Table 1. Effect of graded doses of ovalbumin administered alone or in combination with carrageenan on the antibody response to ovalbumin

Ovalbumin dose (µg) $\pm$ 1 mg carrageenan	Reaginic antibody response (PCA Index)					
	day 7	day 14	day 21	day 63 +	day 70	day 77
0.0 - Cgn	0.0	0.0	0.0	0.0	0.0	275 $\pm$ 75
0.1 + Cgn	0.0	0.0	0.0	0.0	245 $\pm$ 40	314 $\pm$ 0.0
0.1 - Cgn	0.0	0.0	0.0	0.0	0.0	0.0
1.0 + Cgn	0.0	294 $\pm$ 60	336 $\pm$ 30	50 $\pm$ 15	441 $\pm$ 26	308 $\pm$ 12
1.0 - Cgn	0.0	0.0	0.0	0.0	0.0	0.0
10.0 + Cgn	0.0	349 $\pm$ 24	370 $\pm$ 27	102 $\pm$ 25	402 $\pm$ 51	304 $\pm$ 22
10.0 - Cgn	0.0	0.0	0.0	0.0	9.5 $\pm$ 15	0.0
100.0 + Cgn	0.0	130 $\pm$ 21	101 $\pm$ 18	39 $\pm$ 23	127 $\pm$ 28	210 $\pm$ 35
100.0 - Cgn	0.0	0.0	0.0	19 $\pm$ 19	142 $\pm$ 67	44 $\pm$ 44
1000.0 + Cgn	0.0	0.0	0.0	0.0	2.5 $\pm$ 2.5	39 $\pm$ 23
1000.0 - Cgn	0.0	0.0	0.0	0.0	0.0	0.0
Agglutinating antibody response (end point titre)						
0.0 - Cgn	0.0	0.0	0.0	0.0	5.00 $\pm$ 0.0	7.00 $\pm$ 0.70
0.1 + Cgn	2.50 $\pm$ 0.28	2.50 $\pm$ 0.28	2.25 $\pm$ 0.25	2.50 $\pm$ 0.28	9.00 $\pm$ 1.00	>14.00
0.1 - Cgn	0.0	2.00 $\pm$ 0.50	2.25 $\pm$ 0.25	2.25 $\pm$ 0.25	7.25 $\pm$ 0.50	11.00 $\pm$ 0.50
1.0 + Cgn	4.00 $\pm$ 0.40	6.00 $\pm$ 0.40	8.25 $\pm$ 0.50	10.25 $\pm$ 0.25	13.25 $\pm$ 0.25	>14.00
1.0 - Cgn	2.00 $\pm$ 0.50	3.20 $\pm$ 0.40	3.20 $\pm$ 0.40	3.5 $\pm$ 0.50	6.50 $\pm$ 0.30	12.50 $\pm$ 0.75
10.0 + Cgn	5.00 $\pm$ 0.00	7.00 $\pm$ 0.00	8.25 $\pm$ 0.50	9.25 $\pm$ 0.25	12.75 $\pm$ 0.25	>14.00
10.0 - Cgn	4.25 $\pm$ 0.25	4.25 $\pm$ 0.25	4.50 $\pm$ 0.50	5.50 $\pm$ 0.30	13.25 $\pm$ 0.25	>14.00
100.0 + Cgn	6.25 $\pm$ 0.25	8.25 $\pm$ 0.25	7.00 $\pm$ 0.70	12.25 $\pm$ 0.25	13.00 $\pm$ 0.50	>14.00
100.0 - Cgn	4.75 $\pm$ 0.61	4.75 $\pm$ 0.61	5.75 $\pm$ 0.91	7.50 $\pm$ 0.60	11.00 $\pm$ 0.50	>14.00
1000.0 + Cgn	7.00 $\pm$ 0.00	7.00 $\pm$ 0.00	9.75 $\pm$ 0.25	12.25 $\pm$ 0.25	14.00 $\pm$ 0.50	>14.00
1000.0 - Cgn	2.75 $\pm$ 0.25	2.75 $\pm$ 0.25	3.75 $\pm$ 0.25	6.25 $\pm$ 0.25	10.25 $\pm$ 0.25	>14.00

Results indicate mean $\pm$ S.E., $n = 6$, (+ day of secondary challenge, 1 µg ovalbumin plus 1000 µg carrageenan) PCA index refers to the area of dye extravasation in mm². Agglutinating antibody response is presented as the log₂ dilution of the end point titre.

Table 2. Effect of administering carrageenan prior to or simultaneously with ovalbumin on the day 21 reaginic and agglutinating anti-ovalbumin antibody-responses

Carrageenan administration*	Reaginic antibody response (PCA index)	Agglutinating antibody response (end point titre)
Day 0 (with antigen)	440 ± 31	7.33 ± 0.32
Day - 1	144 ± 35	2.00 ± 0.00
Day - 3	60 ± 5	2.00 ± 0.00
Day - 7	143 ± 32	4.66 ± 0.87
Not administered	No response	4.25 ± 0.70

*1000 µg carrageenan was administered i.p. simultaneously with, 1 day, 3 days or 7 days prior to 10 µg ovalbumin. Animals receiving antigen alone served as negative controls. Results indicate mean ± S.E. based on five animals. The reaginic antibody response is presented as the mean PCA index (area of dye extravasation mm²). The agglutinating antibody response is presented as the mean end point titre expressed as the log₂ dilution.

Statistics

Where appropriate, data were analysed using Student's *t*-test, values of *P*>0.05 being taken as significant.

RESULTS

Effect of carrageenan on the anti-ovalbumin response

The first series of experiments were designed to elucidate the nature and kinetics of the antibody response in rats injected intraperitoneally with 1 µg ovalbumin or 1 µg ovalbumin admixed with 1000 µg carrageenan.

The injection of 1 µg antigen alone produced a low level serum anti-ovalbumin response (Fig. 1a) without eliciting the production of reaginic antibody (Fig. 1b). The injection of ovalbumin in association with carrageenan produced an enhanced anti-ovalbumin agglutinin response and simultaneously elicited *de novo* reaginic anti-ovalbumin antibody production. The agglutinin response increased gradually over the 28 day test period (Fig. 1a) whereas the reaginic antibody response presented a characteristic profile with maximal reactivity observed at 21 days, followed by a decline in antibody titre which approached extinction within 60 days (Figs 1b and 2).

Once rats were primed, a secondary reaginic antibody response could be elicited by a second injection of ovalbumin/carrageenan (Fig. 2). This effect occurred only in animals primed with the ovalbumin/carrageenan complex, since rats initially immunised with alum-precipitated ovalbumin (1 µg ovalbumin/1000 µg alum) failed to produce a significant secondary reaginic antibody response on secondary challenge with the same preparation.

Analysis of the anti-ovalbumin response induced by the ovalbumin-carrageenan complex

Isotype analysis of the test serum revealed the presence of both IgG and IgE anti-ovalbumin antibodies (Fig. 3). The IgG anti-ovalbumin response profile resembled the total anti-ovalbumin agglutinin profile (Fig. 1a) both in the time of initiation and the kinetics of the reaction. The IgE anti-ovalbumin response, although of lower titre than the IgG response, presented a similar profile. Despite quantitative differences between the methods of assessment, comparison of the total IgE anti-ovalbumin profile (Fig. 3) with that of the reaginic antibody profile (Fig. 1b) suggests that non-reaginic IgE anti-ovalbumin antibodies are also produced during this response.

Analysis of the conditions governing the magnitude of the anti-ovalbumin response.

Further experiments were designed to investigate the effects of varying the ratio of carrageenan to ovalbumin in the injection mixture on the magnitude and nature of the anti-ovalbumin antibody response. It was clear that reaginic antibody production depended on the presence of carrageenan, with increasing amounts of carrageenan favouring greater responses (Fig. 4b). The low level cut off point for reaginic antibody production was 1–10 µg carrageenan per 1 µg ovalbumin.

The appearance of agglutinating anti-ovalbumin antibodies followed a similar dose dependent progression with increasing amounts of carrageenan causing increasing levels of antibody production (Fig. 4a).

In the second part of this study, groups of animals received graded amounts of ovalbumin (0.1–1000 µg) either alone or in combination with 1000 µg carrageenan. Animals were injected intraperitoneally

and the antibody response monitored weekly, a further injection consisting of 1 µg ovalbumin admixed with 1000 µg carrageenan was given on day 63.

The injection of ovalbumin alone at doses in the range 0.1–1000 µg failed to initiate a reaginic antibody response (Table 1). The injection of the ovalbumin in association with carrageenan however induced reaginic antibody production at the 1 µg, 10 µg and 100 µg dose level. Reaginic antibodies were not produced when the ovalbumin component of the mixture was reduced to 0.1 µg or increased to 1000 µg. The second injection (1 µg ovalbumin plus 1000 µg carrageenan on day 63) initiated a secondary reaginic antibody response, the magnitude of which appeared to be influenced by initial priming dose of ovalbumin. An increased response occurred in all groups except those initially primed with 1000 µg of ovalbumin plus 1000 µg carrageenan. It was also apparent that exposure to antigen alone prior to the day 63 ovalbumin-carrageenan injection clearly modified the later phase of the reaginic antibody response. Animals previously injected with 0.1, 1.0 or 1000 µg ovalbumin failed to produce reaginic antibody following the day 63 challenge, whereas animals receiving 10 or 100 µg ovalbumin initiated a weak reaginic antibody response.

Examination of the agglutinating anti-ovalbumin antibody response revealed that the injection of increasing amounts of ovalbumin up to 100 µg produced a dose dependent increase in the serum level of agglutinating antibody. The administration of 1000 µg ovalbumin proved to be in excess of that required for optimal stimulation. The addition of 1000 µg carrageenan to the immunisation mixture resulted in increased anti-ovalbumin production at all dose levels of ovalbumin greater than 0.1 µg. The day 63 secondary challenge was followed by an increased serum level of antigen specific antibody in all cases.

Effect of time of carrageenan administration relative to the time of antigen challenge

The final series of experiments examined the effect of administering carrageenan at various times prior to ovalbumin. Groups of rats received 1000 µg carrageenan intraperitoneally, 7, 3, or 1 day prior to the i.p. injection of 10 µg ovalbumin. Animals receiving ovalbumin and carrageenan simultaneously (day 0) served as positive controls, animals injected with ovalbumin alone served as negative controls.

Table 2 shows the reaginic and agglutinating anti-ovalbumin antibody titres on day 21. Maximum

antibody titres were observed in animals injected with carrageenan and ovalbumin simultaneously (day 0). A graded decrease in reaginic antibody titre occurred in rats injected with carrageenan 1 day prior (32% of the control) or 3 days prior (13% of the control) to ovalbumin administration. Some degree of recovery in PCA reactivity was apparent in animals injected 7 days prior to antigen. As expected the injection of ovalbumin alone failed to elicit reaginic antibody production (negative control).

The administration of carrageenan either 1 day or 3 days prior to antigen significantly suppressed the agglutinating anti-ovalbumin response ($P < 0.001$ compared to positive control, $P = 0.05 - 0.01$ compared to negative control). Animals injected with carrageenan 7 days prior to ovalbumin presented an anti-ovalbumin response within the negative control range but remained suppressed compared to the positive control response ($P = 0.01 - 0.001$).

DISCUSSION

The results of the present paper confirm that carrageenan can under specific conditions function as an efficient adjuvant and in addition demonstrate the ability of carrageenan to facilitate antigen-specific reaginic antibody production. This effect is not restricted to the rat since Mancino & Minucci (1983) obtained similar effects in mice.

The presence of both IgG and IgE class antibodies with specificity for ovalbumin demonstrated that the adjuvant properties of carrageenan are not restricted to a single immunoglobulin isotype. It was also apparent on comparison of the IgE response profile with that of the reaginic antibody profile that not all IgE class anti-ovalbumin antibodies were necessarily reaginic. This result concurs with earlier studies which demonstrate the functional heterogeneity in the binding capacity of various IgE molecules for mast cell receptors (Godfrey, Gradidge, Hawksley & Elliot, 1978; Lehrer, McCants, Farris & Bazin, 1981). Nevertheless, the reaginic antibody response was responsive to further antigenic stimulation in that the carrageenan immunisation regime (unlike the alum precipitated ovalbumin regime) primed the rats for secondary reaginic antibody production following subsequent challenge.

It is well known that the inclusion of an adjuvant into an immunisation regime normally leads to an increased capacity to produce antigen-specific IgG class antibodies. However, the effect of adjuvant on IgE antibody responses clearly depends on the

particular adjuvant used. It was apparent from the present study that alum precipitated ovalbumin although capable of inducing a primary reagenic antibody response, failed to elicit an effective secondary reagin response. Furthermore, the use of Freund's complete adjuvant in a similar situation has been reported to suppress antigen-specific IgE titres (Hamaoka, Newburger, Katz & Benacerraf, 1974) as has muramyl dipeptide (Kishimoto, Hiria, Nakanashi, Azuma, Asho & Yamamura, 1979). Carrageenan therefore appears to possess particularly interesting adjuvant properties in that it elicits both a potent primary reagenic antibody response and at the same time renders animals sensitive to secondary stimulation.

The nature and magnitude of this response was influenced by both the dose of ovalbumin administered and whether it was given mixed with carrageenan prior to the secondary challenge. Primary and secondary responses were demonstrated in all animals immunised with 1–100 μ g ovalbumin if injected in association with 1000 μ g carrageenan, animals injected with 0.1 μ g ovalbumin plus carrageenan gave no primary response, however secondary challenge elicited a reaction similar in nature and degree to the primary response, thus indicating that this dose was below the critical level of antigen required for immunisation. Animals initially primed using 1000 μ g ovalbumin and 1000 μ g carrageenan also failed to produce reagenic antibodies and on secondary challenge produced only a low level response. In this instance primary immunisation using 1000 μ g ovalbumin apparently exceeds the dose of antigen required for optimal reagenic antibody production.

The injection of graded doses of ovalbumin alone prior to challenge, was also shown to influence the nature of the subsequent antibody response. The initial treatment using doses less than or greater than 100 μ g ovalbumin blocked subsequent reagenic antibody production. At the same time ovalbumin given alone at doses up to 100 μ g produced a stepwise increase in the circulating level of non-reagenic antibody. The injection of ovalbumin at doses > 1 μ g in association with carrageenan resulted in an enhanced antibody response in all cases.

The fact that prior exposure to antigen in the absence of carrageenan blocked only the reagenic component of the anti-ovalbumin response indicates that reagenic antibody production can be subject to selective internal suppression. A similar phenomenon has been reported following successive injections of antigen into antigen primed mice (Maia, Vaz & Vaz 1974). Ishizaka, Okudaira & King (1975)

also demonstrated that the administration of urea-denatured ovalbumin would selectively suppress the anti-ovalbumin IgE response in mice. Various immunisation regimes can induce suppressor T cells which are capable of precisely regulating reagenic antibody responses (Ishizaka, Yodoi, Suemura & Hirashima 1983). Clearly a similar phenomenon could be operative in the system presented above. At the same time it is possible that IgG mediated antibody feedback control may also be involved. It has been demonstrated that reagenic antibody production in several species can be modulated by an IgG antibody response directed against the same antigen (Stranegard & Belin, 1970; Tada & Okumura, 1972; Ishizaka & Okudaira, 1972). The decline in reagenic anti-ovalbumin antibody levels following secondary challenge and the blocking effect mediated by the injection of ovalbumin prior to ovalbumin-carrageenan challenged could therefore be due to the inhibitory effect of an IgG anti-ovalbumin antibody on reagin secreting plasma cells.

These forms of suppression are of course not necessarily mutually exclusive, and in view of the dose dependent nature of the presented data, it is possible that the type of suppression induced is influenced by both the dose of antigen given and the conditions of exposure.

Regardless of the mechanism, the suppression phenomenon could represent a natural defense system designed to block the inappropriate initiation of reagenic antibody production and thereby prevent a potentially damaging hypersensitivity state becoming established. Nevertheless, it is clear that the suppression mechanism(s) can be by-passed if antigen is presented mixed with carrageenan and low levels of reagenic antibody could be induced even when the carrageenan was administered 1, 3 or 7 days prior to antigen. Thus carrageenan does not necessarily have to function as an antigen carrier in order to promote reagenic antibody production. Under the same conditions however, it was apparent that the non-reagenic antibody response was significantly suppressed if the carrageenan was given 1 or 3 days prior to antigen administration. The capacity of carrageenan to suppress conventional antibody responses when given prior to antigen has been reported by many authors (see Introduction) and in the main has been attributed to the selective toxic effects of carrageenans on antigen processing macrophages. If macrophage toxicity is accepted as a possible explanation for the depressed non-reagenic antibody response then we must assume that the reagenic antibody response is less dependent on the

macrophage for antigen processing. It is at the same time clear from the literature that the carrageenan/macrophage interaction is more complex than a simple toxic event, recent studies for example demonstrate that carrageenan treated macrophages can modify immune responses by the release of soluble mediators, which depending on culture conditions either enhance (Eveleigh, Clark & McCandless, 1982) or suppress (Rumjanek, Watson & Sljivic, 1977) *in vitro* antibody responses. It is clearly of great importance to determine whether similar factors are produced *in vivo*, since the production of immunoregulatory factors at appropriate phases of an immune response could be directive in determining the type of response produced.

A more detailed knowledge of the interaction between carrageenan and the various components of

the immune response is obviously central to our understanding of the complex interactions demonstrated in the present report. However, although it is clear that carrageenan can, under defined conditions, function as an efficient adjuvant for reagenic antibody production, the precise inter-relationships that occur between carrageenan and the natural regulatory mechanisms within this specific immune response remain to be determined.

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