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Antigenic competition in IgE antibody production

I. ESTABLISHMENT OF PARAMETERS INVOLVED IN PRIMARY AND SECONDARY RESPONSES

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Summary. Antigenic competition was demonstrated in IgE antibody response in mice immunized with ovalbumin or DNP-ovalbumin associated with several non-related proteins: DNP-Ascaris, DNP-keyhole limpet haemocyanin or Ascaris. Simultaneous injection of two antigens caused a suppression of IgE antibody production to the test antigen, IgG1 antibody formation being only diminished under certain conditions. Competition was dose-dependent and effective only in the primary response. However, the secondary response could be also partially suppressed if the competitor antigen was given in both first and second antigenic stimulation. Competition was abolished by irradiation prior to immunization.

INTRODUCTION

The peculiar regulation of IgE antibody production has been noticed over the last few years by us (Macedo, Braga & Mota, 1976; Macedo & Catty, 1977) and other investigators (Tada, 1975; Ishizaka, 1976; Katz, 1978). During such studies we demonstrated an enhancing effect of X-irradiation on the IgE response in mice immunized with DNP-Ascaris, but not on IgG1. The results of other treatments indicated also that these two classes of antibodies are probably under the control of separate mechanisms. In order to understand such mechanisms better, other antigens and experimental protocols were used, which led us to the observation that antigenic competition in the IgE antibody production could be obtained when a mixture of two non-related proteins were injected in the animals.

Antigenic competition or non-specific antigen-induced suppression has been extensively investigated in relation to IgG and IgM antibody formation and cellular immunity (reviewed in Pross & Eidinger, 1974), but nothing was reported about IgE antibody formation.

Thus, a study of the parameters involved in this phenomenon was carried out in the present work, comparing the effects of antigenic competition on the IgE and IgG1 antibody formation. Some of these results have been previously published (Macedo, 1979).

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MATERIAL AND METHODS

Animals

(a) Six- to eight-week-old mice of the inbred strain A/Sn, of either sex, were used for immunization. (b) Four- to six-week-old A/Sn mice and Lou/M rats,

weighing between 120 and 150 g, of either sex, were used for skin tests.

Antigens

Ascaris suum extract (Asc) was prepared according to a slight modification of the method of Strejan & Campbell (1967). Live Ascaris, obtained from the intestine of pigs, were washed several times with saline, mixed with an equal volume of borate-buffered saline (BBS) pH 8·0 and homogenized in an Ultra-Turrax apparatus (Janke and Kunkel, Staufen, Germany). After centrifugation at 10,000 r.p.m. for 1 h the precipitate was resuspended in 400 ml of BBS and stirred overnight at 4°. This suspension was centrifuged again and the supernatant dialysed against distilled water overnight at 4°. The clear supernatant finally obtained after centrifugation at 10,000 r.p.m. for 2 h was lyophylized in aliquots. Dinitrophenylated Ascaris (DNP-Asc), dinitrophenylated bovine serum albumin (DNP-BSA) and dinitrophenylated ovalbumin (DNP-Oa) were prepared as described by Eisen, Belman & Carsten (1953). Dinitrophenylated keyhole limpet haemocyanin (DNP-KLH) and crystalline ovalbumin (Oa) were purchased from Calbiochem, Los Angeles, Ca. The hapten–protein conjugates contained $5{\cdot}4 \times 10^{-4}$ mol DNP/mg Ascaris, 62 mol DNP/10^5 mol. wt units of KLH, 23 mol DNP/mol BSA and 3·9 or 7·2 mol DNP/mol Oa.

Irradiation

Whole-body irradiation was performed in a Stabilipan Siemens X-ray machine under the following physical conditions: 120 kV, 20 mA, focal distance 50 cm, 0·2 mm copper filter, 53 rad/min. The animals received a total dose of 400 rad 1 day before immunization and were treated with antibiotics [1 g terramycin (Pfizer) per litre of drinking water] from 3 days before irradiation until the end of the experiment.

Immunization

Groups of eight to ten animals were immunized with the following. (a) Oa: the control group was injected intraperitoneally (i.p.) with 500μg Oa whereas the experimental groups received the same dose of Oa mixed with 500 μg of DNP-Asc, DNP-KLH or Asc. All groups received in addition 7·5 mg aluminium hydroxide [$Al(OH)_3$] as an adjuvant. Five days after the primary immunization, all the animals were injected with 100 μg Oa i.p. and the experimental groups received in addition 100 μg DNP-Asc, DNP-KLH or Asc. Two other groups were immunized with a low dose of Oa. One was injected with 50 μg Oa in the same amount of adjuvant whereas the other received the same amount of Oa plus 50 μg Asc. Thirty days later, one group received a boost of 1 μg Oa and the other 1 μg Oa plus 1 μg Asc i.p. (b) DNP-Oa: all the groups were injected with 50 μg DNP-Oa in 7·5 mg of $Al(OH)_3$ i.p., 30 days later they received 1 μg of DNP-Oa i.p. In some groups, 10, 50 or 250 μg Asc were mixed with DNP-Oa in the first injection. Other groups received 10 or 100 μg Asc with or without 7·5 mg adjuvant mixed with DNP-Oa in the second injection. All the groups were bled with a week or 10 day interval in the primary response and 7 days after the booster. The blood was collected in a graduated, heparinized Pasteur pipette and diluted immediately 1:5 with cold saline. Because plasma samples were routinely pooled in each group of mice to provide sufficient material for estimation, the variation in response between individuals was not determined. The pools were tested for homocytotropic antibody by passive cutaneous anaphylactic reaction. When a mixture of two antigens was used for immunization, the pool was tested for both antibodies.

Passive cutaneous anaphylaxis

Passive cutaneous anaphylactic (PCA) reactions in mice were performed according to the technique described by Ovary (1958). For the estimation of IgG1 antibodies, aliquots of pooled plasma were inactivated at 56° for 1 h to destroy any activity due to IgE antibodies. The animals were shaved on the back and two or three intradermal injections (0·05 ml) of different dilutions of pooled plasma were given on each side of the dorsal skin. Two hours later, they were challenged intravenously with 0·5 ml of 0·25% Evans blue solution in 0·15 M NaCl containing 0·25 mg antigen. For the titration of anti-DNP antibodies, a conjugate of hapten on heterologous carrier (BSA) was used as antigen. It has been shown that PCA reactions with a sensitization period of 24 h induced in rat skin with mouse antiserum is due to mouse IgE (Mota & Wong, 1969). Thus mouse IgE was assayed by PCA reaction in rat skin using a similar technique as described for the mouse. The animals received four to eight intradermal injections (0·1 ml) in each side of the back and were challenged 24 h later with 1 ml of 0·25% Evans blue in saline containing 0·5 mg antigen. Twenty to thirty minutes after challenge, mice and rats were killed and lesion diameters read on the inverted skin. Tests in quadruplicate and triplicate were done on mice and rats respectively. The PCA titre was

expressed as the reciprocal of the highest dilution that gave a lesion of more than 5 mm in diameter. Dilutions of samples to be compared were injected into the same animals. Since the variation of the titre for the same sample was two-fold or less, only differences of two or more than two-fold dilutions were considered significant.

RESULTS

Antigenic competition in simultaneous injection of ovalbumin and DNP-Ascaris

In our experience, immunization of A/Sn mice with high doses of DNP-Asc or Oa results in quite different IgE antibody levels, low against the former and high against the latter. It was interesting, therefore, to see what could result from the simultaneous injection of these two antigens.

Thus, three groups of mice were immunized with 500 μg Oa, 500 μg DNP-Asc or a mixture of the same amounts of both antigens. Five days later, they received 100 μg of either Oa, DNP-Asc or of both antigens. The kinetics of homocytotropic antibody response of these groups can be compared in Fig. 1. The IgE a-Oa antibody response of the group injected with the mixture of antigens was four to sixteen times lower than the group injected with Oa alone. The IgE a-DNP antibody response, however, was the same when compared to the control immunized only with DNP-Ascaris. The suppression of anti-Oa response was also restricted to the IgE class. The IgG1 a-Oa and a-DNP antibody responses were not altered significantly.

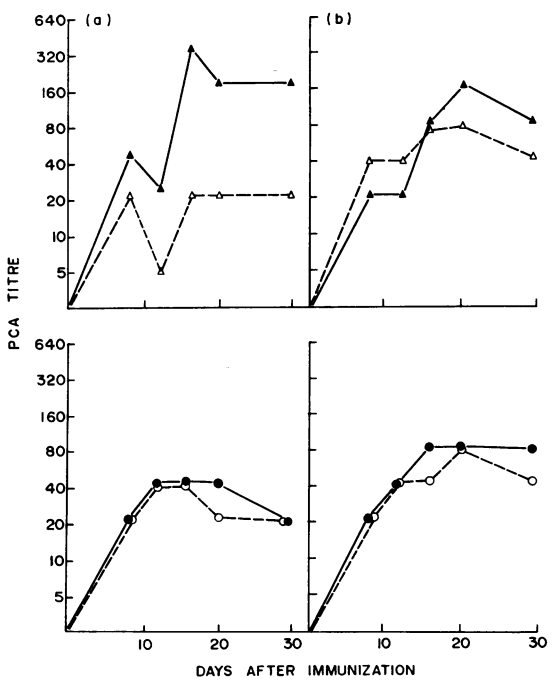

Figure 1. Antigenic competition between Oa and DNP-Asc. Homocytotropic antibody production in mice immunized with 500 μg Oa (▲), 500 μg DNP-Asc (●) or a mixture of 500 μg Oa plus 500 μg DNP-Asc: (△) a-Oa and (○) a-DNP antibody response. PCA reactions elicited with Oa or DNP-BSA. (a) IgE; (b) IgG1.

Antigenic competition between ovalbumin and other non-related proteins

In order to verify if other proteins would also compete with ovalbumin in the induction of an IgE antibody response, three groups of mice were immunized with Oa mixed with either DNP-Asc, DNP-KLH or Asc in the same proportion and their anti-Oa antibody response compared to that of a control group injected with Oa alone. The production of specific IgE and IgG1 a-Oa antibodies by these groups at various times is shown in Fig. 2. All the proteins used induced a marked suppression of IgE antibody formation, but only the group injected with Ascaris showed a significant decrease in IgG1 antibody response over the 30 days (32 ×).

Effect of X irradiation prior to immunization

We have shown previously (Macedo *et al.*, 1976;

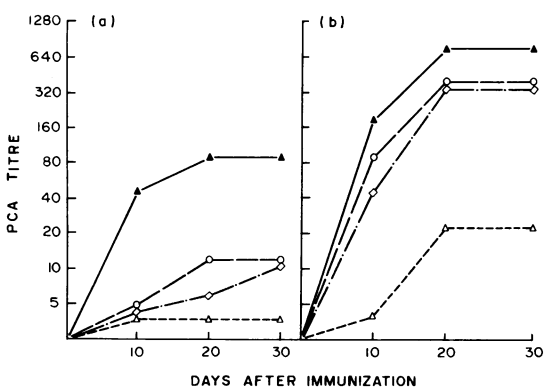

Figure 2. Antigenic competition induced with different competitor antigens. Homocytotropic anti-Oa antibody responses in mice after immunization with 500 μg Oa in adjuvant alone (▲) or with 500 μg DNP-Asc (○), DNP-KLH (◇) or Asc (△). (a) IgE; (b) IgG1.

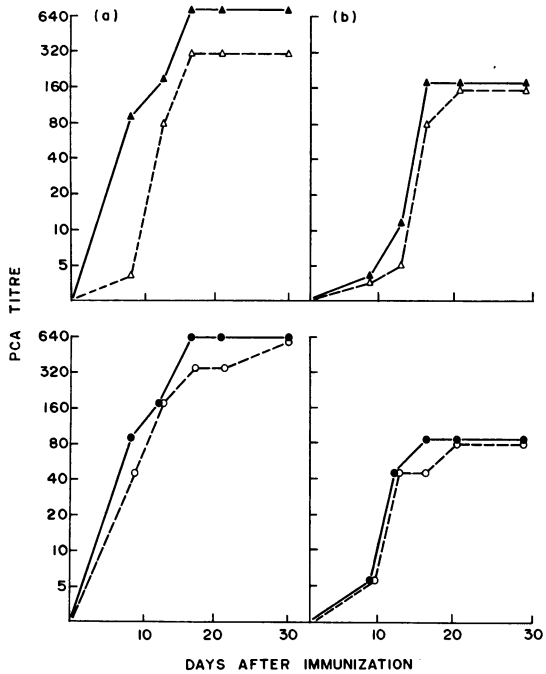

Figure 3. Effect of irradiation on antigenic competition. IgE (a) and IgG1 (b) antibody production in mice irradiated with 400 rad 24 h before immunization with 500 μg Oa (▲), 500 μg DNP-Asc (●) or equal amounts of both antigens: (△) a-Oa and (○) a-DNP response. PCA reactions elicited with Oa or DNP-BSA.

Macedo & Catty, 1977) that irradiation prior to immunization enhances the IgE antibody response and partially suppresses the IgG1 antibody formation. Therefore, a group of mice was irradiated with 400 rad 24 h before immunization with a mixture of Oa and DNP-Asc. Two other irradiated groups were immunized with each antigen alone. As expected, irradiation caused an enhancement of IgE a-Oa and a-DNP antibody production in the control groups (Fig. 1 *vs* Fig. 3) and a suppression of IgG1 in the initial phase of the response. On the other hand, the suppression of IgE a-Oa due to antigenic competition observed in the group injected with the two antigens (Fig. 1) was abolished by this treatment and both responses (a-Oa and a-DNP) increased in the same proportion (about 16 ×) in these animals (Fig. 3). Once again, the IgG1 antibody response was comparable to the controls.

Effect of antigenic competition on anti-hapten and anti-carrier antibody response

It is well known that low doses of antigen induce a prolonged primary and secondary homocytotropic antibody response in mice (Vaz, Vaz & Levine, 1970). At the same time, when a hapten–carrier conjugate is used as an antigen, a response against each part of the molecule can be assessed. Thus, a group of mice was immunized with a low dose of 50 μg of $DNP_{7\text{-}2}$-Oa plus 50 μg Asc and boosted after 30 days with 1 μg of each of these antigens. Another group was injected only with DNP-Oa. As can be seen in Fig. 4, the primary IgE anti-hapten (a-DNP) and anti-carrier (a-Oa) antibody responses were similarly suppressed in the group injected with both antigens. The primary IgG1 a-DNP antibody response was also depressed but no anti-Oa antibodies could be detected. The suppressive effect of competition was extended to both secondary responses too. Simultaneously an experiment was performed using a low dose of Oa and Asc and in this case also, both primary and secondary a-Oa IgE and IgG1

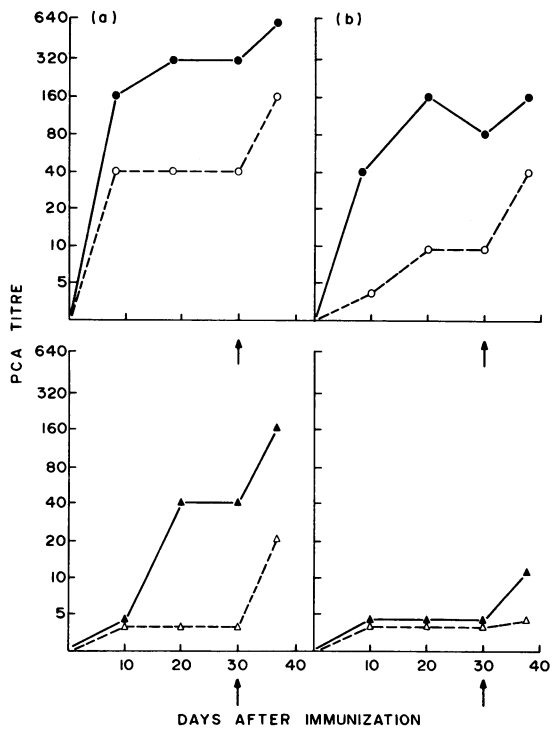

Figure 4. Effect of antigenic competition on antibody response to hapten and carrier. Homocytotropic antibody formation in mice immunized with 50 μg DNP-Oa: (●) a-DNP and (▲) a-Oa response of a group injected with DNP-Oa alone; (○) a-DNP and (△) a-Oa response of a group injected with DNP-Oa plus 50 μg Asc. Arrows represent boost of DNP-Oa or DNP-Oa + Ascaris. PCA reactions elicited with DNP-BSA or Oa. (a) IgE; (b) IgG1.

responses were dampened by competition (data not shown).

Influence of dose of competitor antigen

Up to now the two antigens were mixed in equal amounts. We decided, then, to see the dose–effect relationship using different proportions of test and competitor antigen. Four groups were immunized with 50 μg of $DNP_{3\cdot9}$-Oa plus adjuvant and 10, 50 or 250 μg of Ascaris were given to three of them. All the groups were boosted with 1 μg of DNP-Oa only on day 28. As we can see in Fig. 5, using 10 μg of Asc, i.e. five times less than DNP-Oa, there was no significant alteration of the primary response. Equal amounts (50 μg) of Asc and DNP-Oa resulted in a partial suppression, while a dose of 250 μg of Asc, five times more than DNP-Oa, abolished both anti-DNP and anti-Oa responses over the 28 days of the primary response. The secondary response, however, did not show any significant difference between the IgE response of the four groups, with a slight suppression of IgG1 in the group which received the highest dose of Ascaris. IgE anti-Ascaris antibodies were not detected in the experimental groups over a period of 35 days. Control groups that had been immunized with 10, 50 or 250 μg of Asc in 7·5 mg of adjuvant showed similar primary responses. However, when they were boosted with 5 μg Ascaris on day 35, a PCA titre of 1:80 was obtained 7 days later only in the group immunized with 250 μg, demonstrating that the animals were primed for this antigen (data not shown).

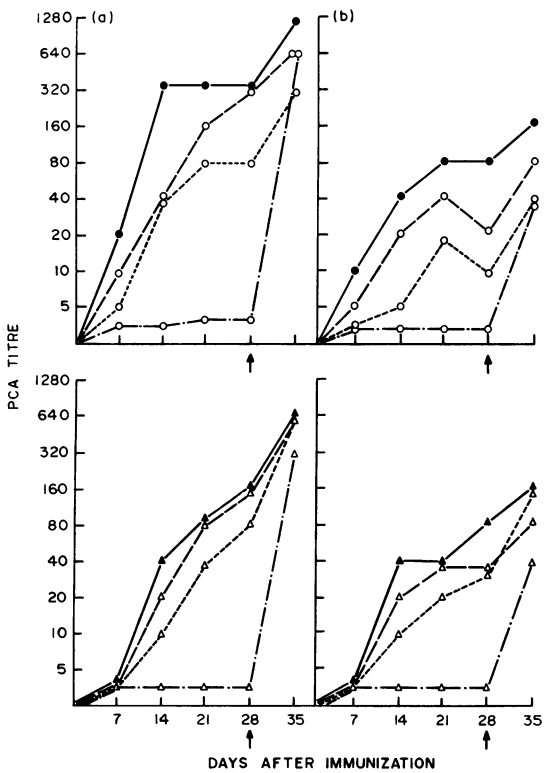

Figure 5. Homocytotropic anti-DNP and a-Oa antibody production in mice immunized with 50 μg DNP-Oa plus different doses of competitor antigen: 10 μg Asc: a-DNP (o——o) and a-Oa (Δ——Δ) response; 50 μg Asc: a-DNP (o · · · o) and a-Oa (Δ . . . . Δ) response or 250 μg Asc: a-DNP (o– · –o) and a-Oa (Δ– · –Δ) response. Control group immunized with DNP-Oa alone: a-DNP (●——●) and a-Oa (▲——▲) response. Arrows represent boost of 1 μg DNP-Oa. PCA reactions elicited with DNP-BSA or Oa. (a) IgE; (b) IgG1.

Effect of antigenic competition on the IgE secondary antibody response

To see the effect of competition on the secondary antibody response, the competitor antigen was given *only* with the boost of test antigen, 28 days after the primary immunization. Six groups of mice were immunized with DNP-Oa as described in Material and Methods and bled after 28 days. Soon after, three groups were injected with 1 μg of DNP-Oa alone or with 10 or 100 μg of Ascaris. The other three groups received the same booster included in 7·5 mg of alum. The PCA titres of these groups, 7 and 28 days after booster, are shown in Table 1. No significant difference was observed between the experimental and the control groups at any time.

DISCUSSION

Several pieces of evidence indicate that IgE antibodies are regulated by a distinct mechanism, probably mediated by class-specific suppressor T cells, through the release of soluble antigen-specific or non-specific factors (Okumura & Tada, 1971; Tada, Okumura & Taniguchi, 1973; Takatsu & Ishizaka, 1976; Suemura, Kishimoto, Hirai & Yamamura, 1977). During our own studies on the regulatory mechanism of IgE antibody production we came across the phenomenon of antigenic competition in IgE antibody formation, which has not yet been reported in the literature. It seemed, therefore, interesting to try to correlate the mechanisms involved in such a phenomenon with the

Table 1. Effect of antigenic competition on the IgE secondary antibody response

Groups*	Booster†	PCA titre‡ a-DNP Day 7§	a-DNP Day 28	a-Oa Day 7	a-Oa Day 28
I	DNP-Oa	640	320	640	640
II	DNP-Oa + 10 μg Asc	640	640	640	1280
III	DNP-Oa + 100 μg Asc	640	640	640	640
IV	DNP-Oa + alum	1280	640	1280	640
V	DNP-Oa + 10 μg Asc + alum	1280	640	1280	1280
VI	DNP-Oa + 100 μg Asc + alum	1280	1280	1280	1280

* Groups of eight mice immunized with 50 μg of DNP-Oa in 7·5 mg of $Al(OH)_3$ i.p. on day 0.

† Booster of 1 μg of DNP-Oa plus the respective dose of Asc in saline or 7·5 mg of $Al(OH)_3$ i.p. on day 28; at this time the PCA titres of anti-DNP and anti-Oa antibody were 1:320 and 1:160, respectively.

‡ Reciprocal of PCA titres after the injection of DNP-BSA or Oa.

§ Days after booster.

information already existent in the field of regulation of this antibody class.

The preliminary experiments which consisted of the simultaneous injection of equal amounts of two non-related proteins (ovalbumin and DNP-Ascaris) led to the conclusion that competition was established only at the level of IgE anti-ovalbumin antibody production. This response was also markedly suppressed by other antigens (Ascaris and DNP-KLH), indicating its susceptibility to regulation by some non-specific mechanism stimulated by these substances. The suppression, however, could be abolished by irradiation prior to immunization. The enhancement of IgE antibody production by X-irradiation has been previously reported by us (Macedo *et al.*, 1976; Macedo & Catty, 1977) and others (Tada, Taniguchi & Okumura, 1971; Watanabe, Kojima & Ovary, 1976; Chiorazzi, Fox & Katz, 1976). This effect is probably due to elimination of class-specific suppressor T cells. In our system, the mechanism involved in competition was similarly radiosensitive and the irradiated animals injected with both antigens showed the same enhancement of IgE antibody production as the irradiated controls injected with each antigen alone. It is interesting to notice that the simultaneous injection of antigens has not always led to antigenic competition in other systems. McArthur, Siskind & Torbecke (1974) observed a decrease in the number of plaque-forming cells against sheep erythrocytes when they were injected 2 days after burro erythrocytes or Brucella antigen, but not when injected simultaneously.

In other aspects, however, the effects of antigenic competition observed in the IgE system resembled those obtained in the production of other antibody classes (Adler, 1964; Pross & Eidinger, 1974). While effective competition could be easily demonstrated in the primary response, the same was not true for the secondary response. In fact, partial suppression of this response could only be obtained when the competitor antigen had been given in the first antigenic stimulation or, in other words, when the primary response was already diminished. On the other hand, if the competitor antigen was not administered in the booster, a typical secondary response was established after 7 days, even in the absence of detectable homocytotropic antibodies in the primary response. These results may indicate the establishment of an active suppressive mechanism dampening the final production and/or secretion of IgE antibodies, but not the priming stage. The observations made for ovalbumin as a test antigen also applied to a hapten–carrier system (DNP-Oa). In this case, competition had the same effect on both anti-hapten and anti-carrier antibody production, which could suggest a preferential action at the level of helper T cells which are common to both responses.

As expected, the degree of competition was directly related to the dose of competitor antigen. Although

some effect could be obtained with injection of both antigens in a 1:1 ratio, much better suppression was observed with the administration of five times more competitor than test antigen. However, once a normal primary response had been mounted, the competitor antigen did not have any effect on the secondary response, even if given in amounts ten- or 100-fold higher than the test antigen. This was so even when the booster was given in adjuvant.

Antigenic competition was restricted to the IgE antibody class excepting when extract of Ascaris was used as competitor antigen, in which case IgG1 antibody was also affected. In this system no antibodies against Ascaris could be detected in the primary response and therefore some relationship might exist between these two facts. It has been emphasized before (Adler, 1964) that the reduction in the response to one antigen was not a direct result of the production of antibody against the second. In fact some 'weak' antigens by the usual criteria, interfered readily with the response of 'good' antigens. Gershon & Kondo (1971) also reported that the amount of interference produced by SRBC on the subsequent response to HRBC did not correlate with the amount of anti-SRBC antibody made.

Several mechanisms have been proposed to explain antigenic competition in general (Pross & Eidinger, 1974). According to Taussig (1975) antigenic competition may be divided into: intramolecular competition, that is a 'B-cell competition' with B-cell populations of different specificities competing for antigen; and intermolecular competition, that is 'T-cell competition'. In mixtures of antigens, T cells would produce co-operative molecules of different specificities, which would compete for macrophage surface space. On the other hand, in sequential administration of antigens, T cells would produce non-specific inhibitors as regulators of the immune response.

It is difficult, however, to fit our results in any of these propositions, since it seems unlikely that irradiation would affect differently the cells with specificity for one or other antigen. Further exploration of our system, which will be reported subsequently, may provide some clarification on this point.

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