Immunology, 1972, **22**, 185.

Studies on Antigenic Competition

II. ABOLITION OF ANTIGENIC COMPETITION BY ANTIBODY AGAINST OR TOLERANCE TO THE DOMINANT ANTIGEN: A MODEL FOR ANTIGENIC COMPETITION

M. J. TAUSSIG* AND P. J. LACHMANN†

Immunology Division, Department of Pathology, Tennis Court Road, Cambridge

(*Received 1st June* 1971)

Summary. Antigenic competition between the Fc and Fab fragments of rabbit IgG in mice could be abolished by passive immunization with antiserum against the dominant antigen (Fc). The induction of tolerance to Fc also eliminated antigenic competition. Anti-Fd production, generally very poor in response to rabbit IgG, was considerably enhanced by these procedures, indicating that it is also subject to antigenic competition with Fc.

A model is proposed to explain antigenic competition which proposes that it is the 'co-operative antibody' produced to the dominant antigen which acts as an inhibitor to antibody production to the suppressed antigen by competing for sites —presumably on macrophage membranes—where co-operation occurs.

The merits and difficulties of such an explanation are discussed.

INTRODUCTION

The conditions under which antigenic competition occurs between the Fc and Fab antigens of rabbit IgG in mice have been described in a previous paper (Taussig, 1971). The experiments which are now reported investigate in more detail the importance of the specific recognition of the dominant antigen (Fc) in competition, and lead to the formulation of a general model for antigenic competition.

Several groups have demonstrated the suppression of the immune response which can be produced by passive administration of antiserum (Brody, Siskind and Walker, 1967; Cerottini, McConahey and Dixon, 1969; Pincus and Nussenzweig, 1969). The effect is determinant specific even with antigens linked together, and is probably due to the competition of antibody with cell-bound receptors, impairing antigen recognition. Passive immunization may thus be used to show whether recognition is a necessary part of dominance in antigenic competition. In the present case, passive administration of anti-Fc has been used to demonstrate the need for recognition of Fc in competition between Fc and Fab.

An alternative approach is to induce a state of tolerance to the dominant antigen and observe the effects on competition. This has so far yielded conflicting results, with some workers finding that tolerance to a dominant antigen abolishes competition (Weigle and High, 1967; Schwartz and Leskowitz, 1969) while others report that it has no effect

* Present address: Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel.
† Present address; Department of Immunology, Royal Postgraduate Medical School, Ducane Road, London, W.12.

(Schechter, 1968). The effect of tolerance to Fc on competition with Fab has therefore been investigated.

MATERIALS AND METHODS

Antigens

Rabbit IgG and the Fab′ and Fc fragments were prepared as described in a previous paper (Taussig, 1971). Light chains were prepared from rabbit γ-globulin by the method of Fleischman, Pain and Porter (1962).

Anti-Fc sera

Two methods were used to raise anti-Fc sera. The first was by hyperimmunization of BALB/c mice with rabbit IgG, followed by adsorption from the serum of anti-Fab′ with an insoluble Fab′ adsorbent. The preparation of the adsorbent was by insolubilization of Fab′ with glutaraldehyde, as described by Avrameas and Ternynck (1969). The anti-IgG serum was adsorbed until anti-Fab′ was no longer detectable by phage neutralization (Taussig, 1970).

An alternative method was by raising antibody to purified Fc, followed by adsorption of any contaminating anti-Fab′ in the same way.

The antibody content of pooled anti-Fc sera obtained by either method was determined by precipitation with rabbit IgG and adjusted to 1 mg/ml with normal mouse serum.

Passive immunization

Anti-Fc, in different dilutions in normal mouse serum, was administered intraperitoneally and was given 1 day before and 4 days after inoculation of rabbit IgG in Freund's complete adjuvant. The doses of anti-Fc serum administered were such as to contain 1, 10, 25, 100 or 250 μg of anti-Fc antibody. The dose of rabbit IgG used for immunization was 25 μg. Thus the ratio of anti-Fc:IgG varied from 1:25 to 10:1 in different groups. Sera were taken at 13, 17 and 21 days after inoculation of rabbit IgG.

Induction of tolerance to Fc

The method used is similar to that described by Dresser (1962) for induction of tolerance to bovine γ-globulin in mice. Purified Fc was centrifuged (20,000 **g** for 30 minutes) to remove particulate matter. A single injection of 50 μg particle-free Fc was given, intraperitoneally. Mice were challenged after 2 weeks with rabbit IgG in adjuvant. Only sera from animals in which the anti-Fc response was shown to be significantly depressed were titrated for anti-Fab.

Assay methods

Titrations for anti-Fab′ and anti-Fc were carried out as described in the preceding paper (Taussig, 1971).

In addition, anti-Fd activity was assayed by agglutination of Fab′-coated sheep erythrocytes in the presence of rabbit light chains (concentration 1 mg/ml) to inhibit agglutination by anti-light chain. Residual agglutinating antibody was assumed to be anti-Fd.

RESULTS

THE EFFECT OF ANTI-FC ON ANTIGENIC COMPETITION BETWEEN FAB′ AND FC

Table 1 and Fig. 1 show a striking effect of passive immunization with anti-Fc on the

TABLE 1

THE EFFECT OF ANTI-Fc ON ANTIGENIC COMPETITION BETWEEN Fc AND Fab'

Immunogen	Day	Anti-Fc dose (μg antibody)					
		0	1	10	25	100	250
IgG	13	2·0 (0·5)	2·4 (0·5)	4·6 (1·0)	5·0 (0·5)	4·8 (1·2)	5·3 (0·5)
	17	2·6 (0·9)	3·0 (0·7)	5·8 (0·7)	5·2 (0·7)	5·4 (0·6)	8·0 (1·0)
	21	5·4 (0·5)	4·8 (0·5)	6·8 (1·2)	6·6 (0·8)	6·6 (0·5)	7·6 (0·8)
$F(ab')_2$	13	7·0 (1·0)	7·2 (0·5)	n.d.	n.d.	n.d.	7·6 (0·7)
	17	8·0 (0·9)	8·1 (0·5)	n.d.	n.d.	n.d.	8·3 (0·5)
	21	8·8 (0·5)	8·6 (1·0)	n.d.	n.d.	n.d.	9·2 (1·2)

Anti-Fab' titres of sera of BALB/c mice preimmunized passively with different doses of anti-Fc serum, and immunized with rabbit IgG or $F(ab')_2$ in Freund's complete adjuvant. Log_2 agglutination titres, arithmetic means of 10, with standard deviation in parentheses.

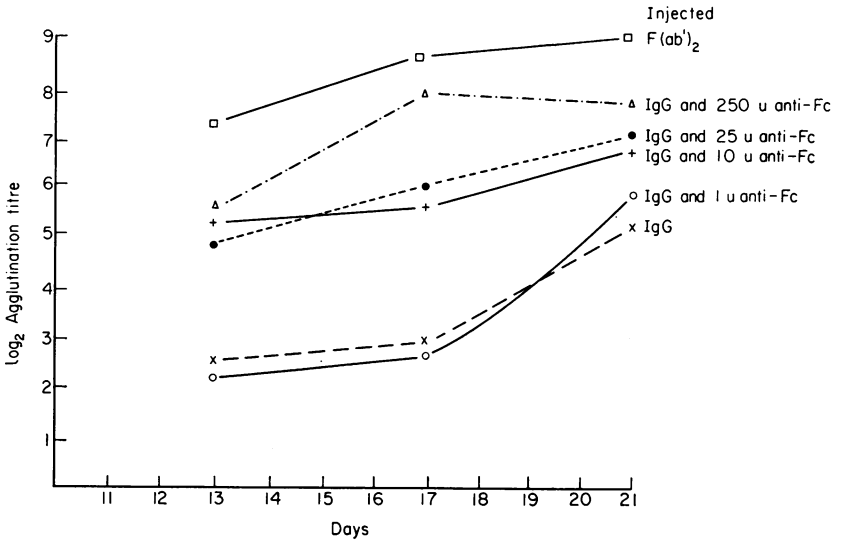

FIG. 1. Anti-Fab' titres (log_2 agglutination titres) of sera of BALB/c mice immunized with rabbit IgG and different amounts of mouse anti-Fc serum, or with rabbit $F(ab')_2$. 1 u anti-Fc serum contained 1 μg anti-Fc antibody.

anti-Fab' response in mice immunized with rabbit IgG. Administration of even small doses of anti-Fc sera (e.g. containing 10 μg anti-Fc antibody) led to significant enhancement of the anti-Fab' titres over the period in which antigenic competition is usually observed. The effect is seen to increase with the dose of anti-Fc used. There was very little difference between the anti-Fab' response of mice which had received $F(ab')_2$ in adjuvant and those receiving IgG and the highest dose of anti-Fc serum (250 μg anti-Fc antibody). Antigenic competition is thus completely abrogated with this level of anti-Fc.

The possibility that these results were simply an enhancement effect induced perhaps by very small amounts of anti-Fab' contaminating the anti-Fc sera, has been ruled out. Control groups which were given anti-Fab' in a wide range of dosage showed no enhancement of the anti-Fab' response on immunization with IgG. Nor did anti-Fc sera increase the anti-Fab' response in mice immunized with $F(ab')_2$ (Table 1).

TABLE 2

THE EFFECT OF ANTI-Fc ON ANTIGENIC COMPETITION BETWEEN Fab AND Fc

Day	Anti-Fc	
	0	250 μg
13	2·3 (±0·6)	6·2 (±0·8)
17	3·1 (±0·8)	7·7 (±0·9)
21	6·1 (±0·9)	9·2 (±0·8)

Anti-Fab′ titres of BALB/c mice preimmunized passively with anti-Fc serum containing 250 μg anti-Fc antibody, and then injected on day 0 with a mixture of Fc and $F(ab')_2$ (molar ratio 6:1, 60 μg Fc with 20 μg $F(ab')_2$) in Freund's complete adjuvant. Titres are $\log_2$ agglutination titres, arithmetic means of 10, ± standard deviation.

A very similar result was obtained with competition between the separate Fc and $F(ab')_2$ fragments, administered as a mixture at a molar ratio of Fc:$F(ab')_2$ of 6:1 (60 μg Fc mixed with 20 μg $F(ab')_2$). Table 2 shows that anti-Fc serum again abolished competition.

The effect of anti-Fc administration on the response to Fc itself was also examined and it was found that there was no significant reduction in the anti-Fc titres. Anti-Fc is thus apparently able to exert its effect on antigenic competition without involving suppression of anti-Fc production.

Anti-Fab response in mice tolerant to rabbit Fc

50 μg centrifuged rabbit Fc was effective in inducing tolerance in many of the mice tested. The anti-Fab′ titres of sera from such mice in response to rabbit IgG are shown in Table 3 and Fig. 2. It will be seen that the effect of antigenic competition is absent in such animals. The anti-Fab′ response resembles that in animals injected with $F(ab')_2$ in the size of titres and the rate of appearance. Tolerance to Fc thus prevents it from acting in antigenic competition.

TABLE 3

ANTIGENIC COMPETITION IN TOLERANT ANIMALS

Day	Anti-Fab′ titre	
	Normal	Fc-tolerant
10	1·6 (±0·8)	3·4 (±0·6)
13	2·3 (±1·0)	4·8 (±0·8)
15	2·5 (±0·7)	6·4 (±0·9)
17	3·1 (±1·1)	7·3 (±0·8)
20	4·6 (±0·8)	8·4 (±1·0)
22	6·9 (±1·2)	7·9 (±0·9)
26	8·3 (±1·0)	8·5 (±1·1)

Anti-Fab′ titres of sera of normal BALB/c mice and mice tolerant to rabbit Fc, after immunization on day 0 with rabbit IgG. Log_2 agglutination titres, ±S.D.

A further group of Fc tolerant mice received an otherwise competitive mixture of Fc: $F(ab')_2$ (5:1). Competition was not observed in tolerant animals.

Antigenic competition and the anti-Fd response

It was of interest to determine whether the anti-Fab antibodies made in response to IgG or $F(ab')_2$ were directed against both light chain and Fd determinants, or whether they were restricted to one of these. Heterologous γ-globulin is known to provoke in general a very poor anti-Fd response.

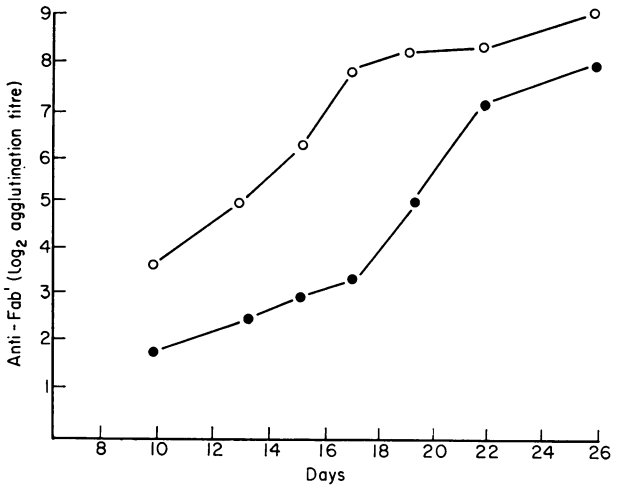

FIG. 2. Anti-Fab' response in normal mice (●) and mice tolerant to rabbit Fc (○) after inoculation of rabbit IgG (in adjuvant) on day 0. Tolerance induced with 50 μg Fc.

TABLE 4

ANTI-Fab' AND ANTI-Fd TITRES, EXPRESSED AS LOG_2 AGGLUTINATION TITRES (±S.D.), OF SERA OF BALB/c MICE IMMUNIZED WITH RABBIT IgG OR RABBIT $F(ab')_2$, TITRES MEANS OF 10

Immunogen	Response	Day	Anti-Fab'	Anti-Fd
IgG	Primary	21	6·3 (±0·5)	2·3 (±0·8)
		28	7·1 (±0·71)	3·9 (±0·5)
	Secondary	14	10·3 (±0·5)	5·6 (±0·7)
$F(ab')_2$	Primary	12	7·4 (±0·9)	6·5 (±0·7)
	Secondary	14	11·5 (±0·5)	10·8 (±0·5)

The anti-Fd activities of primary and secondary antisera raised to IgG or $F(ab')_2$ are given in Table 4. The anti-Fd response to rabbit IgG is indeed very small and constituted no more than 10 per cent of the total anti-Fab response obtained. Even in the secondary response to IgG, the anti-Fd titre was very low. With $F(ab')_2$ as antigen, however, the antisera could be only slightly inhibited by free light chains and must therefore have contained a considerable proportion of antibodies directed against Fd.

A possible explanation for this is that for structural reasons in the whole IgG molecule

Fd is inaccessible to cellular receptors, but becomes more available, and thus more immunogenic after enzymatic digestion. On the other hand, the lack of anti-Fd response could be the result of antigenic competition, more potent here than against the light chain. In this case, conditions under which antigenic competition is prevented should stimulate the expression of Fd as antigen.

Table 5 shows the effect on the anti-Fd response, to IgG as antigen, of passive immunization with anti-Fc and tolerance to Fc, conditions shown above to abolish antigenic competition. It will be seen that with low doses of anti-Fc serum (containing 10 μg and 25 μg anti-Fc antibody) the anti-Fab' agglutinating activity of sera could be almost completely inhibited by rabbit light chains. However, with the highest dose of anti-Fc (250 μg), anti-Fd was now present and the sera resembled those obtained when Fab' itself was the immunogen. Furthermore, in mice tolerant to Fc, the sera produced in response to IgG contained a high proportion of anti-Fd.

Thus both anti-Fc serum and Fc tolerance, have the effect of 'releasing' the Fd determinants and allowing the expression of their immunogenicity. The absence of an anti-Fd response to rabbit IgG in normal mice therefore seems to be the result of antigenic competition with Fc.

TABLE 5

ANTI-Fab' AND ANTI-Fd TITRES OF SERA OF BALB/c MICE EITHER PRE-IMMUNIZED WITH MOUSE ANTI-RABBIT Fc, OR TOLERANT TO RABBIT Fc, AND IMMUNIZED ON DAY 0 WITH RABBIT IgG IN FREUND'S COMPLETE ADJUVANT

Treatment of mice		Anti-Fab'	Anti-Fd
(a) Dose anti-Fc antibody (μg)	0	5·4 (±0·5)	1·2 (±0·6)
	10	6·8 (±1·2)	1·3 (±0·6)
	25	6·6 (±0·8)	0·8 (±0·5)
	250	7·6 (±0·8)	6·4 (±0·8)
(b) Tolerant to Fc		8·2 (±1·0)	7·1 (±1·0)

Sera taken after 21 days. Titres as arithmetic means of $\log_2$ agglutination titres, ±S.D.

DISCUSSION

The first group of results in this paper show that it is possible to abolish the effects of antigenic competition with antibody directed against the dominant antigen. A significant effect could be achieved with very small amounts of anti-Fc antibody, the limit in the present case being with a molar ratio of anti-Fc:IgG of 1:2·5. It should be noted that simultaneous suppression of the anti-Fc response was not achieved with any of the anti-Fc doses used; it is possible that larger amounts of anti-Fc would achieve suppression of the anti-Fc response with even greater anti-Fab levels. However, the fact that anti-Fc production is not inhibited shows that antibody production to the two antigens can, in these circumstances, occur simultaneously. This tends to rule out competition for a non-specific factor, such as lymphoid space, supply of metabolites, etc., as an explanation for antigenic competition.

The experiments in which competition was abolished in animals rendered tolerant to Fc, the normally dominant antigen, lead again to the conclusion that antigen recognition is necessary for dominance in competition. It may be pointed out that in the Fc tolerant

animals the response to Fc was not completely abolished; several animals showing up to 10 per cent of the normal response. It would not be correct, however, to conclude that it was simply the smaller size of the anti-Fc response that was responsible for loss of competition, since it is well documented that the size of a response does not necessarily relate to success in competition (e.g. Ben-Efraim and Liacopoulos, 1967) and indeed it has been shown previously with this system that optimal anti-Fc and anti-Fab responses can take place together. The anti-Fab and anti-Fc responses can, in other words, vary independently. Rather, the results imply that it is the extent to which the Fc antigens are recognized that determines whether or not competition will occur. Both passive administration of anti-Fc and specific tolerance to Fc can then be considered as affecting the efficiency of recognition of Fc, even though in the former case the anti-Fc response is very little affected and in the latter it is very efficiently inhibited.

The implication of the observation that the anti-Fab and anti-Fc responses can thus be made to vary independently is that competition is the result of a phase of the response to Fc earlier than cellular proliferation or antibody production and one which can be affected by anti-Fc. If, as seems likely, anti-Fc is affecting the recognition of Fc by competing for antigen with cell receptors, the results suggest that there may be two stages to the recognition of Fc, one responsible for antigenic competition while the other leads to antibody production, the first of these being more susceptible to inhibition by anti-Fc.

A similar abrogating effect of passive antibody on antigenic competition was observed by Brody *et al.* (1967), who raised the possibility that this might be a new aspect of the control of antibody production. They pointed out that the natural antigens to which a response is normally mounted are multi-determinant, and it is very likely that antigenic competition exists between the determinants. If the first-formed antibody to the dominant antigens was not effective in preventing the spread of a pathogen, for example, it would be important to have a mechanism to 'release' antibody formation to the suppressed antigens. An effect of circulating antibody would then be to specifically inhibit antibody production to the dominant antigen, while promoting antibody formation to those determinants which were initially suppressed by antigenic competition.

The effects of anti-Fc and Fc tolerance in enhancing the anti-Fd response with IgG as immunogen, strongly suggest that antigenic competition with Fc is responsible for the poor anti-Fd production usually obtained in normal animals. Inaccessibility of the Fd determinants seems to be a factor of secondary importance only although this may act to increase further the effects of competition. These observations also raise the possibility that the lack of response to other so-called 'immunosilent' determinants, may be a result of antigenic competition. Confirmation of this awaits further investigation.

Certain inferences regarding the mechanism of competition may be drawn from these results. A favourite subject of speculation on this point is the role of 'antigen processing' and the macrophage. Brody and Siskind (1969), for example, suggest that antigen processing could be the 'rate determining step' of antibody production where competition might occur; Amkraut, Garvey and Campbell (1966) suggested a similar explanation for their results. The data given here, with Fc/Fab competition, are, however, not consistent with such a hypothesis, if 'processing' is regarded as non-specific antigen handling by macrophages after phagocytosis. Such a stage or cell would have to be missing in the tolerant animal, but all the evidence is that macrophage function is not affected by tolerance (Mitchison, 1969). A more specific event, and one which would be missing in tolerance, must be involved.

Antigenic competition seems rather to be a result of an early recognition event of the dominant antigen, which inhibits the early phases of immune induction to the suppressed antigen. This conclusion agrees with the results of similar experiments on the effect of tolerance on antigenic competition of Weigle and High (1967) and Schwartz and Leskowitz (1969); the findings of Schechter (1968), however, are at odds with this result. Schechter (1968) showed that polyphenylalanine-RSA was able to suppress the response to polyalanine-RSA even when the animals were tolerant to polyphenylalanine-RSA. There is no clear explanation for the contradiction in these results of different groups. However, tolerance can be induced in both the T cell and B cell lines and it is possible that the systems studied differed in which cell line was tolerant.

It is appropriate to consider here possible models for antigenic competition. An all-embracing explanation for this phenomenon has yet to be found, but several possible mechanisms have been proposed. The models which have been suggested are of two general types, which may be termed the 'competitive' and the 'inhibitory' hypotheses. The first of these proposes that the antigens compete with each other, either at a rate-determining 'processing' step, or at the level of cellular commitment; the inhibitory hypothesis, on the other hand, is that an immune response to the dominant antigen produces an inhibitor effective against the suppressed antigen. Both of these can be tested against the results obtained with competition between Fc and Fab.

The competitive hypothesis, in the forms in which it has been proposed by several workers, is not adequate to account for the results obtained in the system described. A common suggestion is that antigen processing is the step at which direct competition between the antigens occurs, this presumably being at the macrophage (Adler, 1964; Amkraut *et al.*, 1966; Brody *et al.*, 1967; Brody and Siskind, 1969). It has already been pointed out that in the Fc/Fab system, the evidence is against competition at a processing step, the strongest argument being that tolerance to the dominant Fc antigens prevents competition. Less conclusive, but supporting, evidence is that Fc suppresses the anti-Fab response almost as efficiently when the antigens are separated, and could thus quite probably reach different processing cells, as when linked together (Taussig, 1971). Furthermore, the efficiency of competition when the antigens are given at widely separated sitet (Taussig, 1971) is also against the hypothesis.

A second possibility as a competitive hypothesis is that competition is for the commitment of a population of pluripotential antigen-sensitive cells, a view that has been argued by Albright and Makinodan (1965), Makinodan and Albright (1966) and Schechter (1968). This explanation has been tested, and found wanting, by Radovich and Talmage (1967) and is also unattractive in view of the large body of evidence in favour of the pre-commitment of antigen-sensitive cells. In fact, the system used by Albright and Makinodan (1965) where competition was demonstrated between non-cross-reacting heterologous erythrocytes when injected at various times after each other, is probably the best evidence for an inhibitory theory of antigen competition. Radovich and Talmage (1967) have also expressed this view.

Another competitive hypothesis which has been suggested is that there is available a limiting amount of lymphoid space, essential metabolites, or other non-specific factors for which cells would be in competition once proliferation has been effected (e.g. Mitchison, 1968a). The evidence against this has been argued above.

One common observation which is in agreement with the prediction of a competitive theory is the dose dependence of antigenic competition. Thus, with the separated antigens,

either Fc and Fab or IgG and Fab, an excess (in molar terms) of Fc over free Fab was required for competition. The dose relationships for different antigens are highly variable. With the Fc/Fab system, a ratio of more than 3/1 will secure competition; with other antigens, a much greater excess may be required to produce an effect. Whether a different mechanism operates in these cases, or whether the doses reflect differences, for example, in the affinities for a competition site is difficult to judge.

The alternative to a competitive hypothesis is an inhibitory one, in which as a result of an immune response to one antigen an inhibitor would be produced to suppress the response to other antigens. An important piece of evidence favouring this is the efficacy of antigen given as the first of two in competition. Thus Radovich and Talmage (1967) found that competition was maximal when horse red cells were given (in mice) 4 days before sheep red cells and point out that this makes it unlikely that antigenic competition is, in fact, competition for anything. They suggest that a humoral feedback inhibition may result from the response to the first antigen. Similar results have been found by Möller and Sjoberg (1970). In competition between Fc and Fab, it is interesting to note that Fab, which is the suppressed antigen when the two are given simultaneously, will itself suppress the anti-Fc response if it is given alone 1–5 days before Fc (Taussig, 1971). There is, however, a difference between the two systems which is important, namely that the heterologous red cells only compete when given at different times and not when administered together, whereas Fab and Fc compete both when injected together and on different days. This may be attributable to the efficiency or the amount of inhibitor produced by different antigens, or there may possibly be a different mechanism involved in the two cases.

A further observation in support of an inhibitory theory in the Fc/Fab system is that the antigens can be given in different sites and still compete, with no greater Fc/Fab ratio required than when the antigens are given together at the same site.

If an inhibitor were playing a part in suppression by antigen competition, what would be its characteristics? A first requisite would be some specificity of action, at least in that it should inhibit the response to other antigens only. This restriction could imply either the existence of a different inhibitor for each antigen; or perhaps that a single inhibitor was produced late in the response to an antigen, but acted at an early stage of the response. As far as the point of action is concerned, it must inhibit the very early stages of response to the competing (suppressed) antigen. The evidence suggesting an early site of action is the almost complete absence of rosettes to Fab in animals in which competition was occurring (M. J. Taussig, unpublished observation) and also the absence of priming for immunological memory during competition (Weigle and High, 1967). As regards the production of the inhibitor, it should be dependent (in amount) on the antigen dose, in order to account for the dose dependence of competition. The most important feature of its production, however, would be its susceptibility to factors influencing antigen recognition, in particular its production must be inhibited by antibody against the antigen in question, and not take place at all in the state of tolerance to the antigen. In brief, an inhibitor would be made at or after the recognition of a dominant antigen, and its site of action would be at or before the recognition of the suppressed antigen.

The nature of a possible inhibitor substance at present remains a matter for speculation. Although one might postulate the production of a substance, by each antigen, of which the sole function was to inhibit the response to other antigens, it seems more likely that the inhibition is a side effect of a substance which plays a more direct role in the immune response. A reasonable supposition, therefore, is that something which plays a part in the

recognition of one antigen could act, incidentally, to inhibit the recognition of other antigens.

Since cell co-operation between bone-marrow derived (B) cells and thymus-derived (T) cells is believed to be important in the recognition process, it is interesting to consider the possibility that competition occurs at this stage. At present, the mechanism of co-operation is uncertain. It has been suggested, for example, that co-operation is required to concentrate antigen sufficiently in order to trigger B cells into proliferation and that the T cells produce a special class of 'co-operating antibody' for this purpose. This mediator of co-operation (which has been termed 'IgX') would then account for the 'carrier effect' and be directed against carrier-specific determinants (Mitchison, 1967, 1968b). It is attractive to speculate that IgX could both mediate cell co-operation and at the same time be the cause of antigenic competition. Such a 'co-operating antibody' would fit the requirements of the inhibitor in antigenic competition, in as much as it is produced at (or after) the recognition step, where it would also act, it would be absent in tolerance and would not be inhibitory to the antigen which caused its production. To account for competition, IgX would however presumably have to be released from the thymus-derived cell, so that a simple scheme such as that of Mitchison (1968b), or Rajewsky, Schirrmacher, Nase and Jerne (1969), in which the antigen simply bridges the thymus and marrow cells would have to be replaced by a more complex one. A scheme suggested by Lachmann (1970) and Lachmann and Amos (1971) could bring together cell co-operation and some aspects of delayed hypersensitivity with antigenic competition. In this, co-operating antibody is identified with an antigen-dependent variety of the macrophage inhibition factors (MIF), which are an *in-vitro* correlate of delayed hypersensitivity (David, 1966; Bloom

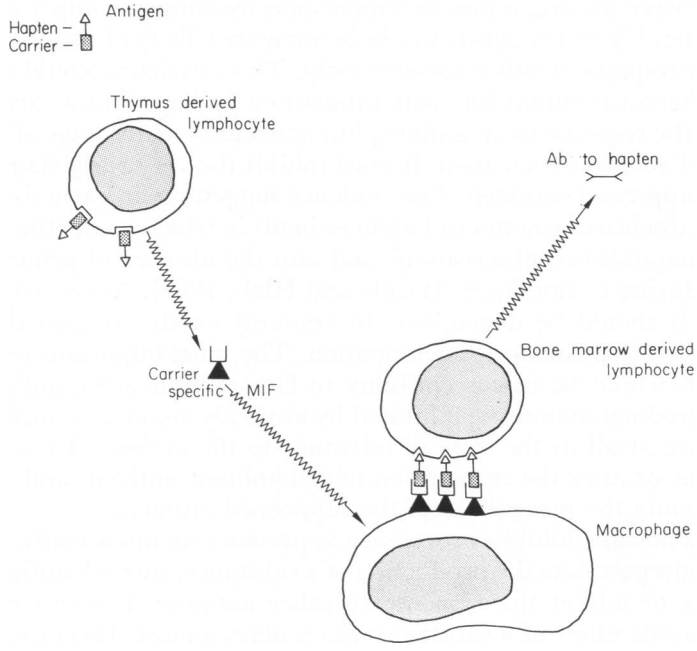

FIG. 3. A model for cell co-operation (from Lachmann, 1970).

and Bennett, 1966; Amos and Lachmann, 1970). In delayed hypersensitivity macrophage inhibition factors act to immobilize macrophages at the site of reaction of thymus-derived cells with antigen. In cellular co-operation, antigen is pictured as being concentrated by antigen dependent MIF at the surface of macrophages for presentation to marrow derived cells (Fig. 3). The site of competition would then presumably be the macrophage surface, with the co-operating antibodies produced by different antigens competing for binding sites on the macrophage. The antigen which produced the greater amount of 'co-operating antibody', or 'co-operating antibody' which was better bound to macrophages would be the dominant one in competition.

This hypothesis—that competition is for the presentation of antigen by co-operating antibody—has advantages. It does not require the antigen-sensitive cells to be pluripotential or contradict the one immunoglobulin–one cell dogma, and it relates antigenic competition to the stage of induction of the antibody response rather than later stages of proliferation or antibody production, so that there is an explanation here for the possible dominance of antigens which provoke little antibody production (Ben-Efraim and Liacopoulos, 1967).

The other observations relating to antigen recognition and competition can also find an explanation here. The effect of tolerance to the dominant antigen in preventing competition would be anticipated if the T cells were tolerant for which there is some evidence (Taylor, 1969). Passive anti-Fc could reduce the amount of 'co-operating antibody' produced, and hence reduce competition, without necessarily affecting the number of B cells stimulated. The hypothesis could account for the dose dependence of competition and the dominance of antigens given first in a sequence. In the latter case, the antigen which stimulated T cells first, would always be expected to have the advantage in competition.

There are, however, also difficulties in regarding competition solely as a failure of a concentration device for the suppressed antigen. There is, for instance, the fact that Fc competes as well against Fab when the two are combined in an IgG molecule as when they are given as a mixture (Taussig, 1971). In this situation, Fab (the suppressed antigen) should be able to act as a hapten using Fc as a carrier and 'co-operating antibody' to Fc would be expected to co-operate in the formation of antibody to Fab—as in the studies of Iverson (1970) on the formation of anti-idiotype antibody in mice—rather than to compete. It has also been found by others that two haptens on the same carrier can compete against each other (Schechter, 1968; Brody and Siskind, 1969). If competition is to be explained on the basis of competition on macrophage or dendritic cell membranes by 'co-operating antibody', then it would seem necessary that the 'co-operating antibody' should function in some way more specifically than as a simple concentrating device. One may postulate that there is some geometric reason why presentation by way of 'co-operating antibody' to particular carrier determinants favours antibody formation to only some of the 'haptenic' determinants in the molecule.

A further prediction from this type of hypothesis is that antigens which are completely thymus-independent (in that they are unable to stimulate T cells at all) should not take part in antigenic competition. The findings of Roelants and Goodman (1970) are at variance with this prediction. They have found that Poly γ D-Glutamic acid will compete against methylated bovine serum albumin, although this substance is apparently incapable of stimulating a T cell response.

It is also difficult to picture that space on macrophages in general should be a limiting factor in antibody formation and it would be necessary to postulate that the sites for which competition occurs are limited in some way.

Recently, Möller and Sjoberg (1970) have put forward an explanation of antigenic competition which resembles the present model in many respects. It differs, however, in considering the possible inhibitor to be a non-specific effector molecule. Whereas this view is adequate to explain competition where one antigen is given first, it would be more difficult to account for the Fc/Fab system and others where competition is equally effective when the antigens are administered at the same time.

Even more recently—and since this manuscript was written—Taylor and Iverson (1971) have proposed an explanation of antigenic competition that is essentially similar to the present model to account for their finding that animals primed with DNP-bovine γ-globulin and challenged with oxazolone-DNP-bovine serum albumin made much less anti-DNP if they were skin-painted with oxazolone before challenge.

REFERENCES

ADLER, F. L. (1964). 'Competition of antigens.' *Progr. Allergy*, **8**, 41.

ALBRIGHT, J. F. and MAKINODAN, T. (1965). 'Dynamics of expression of competence of antibody-producing cells.' *Molecular and Cellular Basis of Antibody Formation* (Ed. by J. Sterzl), p. 427. National Academy of Science. Prague.

AMKRAUT, A. A., GARVEY, J. S. and CAMPBELL, D. H. (1966). 'Competition of haptens.' *J. exp. Med.*, **124**, 293.

AMOS, H. E. and LACHMANN, P. J. (1970). 'The immunological specificity of a macrophage inhibition factor.' *Immunology*, **18**, 269.

AVRAMEAS, S. and TERNYNCK, T. (1969). 'The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbants.' *Immunochemistry*, **6**, 53.

BEN-EFRAIM, S. and LIACOPOULOS, P. (1967). 'Inhibition, no effect, or enhancement of immune response following injections of mixtures of immunogenic and non-immunogenic synthetic polypeptides.' *Immunology*, **12**, 517.

BLOOM, B. R. and BENNETT, B. (1966). 'Delayed hypersensitivity *in vitro*. The mechanism of inhibition by antigen of cell migration.' *Science*, **153**, 80.

BRODY, N. I. and SISKIND, G. W. (1969). 'Studies on antigenic competition.' *J. exp. Med.*, **130**, 821.

BRODY, N. I., SISKIND, G. W. and WALKER, J. G. (1967). 'Studies on the control of antibody synthesis. Interaction of antigenic competition and suppression of antibody formation by passive antibody on the immune response.' *J. exp. Med.*, **126**, 81.

CEROTTINI, J. C., MCCONAHEY, P. and DIXON, F. J. (1969). Specificity of the immunosuppression caused by passive administration of antibody.' *J. Immunol.*, **103**, 268.

DAVID, J. R. (1966). 'Delayed hypersensitivity *in vitro*. Its mediation by cell-free substances formed by lymphoid cell-antigen interactions.' *Proc. nat. Acad. Sci. (Wash.)*, **56**, 72.

DRESSER, D. W. (1962). 'Specific inhibition of antibody production. II. Paralysis induced in adult mice by small quantities of protein antigen.' *Immunology*, 5, 378.

FLEISCHMAN, J. B., PAIN, R. H. and PORTER, R. (1962). 'Reduction of γ-globulins.' *Arch. Biochem.*, Suppl. 1, 174.

IVERSON, G. M. (1970). 'Ability of CBA mice to produce anti-idiotypic sera to 5563 myeloma protein.' *Nature (Lond.)*, **227**, 273.

LACHMANN, P. J. (1970). 'Lymphocyte co-operation.' *Proc. Roy. Soc.* B, **176**, 425.

LACHMANN, P. J. and AMOS, H. E. (1971). 'Soluble factors in the mediation of the co-operative effect.' *Immunopathology*, **6**, (Ed. by P. A. Miescher), p. 65. Schwabe & Co, Basel.

MAKINODAN, T. and ALBRIGHT, J. F. (1966). Proliferative and differentiative manifestations of cellular immune potential.' *Progr. Allergy*, **10**, 1.

MITCHELL, G. F. and MILLER, J. F. A. P. (1968). 'Cell–cell interaction in the immune response. II. The source of haemolysin-forming cells in irradiated mice given bone-marrow and thymus or thoracic duct lymphocytes.' *J. exp. Med.*, **128**, 821.

MITCHISON, N. A. (1967). 'Antigen recognition responsible for the induction *in vitro* of the secondary response.' *Cold Spring Harb. Symp. quant. Biol.*, **32**, 431.

MITCHISON, N. A. (1968a). 'Recognition of antigen'. In: *Differentiation and Immunology* (Ed. by K. B. Warren). Academic Press, New York.

MITCHISON, N. A. (1968b). 'Antigen dosage in relation to responsiveness and non-responsiveness.' In: *Immunological Tolerance* (Ed. by M. Landy and W. Braun). Academic Press, New York.

MITCHISON, N. A. (1969). 'The immunogenic capacity of antigen taken up by peritoneal exudate cells.' *Immunology*, **16**, 1.

MÖLLER, G. and SJOBERG, O. (1970). 'Effect of antigenic competition on antigen-sensitive cells and on adoptively transformed immunocompetent cells.' *Cellular Immunology*, **1**, 110.

PINCUS, C. Y. and NUSSENZWEIG, V. (1969). 'Passive antibody may simultaneously suppress and stimulate antibody formation against different portions of a protein molecule.' *Nature (Lond.)*, **222**, 594.

RADOVICH, J. and TALMAGE, D. W. (1967). Antigenic competition. Cellular or humoral.' *Science*, **158**, 512.

RAJEWSKY, K., SCHIRRMACHER, V., NASE, S. and JERNE, N. K. (1969). 'The requirement of more than one antigenic determinant for immunogenicity.' *J. exp. Med.*, **129**, 1131.

ROELANTS, G. E. and GOODMAN, J. W. (1970). 'Tolerance induction by an apparently non-immunogenic molecule.' *Nature (Lond.)*, **227**, 175.

SCHECHTER, I. (1968). 'Antigenic competition between polypeptidyl determinants in normal and tolerant rabbits.' *J. exp. Med.*, **127**, 237.

SCHWARTZ, H. and LESKOWITZ, S. (1969). 'Antigenic competition in the development of delayed hyper-

sensitivity to the azobenzenearsonate group.' *Immunochemistry*, **6**, 503.

TAUSSIG, M. J. (1970). 'Bacteriophage-linked assays for antibodies to protein antigens.' *Immunology*, **18**, 323.

TAUSSIG, M. J. (1971). 'Studies on antigenic competition. I. Antigenic competition between the Fc and Fab fragments of rabbit IgG in mice.' *Immunology*, **21**, 51.

TAYLOR, R. B. (1969). 'Cellular co-operation in the antibody response of mice to two serum albumins: specific function of thymus cells.' *Transpl. Rev.*, **1**, 114.

TAYLOR, R. B. and IVERSON, G. M. (1971). 'Hapten competition and the nature of cell co-operation in the antibody response. *Proc. Roy. Soc.* B, **176**, 393.

WEIGLE, W. O. and HIGH, G. J. (1967). 'The effect of antigenic competition on antibody production to heterologous proteins, termination of immunologic unresponsiveness and induction of autoimmunity.' *J. Immunol.*, **99**, 392.