THE EFFECT OF ANTIGENIC COMPETITION ON VARIOUS MANIFESTATIONS OF HUMORAL ANTIBODY FORMATION AND CELLULAR IMMUNITY*

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(Received for publication 1 July 1968)

Since the initial description by Michaelis over a half century ago, of the effects of competition of antigens on the immune response (1), interest in this area has waxed and waned concomitant with the current views and interests of investigators in medicine and biology. Initially, workers were motivated in their studies by a practical purpose, since antigenic competition played a vital role in determining the immune responses to vaccines containing a multiplicity of antigens (2–4). In modern times, antigenic competition has been employed for diverse experimental purposes. The model has been employed as a useful tool for the study of the theory of clonal selection (5), the investigation of the immune responses to two haptens placed on the same or differing carrier molecules (6), the inhibition of the immune response to haptenic determinants bound to a single carrier or multiple carriers by passively administered antibody (7), and the maintenance of immunological tolerance and the induction of experimental autoimmunity (8).

Previous work by this investigator as well as by a number of other investigators, provided evidence for the sequential production of 19S and 7S immunoglobulins by cells arising from differing progenitors (9–12). These findings implied that the induction phase of these two events in the immune response might possess important biological differences which could be delineated by a model of antigenic competition. In particular, questions were raised pertaining to the characteristics of the immune response to two antigens administered in appropriate sequence, such that the induction of the 19S or 7S response to the initial antigen coincided with the period of induction of 19S response to the second antigen.

During the initial phase of the present work, experiments were performed to evaluate the susceptibility of both the 19S and 7S antibody forming mechanism to the influence of antigenic competition. Subsequently, the effects on several parameters of the primary and secondary immune responses to a number of antigens administered in sequence were investigated. Finally, the relationship between induction of cellular immunity exemplified by development of trans-

* This investigation was supported by Grant MA-2961 from the Medical Research Council of Canada.

plant immunity and induction of humoral antibody formation was studied. For this purpose, an experimental model of antigenic competition was designed to assess the influence of humoral antibody formation upon the development of immunity to a skin graft.

Materials and Methods

Animals.—A noninbred strain of female Swiss white mice, weighing 20–25 g at the onset of the experiments, was used in all experiments involving induction of humoral antibody formation. Experiments requiring skin grafting were performed employing 6-wk-old females of two inbred strains, C57 BL/6J, and A/J.[1]

Immunization.—Individual groups of 12 mice were immunized with 0.6 mg of hemocyanin[2] or with 0.2 cc of 10% washed erythrocyte suspensions (RBC) intravenously via the tail vein, except when stated otherwise. Suspensions of RBC were prepared in a standard fashion employing determinations of hematocrit following centrifugation at 450 *g* for 10 min.

Serological Procedures.—Groups of mice were bled from the retro-orbital sinus, and hemagglutination tests were performed on the pooled antisera. In addition, all antisera were assayed after treatment with 0.1 M 2-mercaptoethanol (2-ME) as previously described (9, 13). Serial twofold dilutions of 0.5 ml in phosphate-buffered saline, pH 7.2 were made in Wassermann tubes, and 0.2 ml of 0.3% washed RBC were added per tube. Racks were shaken and the patterns read after the suspension had settled for 3–4 hr at room temperature.

The antisera were tested for cross-reactivity by titrating selected pooled antisera against all of the other erythrocyte suspensions employed in the present work. In only one instance, namely between rat RBC and sheep RBC, was evidence for cross-reactivity detected. The results of these experiments will be reported elsewhere.

Ultracentrifugation.—A 7–40% linear sucrose gradient was prepared employing a Buchler linear gradient former (Buchler Instruments, Inc., Fort Lee, N.J.). 0.3 ml samples of selected antisera were layered onto the gradient yielding a total volume of 4.6 ml. The gradient was spun in a Spinco Model L ultracentrifuge (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.) at 30,000 rpm (73,500 *g*) for 16 hr at 4°C. Fractions from the gradient were collected by puncturing the bottom of the centrifuge tube with a 22 gauge hypodermic needle and collecting the drops. Each fraction contained 20 drops and approximately 16 fractions were collected. The fractions were titrated as previously described.

Throughout the text of this work, reference will be made at intervals, to statements pertaining to the levels of 19S and 7S antibody. These statements were made according to the following criteria. Representations of 19S antibody were derived (*a*) from the titration of the antisera before and after treatment with 2-ME, and demonstration of the total loss of activity following this treatment; (*b*) the appearance of antibody activity contained in the dense fractions on sucrose gradient density ultracentrifugation, the activity of which was totally abolished by treatment with 2-ME. The antibody levels were designated as 7S antibody when the following criteria were satisfied. (*a*) Antibody activity remaining in antisera after treatment with 2-ME was within two tubes of the total antibody level prior to such treatment. (*b*) The antibody activity present in the less dense fractions on gradient centrifugation contained the major proportion of antibody activity, which was not affected by treatment with 2-ME.

Grafting Procedures.—The A/J strain mice were grafted with 1 cm pinch grafts from C57 BL/6J mice placed on the dorsum. Autografts were also prepared on an adjacent site as a control. The grafts were covered with a vaseline gauze and protected with casts molded of Gypsona (Smith & Nephew, Ltd., Lachine, Quebec) for an initial period of 10 days. The criteria for skin graft rejection were those of Billingham (14).

[1] Obtained from Jackson Memorial Labs., Bar Harbor, Maine.

[2] Obtained from Mann Research Laboratories, New York, N.Y.

RESULTS

Effect of Competition of Antigens in the Primary Immune Response.—The study of the effect of a primary immune response to an initial antigen upon the induction of a primary immune response to a second antigen administered in sequence, was undertaken. The initial phase of the work was carried out to determine the optimum time interval for administration of the sequence of anti-

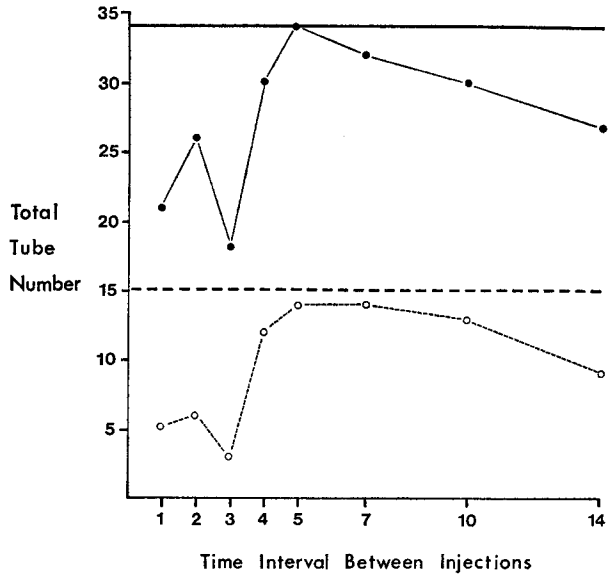

FIG. 1. Representation of the sum total of tubes containing anti-rat hemagglutinins upon titration of pooled antisera derived from bleedings on days 3, 5, 7, 12 and 20. Individual groups of 12 animals were immunized with rat RBC at intervals of 1–14 days subsequent to injection of hemocyanin. ——, total antibody levels of the control group; – – – –, 2-ME–resistant control antibody levels; ●——●, total experimental antibody levels; O– – – –O, 2-ME–resistant experimental antibody levels.

gens. In addition, several combinations of antigens were tested for their effects in eliciting the characteristics of antigenic competition. Antigens were administered intravenously except when noted otherwise.

The effect of variation in the interval of time between the administration of the sequence of the first and second antigens was studied in groups of mice at intervals of 1–14 days. The degree of suppression was expressed by comparing the number of positive hemagglutination tubes in the experimental group with control groups immunized with either the first or second antigen alone. For this purpose, the number of positive hemagglutination tubes derived by titrating antisera obtained from trial bleedings of the groups of animals on days 3, 5, 7,

12, and 20, were summated and used as the estimation of production of both total and 2-ME resistant, 7S antibody.

Fig. 1 illustrates the data of a representative experiment of anti-rat hemagglutinins contained in antisera derived from groups of animals immunized with rat RBC at intervals of 1–14 days subsequent to immunization with hemocyanin. Several features may be noted which were common to other pairs of antigens. First, the initial phase of maximum suppression to the second antigen

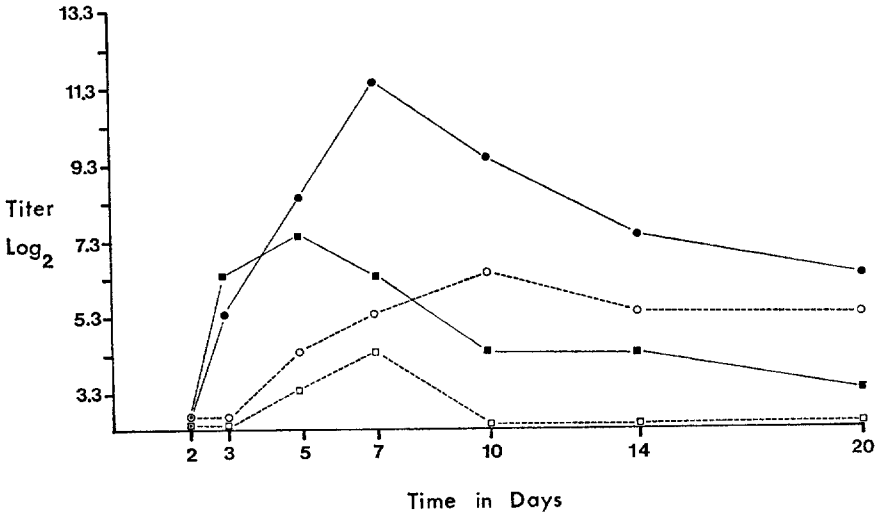

FIG. 2. Comparison of total and 2-ME–resistant antibody levels in control group of 12 animals immunized with rat cells alone, and an experimental group of 12 animals immunized with rat cells 3 days following administration of hemocyanin. Titrations were performed on pooled antisera. ●——●, total antibody levels of the control group, ○- - - -○; 2-ME–resistant 7S antibody levels of the control group; ■——■, total antibody levels in experimental group; □- - - -□, 2-ME–resistant 7S antibody levels in experimental group.

was observed when the time interval between the sequence of injections was 1–3 days. Secondly, this was followed by a sharp decrease in suppression. Finally, this phase was followed by a gradual increase in suppression of the response to the second antigen which was sustained throughout the period of study.

Immunological Characteristics of the Suppressed Responses to the Second Antigen of the Sequence.—Figs. 2 and 3 illustrate the curve of development of rat and goose hemagglutinins in animals immunized with these antigens 3 days following the administration of hemocyanin. The initial exponential increase in 19S antibody production possessed similar characteristics in both the control group receiving rat or goose cells alone and in the experimental groups receiving rat or goose RBC after injection of hemocyanin. Subsequently, however, the 19S

response appeared to decrease to lower levels than those of the controls, indicating deficient, continuing 19S antibody production. The characteristics of production of 2-ME–resistant antibody differed from the control group throughout the period of study, in that the maximum levels attained in the experimental group were markedly deficient during both the initial and later phases of the response.

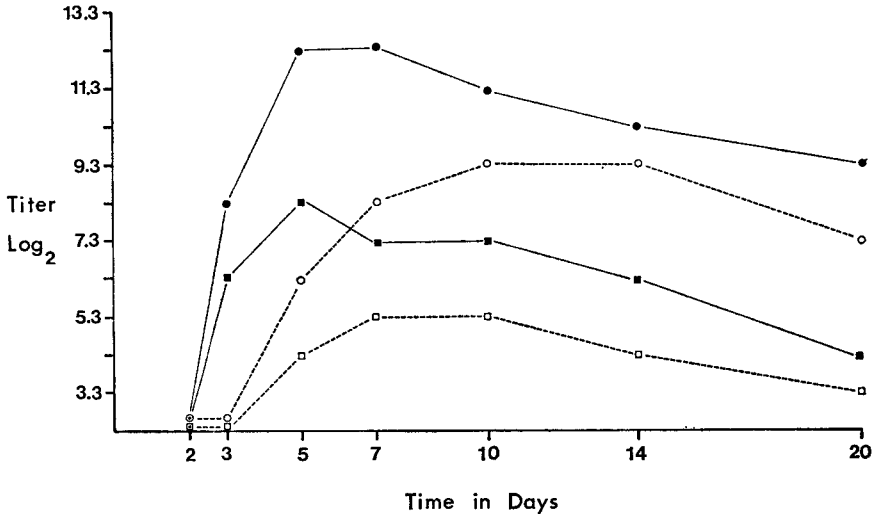

FIG. 3. Comparison of total and 2-ME–resistant antibody levels in a control group of 12 animals immunized with goose cells alone, and an experimental group of 12 animals immunized with goose cells 3 days following administration of hemocyanin. ●——●, total antibody levels of the control group; ○- - - -○, 2-ME–resistant 7S antibody levels of the control group; ■——■, total antibody levels in experimental group; □- - - -□, 2-ME–resistant 7S antibody levels in experimental group.

Immunological Characteristics of the Suppressed Responses to the First Antigen of the Sequence.—Figs. 4 and 5 illustrate the capacity of the response to the second antigen (rat RBC) to suppress the induction of both 19S and 7S antibody production to the first antigen (goose RBC). Fig. 4 illustrates the characteristics of suppression of total antibody formation in general and of the induction of 19S antibody synthesis in particular when the interval between administration of antigens was 1–3 days. Since the titer at 3 days represents 19S antibody exclusively on the basis of parameters enumerated above, it may be seen that the administration of rat RBC 1 day following goose RBC suppressed the production of 19S antibody to goose cells, as denoted by the reduced levels of 19S antibody on the 3rd day. When intervals of 2 and 3 days were employed, no effect on the 19S antibody production to goose RBC was demon-

strated on day 3, although the reduced titers of antisera obtained on day 5 indicated a suppression of subsequent 19S antibody formation.

Fig. 5 illustrates the unequivocal suppression of the over-all 7S response to goose RBC by the administration of rat RBC 1–3 days subsequent to the initial antigen. The degree of immunosuppression was maximal when the administration of antigens was separated by an interval of 2 days, although a significant

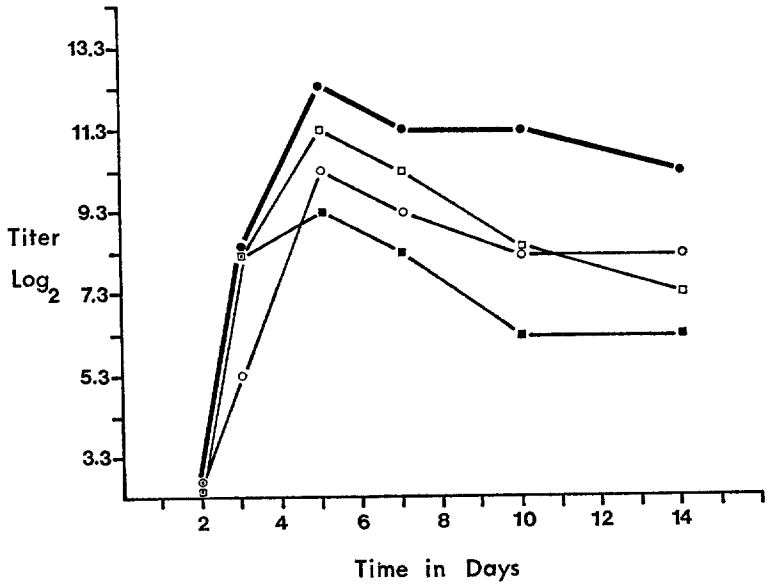

FIG. 4. Comparison of the total antibody levels in control animals immunized with goose RBC alone, with experimental groups immunized with goose RBC, 1, 2, and 3 days *prior to* immunization with rat RBC. Titrations were performed on pooled antisera. ●——●, control total antibody levels of anti-goose hemagglutinins; ○——○, total anti-goose RBC antibody levels obtained with a 1 day interval between injection of goose RBC and rat RBC; ■——■, total anti-goose RBC antibody levels at an interval of 2 days between injection of the two antigens; □——□, total anti-goose RBC antibody levels when an interval of 3 days separated injections of the two antigens.

suppression was also observed when time intervals of 1 and 3 days were employed.

The Effect of a Secondary Immune Response to the Initial Antigen upon Induction of a Primary Response to the Second Antigen.—Figs. 6 and 7 contrast the primary immune response to goose RBC obtained in control groups of mice with experimental groups immunized with goose RBC, 2 and 5 days respectively, following either a primary response to rat RBC, or a secondary response to rat RBC. The latter group had been primed 30 days previously with this antigen.

Several differences in the primary response to the goose cells were observed. The sequential induction of 2 primary immune responses at a 2 day interval was characterized by similar features of immunosuppression noted previously, namely, a moderate reduction in both 19S and 7S antibody synthesis (compare Figs. 2, 3, and 6). In contrast, the effect of a secondary response to the initial antigen (rat RBC) upon a subsequent primary response to goose RBC was asso-

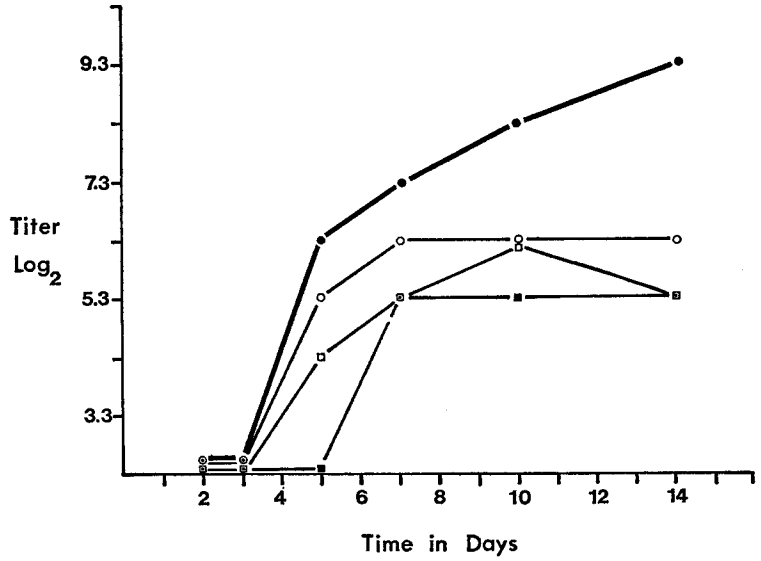

FIG. 5. Comparison of 2-ME–resistant 7S antibody levels in animals immunized with goose RBC alone with experimental groups immunized with goose RBC, 1, 2, and 3 days *prior to* immunization with rat RBC. ●——●, 2-ME–resistant 7S antibody levels of anti-goose hemagglutinins; ○——○, total anti-goose RBC 7S antibody levels obtained with a 1 day interval between injections of goose RBC and rat RBC; ■——■, total anti-goose RBC 7S antibody levels at an interval of 2 days between injections of the two antigens; □——□, total anti-goose RBC 7S antibody levels when an interval of 3 days separated injections of the two antigens.

ciated with a marked suppression of both 19S and 7S antibody production employing a 2 day interval between the sequence of injections.

Consecutive induction of two primary responses at a 5 day interval (Fig. 7) was characterized by less suppression of the immune response to the second antigen than the group receiving goose RBC 2 days following immunization with rat RBC (Fig. 6). These results were similar to those obtained employing hemocyanin and rat RBC as a combination (Fig. 1). However, the group receiving goose RBC 5 days following a secondary injection of rat RBC failed to demonstrate any 19S or 7S antibody formation during the period of observation, as

manifested by failure of the antisera to agglutinate suspensions of goose RBC at a dilution of 1/5.

The Effect of Antigenic Competition upon Priming and Immunological Memory.—The striking suppression of the immune response (noted above) raised the possibility that a model of antigenic competition could be employed

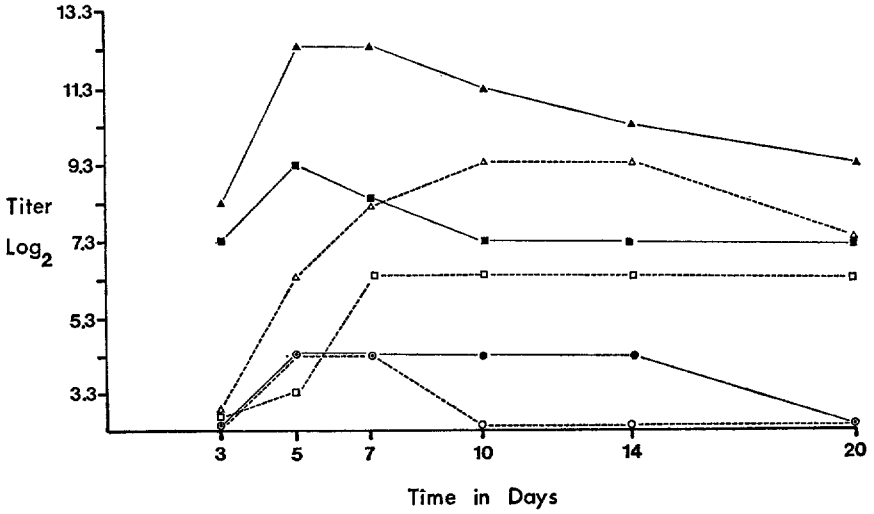

FIG. 6. Comparison of the total and 2-ME–resistant antibody levels in a control group immunized with goose RBC alone, an experimental group immunized with goose RBC 2 days after a primary injection of rat RBC, and a group immunized by a primary injection of goose RBC 2 days following a secondary injection of rat RBC. Titrations were performed on pooled antisera. ▲——▲, total anti-goose antibody levels in the control group immunized with goose cells alone; △----△, 2-ME–resistant 7S antibody levels in the groups immunized with goose RBC alone; ■——■, total antibody levels in the experimental group immunized with goose RBC after primary immunization with rat RBC; □----□, 2-ME–resistant 7S antibody levels in the experimental group immunized with goose RBC following primary immunization with rat RBC; ●——●, total antibody levels in the experimental group immunized with goose RBC 2 days following a secondary response induced to rat RBC; ○----○, 2-ME–resistant 7S antibody levels in the experimental group immunized with goose RBC 2 days following a secondary response induced to rat RBC.

to produce a deficiency in priming and immunological memory. For this purpose, the groups exhibiting total suppression of 19S and 7S antibody synthesis to goose RBC following the secondary response to rat RBC were immunized intravenously with goose RBC, 30 days following the primary immune response to this antigen.

Fig. 8 contrasts the characteristics of suppression of the secondary immune response in comparison to control groups immunized secondarily with goose RBC alone. Titrations were performed on antisera derived from individual

animals bled at intervals for 14 days. It may be seen that 19S response was delayed in onset and of a lower magnitude than the control group. The 7S response was markedly deficient and in 6 of the 10 animals was undetectable at dilutions of 1/10. Sucrose density gradient analysis performed on a pooled serum sample demonstrated antibody activity exclusively in the dense 19S fractions.

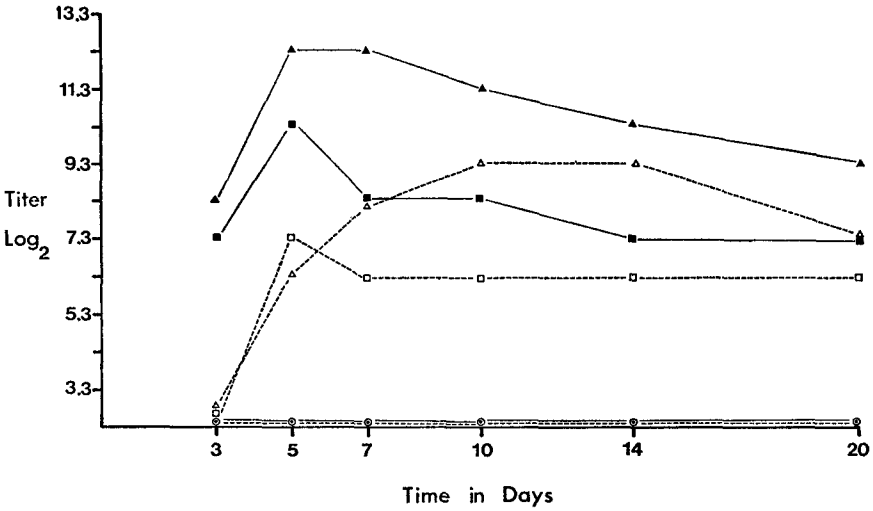

FIG. 7. Comparison of the total and 2-ME-resistant antibody levels in a control group immunized with goose RBC alone, an experimental group immunized with goose RBC 5 days after a primary injection of rat RBC, and a group immunized by a primary injection of goose RBC 5 days following a secondary injection of rat RBC. ▲——▲, total anti-goose antibody levels in the control group immunized with goose cells alone; △----△, 2-ME-resistant 7S antibody levels in the group immunized with goose RBC alone; ■——■, total antibody levels in the experimental group immunized with goose RBC after primary immunization with rat RBC; □----□, 2-ME-resistant 7S antibody levels in the experimental group immunized with goose RBC following primary immunization with rat RBC; ●——●, total antibody levels in the experimental group immunized with goose RBC 5 days following a secondary response induced to rat RBC; ○----○, 2-ME-resistant 7S antibody levels in the experimental group immunized with goose RBC 5 days following a secondary response induced to rat RBC.

These results were interpreted as indicative of deficient priming and immunological memory to goose RBC in the suppressed group, although the possibility of development of immune tolerance of a split type involving the population of 7S antibody forming cells was also suggested by these experiments. In this regard, it should be emphasized that the characteristics of both 19S and 7S antibody formation differed from those expected in a normal *primary* immune response to goose RBC (Compare Figs. 8 and 3), since the production of both

19S and 7S antibody was depressed below primary levels even in the 4 of 10 animals in which 7S antibody formation had been induced.

Effect of Alteration in Route of Administration of Antigens upon the Development of Antigenic Competition.—In the past, antigenic competition has been attributed in part to a competition for phagocytic cells, or alternatively, to reticuloendothelial cell blockade (15). Previous work in this laboratory had

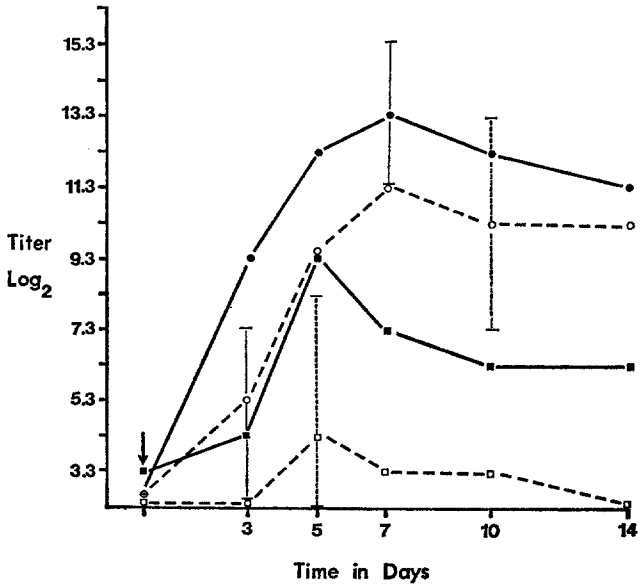

FIG. 8. Comparison of a control secondary anti-goose RBC response with an experimental group secondarily stimulated with goose RBC following total suppression of both 19S and 7S primary immune response by competition of antigens (see Fig. 7). Levels represent an average of individual titrations of antisera from 10 animals of which 6 showed no 7S response (titer < 1/10). ●——●, control total antibody levels; ○----○, control 2-ME–resistant 7S antibody levels; ■——■, total anti-goose RBC antibody levels in the suppressed experimental group; □----□, total 2-ME-resistant 7S antibody levels in the experimental group.

demonstrated that the predominantly active tissue in response to an intravenous injection of sheep erythrocytes was contained in the spleen, whereas, the active proximal lymphoid tissue following footpad administration of antigen was the draining popliteal lymph node (9).

Consequently, although considered unlikely in view of previous evidence (16), these parameters were investigated in the present work by administration of the second antigen into the right hind footpad following an intravenous suppressing dose of the initial antigen. Fig. 9 indicates the results obtained following administration of 0.1 cc of 10% rat RBC into this site 7 days following intravenous

injection of 0.2 cc of 10% goose RBC. The production of 19S antibody was suppressed in comparison to the control group immunized with rat RBC into the footpad alone. The suppression of 19S antibody production was more sharply delineated in this group than in that observed when the pair of antigens were both administered intravenously. The levels of 7S 2-ME resistant antibody

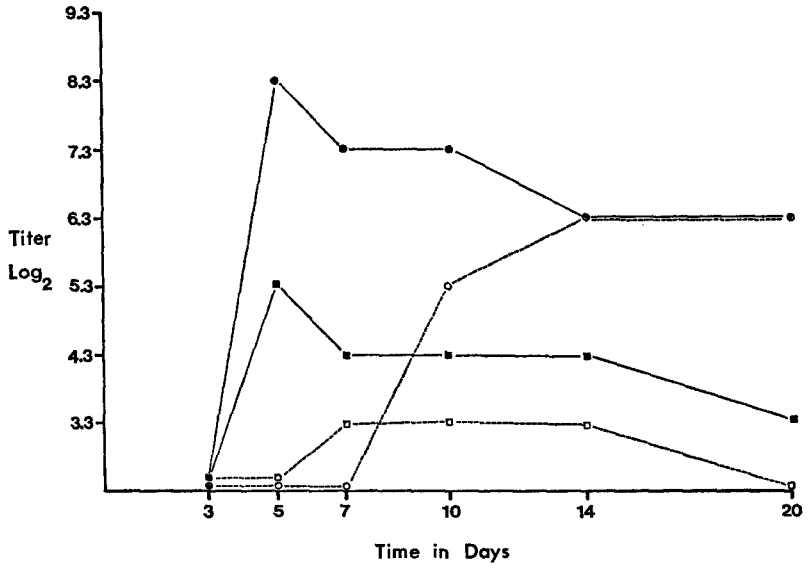

FIG. 9. Comparison of the anti-rat RBC antibody levels following an injection of rat RBC into a single right hind footpad in control animals with an experimental group immunized in the right hind footpad with rat RBC 7 days following an intravenous injection of goose RBC. Values represent titrations of pooled antisera derived from individual groups containing 12 animals. ●——●, total anti-rat hemagglutinins in the control group; ○----○, 2-ME-resistant 7S hemagglutinins in the control group; ■——■, total anti-rat hemagglutinins in the experimental group; □----□, 2-ME-resistant 7S hemagglutinins in the experimental group.

attained were markedly deficient indicating decreased production of this class of immunoglobulins.

The Effect of Induction of Humoral Antibody Formation upon the Development of Cellular Immunity.—The effect of induction of a primary immune response upon the development of cellular immunity as manifested by the rejection of a skin graft was undertaken. Initial experiments demonstrated that the injection of 0.2 cc of 10% goose RBC produced significant prolongation of survival of C57 BL/6J pinch grafts on A/J recipients. Maximum skin graft survival was achieved when the skin grafts were placed 7 days following the intravenous administration of antigen (Table I).

The results of experiments denoting several additional features of the pattern response are also summarized in Table I. Such features as the variation in response with the type of antigen used for induction of humoral antibody formation, the efficacy of combinations of antigen administered prior to skin grafting, and the effect of alteration in the sequence of immune stimuli were studied. The findings indicated that several antigens were effective in prolonging graft survival, although the best single antigen appeared to be goose erythrocytes. A combination of antigens made up of 0.3 mg of hemocyanin, and 0.2 cc of 10% rat erythrocytes contained in a 0.2 cc volume, administered intravenously as a mixture 7 days prior to skin grafting, greatly increased the percentage survival on the 12th day, as well as the prolongation of individual grafts. Finally, the administration of antigen subsequent to skin grafting also prolonged graft survival, indicating interference with the continuity of induction of cellular immunity.

TABLE I

The Effect of Induction of a Primary Immune Response Upon the Development of Cellular Immunity Initiated in Sequence

Group No.	Stimulus A	Stimulus B	Time intervals	No. of mice	No. surviving at 12 days	Longest survival
			days			*days*
I	—	—	—	45	4	13
II	Goose RBC	Skin graft	1	12	0	—
III	Goose RBC	Skin graft	2	15	1	12
IV	Goose RBC	Skin graft	3	9	1	12
V	Goose RBC	Skin graft	4	9	3*	14
VI	Goose RBC	Skin graft	7	11	6*	17
VII	Goose RBC	Skin graft	10	10	3*	15
VIII	Sheep RBC	Skin graft	7	20	4	15
IX	Rat RBC	Skin graft	7	18	5*	14
X	Hemocyanin	Skin graft	7	40	9*	18
XI	Skin graft	Sheep RBC	7	21	9‡	14
XII	Skin graft	Goose RBC	7	15	8‡	16
XIII	Hemocyanin and rat RBC	Skin graft	7	20	14‡	19

* Statistically significant at a 5% level.

‡ Statistically significant at better than a 1% level.

DISCUSSION

In the past, the impetus for the investigation of antigenic competition stemmed from the concern of many investigators about the role of competition in the development of protective immunity following immunization with vac-

cines containing a mixture of antigens. The work revolved mainly about the investigation of the immune response to diphtheria toxoid administered in combination with other immunogens. It was shown that the primary immune response to diphtheria toxoid was suppressed by the simultaneous elicitation of a primary response to a second antigen administered in combination (17), or by a primary immune response to an antigen administered prior to diphtheria toxoid (2), or more strikingly, by the development of a secondary immune response to an unrelated antigen concomitant with the induction of a primary immune response to the diphtheria antigen (18). More recently, the descriptive aspects, and the underlying mechanism of suppression of the immune response by antigenic competition have received renewed interest (19, 20). Many of these experiments have been reviewed recently by Adler (15).

The initial description of the phenomenon was referred to as a "crowding-out" of the response by competition (2). Subsequently, the effect was considered to be mediated at an early inductive stage of the immune response but probably not at the level of the phagocytic cell (16). Very recently, it has been suggested that antigenic competition may be mediated at the level of the antigen sensitive cell, a premise that would mitigate against the validity of the clonal selection theory (19, 20).

At the onset of the present work, it was tacitly assumed in the light of a large body of literature that the effect of competition of antigens was manifested during the induction phase of the immune response. Previous work had established that the parameters of induction of 19S and 7S antibody formation might possess basic differences which were accountable by the origin of these immunoglobulins from differing precursors (9–12). This view led to an investigation of the differential effect of antigenic competition upon the induction of these two phases of the primary immune response leading to humoral antibody formation.

The results of the present work would suggest that antigenic competition mediated by the antagonism of two intervening primary immune responses is associated with moderate suppression of induction of both 19S and 7S antibody formation. This was particularly clear in the group receiving the second of the pair of antigens in the footpad rather than intravenously.

In contrast, however, the elicitation of a secondary immune response exerted a very much greater suppression of a subsequent primary immune response than that observed by a competitive induction of two primary immune responses. These differences were strikingly apparent in the group receiving an initial dose of goose erythrocytes 5 days following the induction of a secondary immune response to rat erythrocytes, since competition in this group was manifested by a total suppression of both 19S and 7S antibody formation. Whatever the mechanism of antigenic competition is, it is clear from these findings that the underlying competitive processes are more profoundly expressed by induction of a secondary immune response upon a subsequent primary immune response. This

is surprising if one considers the potential of primed antigen sensitive cells which carry immunological memory and immune specificity for the initial antigen. Such cells comprise antigenically derived and selected clones which would not be utilized in the response to a new antigenic exposure. This may be taken as evidence for the assertion that competition of antigens is expressed at least in part, prior to recruitment of the antigen sensitive cell, and perhaps at the level of a processing cell.

Cells capable of phagocytosis of antigen clearly fall into the realm of a processing cell. Use of particulate antigens injected via various routes initiates an immune response in proximal lymphoid tissues (9), which would tend to rule out competition for space or phagocytic cells. In the present work, the possibility that competition was mediated at the level of a phagocytic cell was investigated by injecting the two antigens in sequence by different routes. It was shown that antigenic competition was equally well expressed employing sequential induction of two primary immune responses in which the second antigen was injected intradermally following injection of the first antigen intravenously. These findings tend to rule out competition for space or phagocytic cells as the underlying mechanism.

Several features of the dynamics of the immune response were also shown to be influenced by antigenic competition. First, the administration of a second antigen in sequence suppressed the induction of both 19S and 7S antibody production to the initial antigen. In this regard, it may be possible to employ a model of antigenic competition as an assay for the existence and timing of the processing of antigen. Secondly, the secondary immune response elicited by injection of the second of the pair of antigens following total suppression of its primary response by a secondary response to the initial antigen, was manifested by deficient production of 19S antibody formation in all animals, associated with either marked suppression or total abolition of 7S antibody synthesis. These results were interpreted as indicating a deficiency of production of primed cells carrying immunological memory. If one attempts to explain the two widely divergent findings as representative of the multiple consequences of a single underlying defect initiated by antigenic competition, the best choice, although by no means the only one, is at the level of processing of either the first or second antigen of the sequence.

The relationship of induction of humoral antibody formation and cellular immunity was also investigated, and the existence of such an intimate relationship demonstrated in the present work. It was shown that induction of humoral antibody formation suppressed the development of cellular immunity to a homograft possessing antigenic differences at the strong H-2 locus. Although the priming of the responses was, of necessity, different in view of the more prolonged antigenic stimulus initiated by a skin graft, the results were nevertheless interpreted as indicative of a relationship within the inductive phase of the two kinds

of immune response, which again could be more readily interpreted on the basis of competition for a processing cell. These experiments are in agreement with the findings of Flax and Waksman (21), and Liacopoulos (22), who demonstrated the suppression of development of delayed hypersensitivity to tuberculin or picryl determinants by administration of bovine serum albumin. However, Weigle and coworkers could not demonstrate an effect of competition upon the induction of an autoimmune response (8).

Recently, Mitchell and Miller employing a different experimental model, have suggested that the antigen-sensitive cell for the production of 19S hemolysin to sheep RBC, which is derived from bone marrow, requires the associated function of a processing (antigen-reactive) cell which is presumably a thymus-dependent cell (23). The results of the present work would appear to provide further evidence for this basic model for the induction of the immune response. The simplest model would contend that the processing cell provides information to the antigen-sensitive cell, which elicits a response possessing a multiplicity of characteristics, namely, induction of humoral antibody formation characterized by different classes of immunoglobulins, and development of cellular immunity. However, relative differences in the ability of a primary or secondary immune response to the first antigen to suppress the induction of 19S and 7S antibody formation to the second antigen, complicates the simplified interpretation of the data. Moreover, antigenic competition has been shown, in the present work, to be capable of inducing a degree of immunological tolerance manifested by failure of 7S antibody production in the secondary immune response to an antigen (goose RBC) in 6 of 10 animals in which priming had been suppressed by a secondary response to a suppressing antigen (rat RBC). In addition, synthesis of both 19S and 7S antibody (in the remaining four animals) was deficient when compared with control levels of synthesis induced by a primary immune response in nonimmunized normal animals. These differences could reflect in part simple quantitative differences manifested by enhanced efficiency in processing of antigen in the secondary immune response. Alternatively, the data may be interpreted in part, on the basis of two populations of antigen-sensitive or antigen-reactive precursor cells to account for the differences in suppression of 19S and 7S immunoglobulin production, each with differing susceptibility to the effects of antigenic competition.

There are many unanswered questions regarding the mechanism of action of antigenic competition. The results of the present work simply provide impetus for further investigation of this immunological phenomenon. The importance of this event in biology and medicine is underscored by consideration of a number of other phenomena of unknown etiology in the light of antigenic competition. These phenomena include the "doctrine of original antigenic sin," wasting disease in graft vs. host reactions and in thymectomized animals, and the manifestations of allergic death following midlethal doses of irradiation (24–27). It might

be argued that the doctrine of original antigenic sin is expressed as a consequence of inhibition of a primary immune response by induction of a concomitant secondary response (15, 24). Part of the spectrum of wasting disease in graft vs. host reactions and in thymectomized animals may reflect commitment of immune cells to an initial immune event resulting in a deficiency of the host to react against infectious agents in the environment. In agreement with this speculation, one might cite the work of Miller and coworkers demonstrating the absence of wasting disease in animals kept in a germ-free environment (25), and the experiments of Goldstein and coworkers who demonstrated that cells involved in graft vs. host reactions exhibit deficient responses to unrelated administered antigens (26). The phenomenon of allergic death in midlethally irradiated mice given F1 hybrid cells (27) might be accountable on the same basis.

SUMMARY

The effect of antigenic competition on various parameters of humoral antibody formation and cellular immunity was studied in mice.

Several pairs of antigens were employed in the investigation of the competitive aspects of induction of humoral antibody formation. Induction of a primary immune response to hemocyanin in Swiss white female mice moderately suppressed the induction of both 19S and 7S antibody formation to goose or rat erythrocytes. Suppression of 7S antibody formation was maximal when a time interval of 1–3 days separated the sequence of injections, although suppression was noted for intervals of up to 14 days. The induction of a primary immune response to rat RBC, the second of the two antigens in sequence, also suppressed the induction of both 19S and 7S antibody formation to goose RBC when appropriate intervals of 1–3 days were employed between injections.

The induction of a secondary immune response to rat RBC totally suppressed the primary induction of both 19S and 7S antibody formation to goose RBC administered in the appropriate time sequence. Subsequently, it was shown that the secondary immune response to the suppressed antigen (goose RBC) elicited 30 days after induction of a primary immune response (5 days after secondary immunization with rat RBC) was characterized by deficient 19S and 7S antibody production. These levels were suppressed even in comparison with a normal primary immune response to this antigen. The results were interpreted in part on the basis of a deficiency of formation of primed cells associated with immunological memory. Alternatively, evidence was obtained for the development of a split type of immunological tolerance in 6 of 10 animals studied, since a total suppression of 7S antibody production was obtained in association with deficient 19S antibody synthesis (titers $< 1/10$).

The induction of a primary immune response to several antigens in A/J female mice suppressed the processes of cellular immunity as manifested by prolonged survival of skin grafts from C57 BL/6J female donors.

These results were interpreted as evidence for the existence and utilization of processing cells by the initial immune stimulus yielding a deficiency of cells available for processing the second antigen administered in sequence.

We should like to thank Miss Donna-Marie Jackson, Mrs. Rita Kennedy, and Miss Shirin McCullough for their excellent technical assistance.

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