REGULATION OF THE IMMUNE RESPONSE

I. Reduction in Ability of Specific Antibody to Inhibit Long-Lasting IgG Immunological Priming after Removal of the Fc Fragment*

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Many observations on the immune response have led to common agreement that there are a number of regulatory mechanisms which may act to either increase, decrease or alter the nature of the immune response. The delineation of these regulatory mechanisms and understanding how they operate represents one of the more important areas for investigation in immunology. One of the most precise routes by which regulation of the immune response takes place is through the specific antibody itself (1). Specific antibody, introduced into an immunologically responding system, may alter the consequent response by suppressing, stimulating or altering the character of the response (2–7). Immunoglobulin G (IgG) antibody usually suppresses responses to immunogenic antigens, but may increase responses to some immunogens (8, 9). That the IgG preparation must contain the specific antibody for the suppression of the immune response to that particular antigen (at least when small amounts of IgG are used) indicates that the regulation involves the binding of antibody to the antigen (2, 5, 10, 11).

Is the binding of antibody to antigen and the consequent masking of antigenic determinants sufficient in itself to induce the suppression of the immune response, or do other events occur which lead to the suppression? In an attempt to answer these questions, the antibody was altered in a way which did not change its ability to bind to antigen. This was accomplished by removing the Fc portion, and the resulting $F(ab')_2$ antibody was tested for its ability to inhibit immunological priming (the establishment of immunological memory). Previous results from this laboratory indicated that $F(ab')_2$ antibody was much less effective than whole antibody in inhibiting the primary hemolysin response to sheep erythrocytes (12), and the experiments reported here indicate

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that $F(ab')_2$ antibody preparations contain approximately one-hundredth to one-thousandth the activity of intact 7S antibody preparations, when both preparations possessed comparable antigen-binding activity (as measured by agglutinin titers).

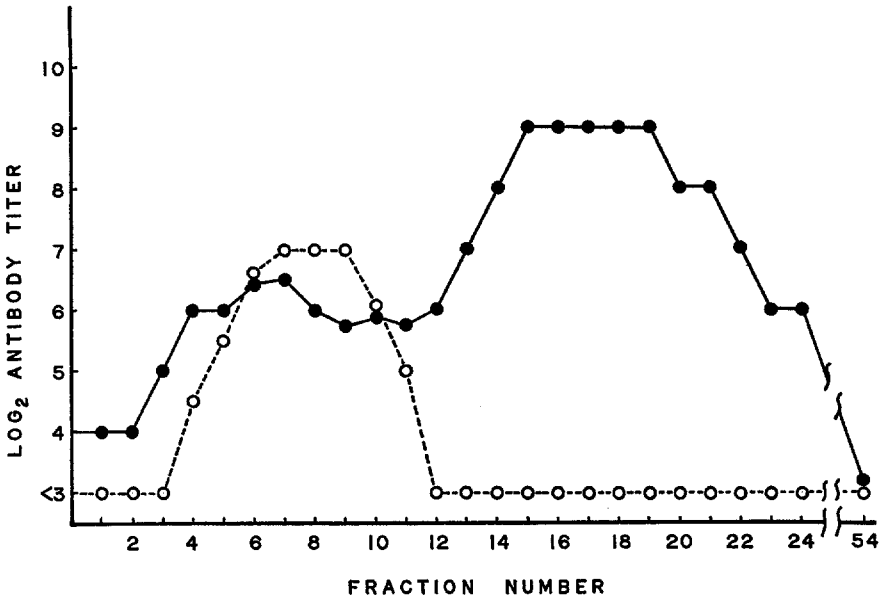

FIG. 1. Sucrose gradient profile of the pepsin digest of IgG antibody. Closed circles (●) indicate the hemagglutinin titer and open circles (○) indicate the hemolysin titer. Fractions are numbered from the bottom of the tube. The antibody with both hemolysin and hemagglutinin activity sedimented to the same distance as 7S antibody not treated with pepsin. The antibody with agglutinin activity only sedimented slightly further than hemoglobulin and albumen. Fractions 15 to 21 were taken for the $F(ab')_2$ antibody preparation. This gradient gave the *least* satisfactory separation of 7S and 5S agglutinins, the other three gradients from which the $F(ab')_2$ preparation was obtained showing a more pronounced trough between the two agglutinin peaks.

Materials and Methods

Mice.—Inbred male and female Swiss mice originally obtained from Carworth Farms, New City, N. Y. were used for all the experiments. The animals were given water and commercial cubed food ad libitum. Mice were weaned and separated according to sex at 1 month of age.

Anti-Sheep Erythrocyte Antibody for Passive Immunization.—Mice were given two injections of 0.1 ml of a 10% suspension of sheep erythrocytes (2×10^8) cells) (Grand Island Biological Co., Grand Island, N. Y.) and the serum was collected 40–60 days after the second injection of antigen. This serum contained no detectable 19S antibody and was considered to be the IgG antibody-containing serum. After three ammonium sulfate precipitations (40%), pepsin digestion was carried out in a 0.07 M sodium acetate and 0.05 M sodium chloride buffer at

pH 4.0 in the presence of 1 mg of pepsin (2650 units per mg, twice crystallized and lyophilized, obtained from Sigma Chemical Co., St. Louis, Mo.) per 100 mg of protein (13). After pepsin digestion or exposure to acetate buffer, antibody samples were dialyzed against phosphate buffered saline (PBS) (0.8% NaCl, 0.1 M phosphate, pH 7.8). Titration of the antibody preparation gave a $\log_2$ hemolysin titer of 11 and pepsin digestion decreased the titer to 4 without altering the hemagglutinin titer. The 5S ($F(ab')_2$) and 7S (whole and undigested) anti-sheep

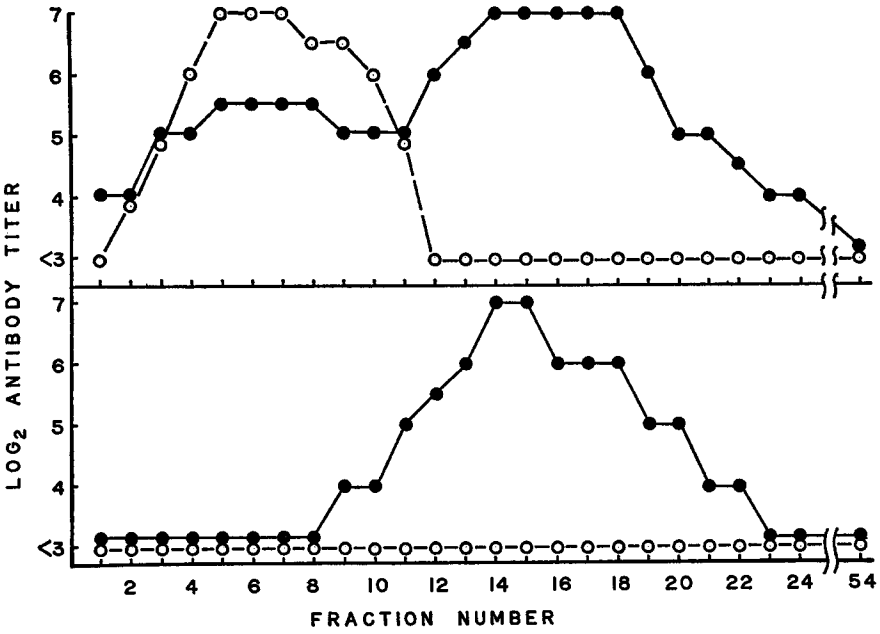

FIG. 2. Sucrose gradient profiles of pepsin digest of IgG antibody (upper graph) and of the 5S agglutinin peak from the first centrifugation (lower graph). Closed circles (●) indicate hemagglutinin activity and open circles (○) indicate hemolysin activity. Fractions are numbered from the bottom of the tube. Fractions numbers 12 to 21 (upper graph) were dialyzed and centrifuged in sucrose gradients to give a single 5S peak (lower graph).

erythrocyte antibodies were separated on 5–20% sucrose gradients in PBS. To resolve the 5S and 7S antibodies into two peaks, the gradients were centrifuged in the SW 40 swinging-bucket titanium rotor which accommodates $\frac{9}{16} \times 3\frac{3}{4}$ inch nitrocellulose tubes. The gradients were centrifuged at 40,000 rpm for 40 hr at 5°C in the Beckman L2-65B ultracentrifuge. The separation of 5S and 7S antibodies can be seen in Figs. 1 and 2. Hemolysin activity was found only in the 7S peak. The 7S peak contained undigested whole antibody, whereas the 5S peak contained pepsin-digested $F(ab')_2$ antibody.

Preparation of Antibody-Erythrocyte Mixtures and Procedure for Priming.—The agglutinin activities of 7S and $F(ab')_2$ antibody preparations were adjusted to a titer of 9. Varying amounts of intact 7S or 5S $F(ab')_2$ antibody were incubated with sheep erythrocytes at a concentration of 10^7 or 5×10^6 erythrocytes per ml. After incubation at 37°C for 1 hr, 0.1 ml of the sheep erythrocyte and antibody suspensions were injected intraperitoneally into inbred

Swiss mice, so that each animal received either 10^6 or 5×10^5 sheep erythrocytes. A period of 60 days was left between this first dose of antigen and reimmunization.

Reimmunization.—Whether or not the priming dose of antigen actually did prime was assessed by reimmunizing with 2×10^8 sheep erythrocytes and observing the responses after this second injection to see if they were primary or secondary. The establishment of criteria for distinguishing between primary and secondary responses is documented in the Results.

Measurement of Serum Hemolysin and Hemagglutinin Activity.—Blood was obtained from the retro-orbital sinus, and diluted 2:1 with 0.9% saline to avoid gel formation in the serum. The serum was collected after centrifugation, and endogenous complement inactivated by incubating the serum at 56°C for 30 min. Serum samples were serially diluted 1:1 with 0.9%

TABLE I

Inhibitory Effect of 5S F(ab')$_2$ on the Expression of Hemolysis by 7S Antibody

F(ab')$_2$ antibody	$\log_2$ hemolysin titers	
	7S antibody not exposed to pepsin	7S antibody surviving exposure to pepsin
None	9–10	11
1/16*	7–8	9
1/32	8	9
1/64	9	10
1/128	9–10	11
1/256	10	11–12
1/512	9–10	11–12

* Final dilutions of F(ab')$_2$ antibody (hemagglutinin titer, $\log_2$ 11).

saline in Microtiter plates (Cooke Engineering Co., Alexandria, Va.) (14). A standard amount of guinea pig complement (Grand Island Biological Co.) was added, and the serum and complement incubated for 30 min at 37°C. The preincubation of serum and complement lowered the incidence of titrations which were negative at low dilutions but positive at higher dilutions. Washed sheep erythrocytes (0.05 ml of a 0.5% suspension) were added and the complete mixture incubated for 2 hr at 37°C and then read, the end point being roughly half hemolysis on visual inspection. The plates were stored overnight at room temperature and read again the following morning. Titers were usually one $\log_2$ unit higher on the second reading. The titers are expressed as the $\log_2$ of dilution. Hemagglutinin titers were determined as above except complement was not added and no preincubation was carried out, and the end point was assessed by the ability of the sheep erythrocytes to form a "button".

Ultracentrifugation.—Pooled serum, layered on a 10–40% linear sucrose gradient made up to 0.9% with respect to NaCl concentration, was centrifuged in Beckman model L, L2-50 and L2-65B ultracentrifuges at 35,000 rpm for 15 hr at 5°C. The pooled serum samples were centrifuged in the type 50 titanium fixed-single rotor, fitted with adapters to accommodate ½ × 2½ inch polyallomer tubes, or in the type 40.3 aluminum fixed-angle rotor. The separation of 19S and 7S hemolysins in these 6.5 ml polyallomer tubes in fixed-angle rotors was slightly better than that obtained in the SW50 swinging-bucket rotor (15). 24 Fractions were collected by puncturing the centrifuge tube at the bottom with a number 18 lumbar puncture needle and each fraction was tested for hemolysin activity.

Calculations.—The calculations of total 19S and 7S hemolysin activity were carried out as previously described (16).

RESULTS

Separation of $F(ab')_2$ from Undigested 7S IgG Antibody.—The method of pepsin digestion used reduced the hemolysin titers from 11 to 4, which was just above the threshold level in our antibody assay system. The hemagglutinin titers were not changed ($\log_2 11$) indicating that antigen-binding activity was not affected by the pepsin digestion. The change in hemolysin titer from 11 to 4 might have been interpreted as a 99% decrease in intact 7S antibody, but

TABLE II

Hemolysin Titers after Injection of 2×10^8 Sheep Erythrocytes into Nonprimed Mice and Mice Primed with 10^6 Sheep Erythrocytes

Days after immunization with 2×10^8 sheep erythrocytes	Nonprimed	Primed
	total hemolysin titers	
4	9.4 ± 0.16* (79)‡	9.0 ± 0.25 (16)
7	8.4 ± 0.1 (79)	10.7 ± 0.35 (16)
11	8.2 ± 0.12 (75)	10.1 ± 0.27 (16)
	19S hemolysin titers	
4	9.4 ± 0.16 (79)	7.4 ± 0.16 (4)
7	7.9 ± 0.09 (14)	6.1 ± 0.7 (4)
	7S hemolysin titers	
4	0 (22)	8.6 ± 0.38 (4)
7	6.6 ± 0.2 (22)	10.3 ± 0.63 (5)

* Arithmetic mean ± standard error of the (reported) mean.

‡ Number of independent determinations: total hemolysin, number of mice; 19S or 7S hemolysin, number of sucrose gradients of pooled serum.

the $F(ab')_2$ antibody, which was being generated through the pepsin digestion, could have competed with whole antibody and prevented lysis of the erythrocytes. After physical separation of the 5S $F(ab')_2$ antibody from the intact 7S antibody remaining after pepsin digestion (Fig. 1), the hemolysin activity at the peak was much higher (approximately 10-fold). Therefore, although 99% loss of intact antibody might have been assumed after pepsin digestion, actually less than a 90% loss of intact antibody had occurred. That $F(ab')_2$ could inhibit erythrocyte lysis by whole antibody was also tested by mixing various amounts of purified $F(ab')_2$ with 7S antibody and observing the effect on the hemolysin titers (Table I). Such experiments demonstrate that the hemolysin titers are lowered by two $\log_2$ units in the presence of $F(ab')_2$ indicating that competition between $F(ab')_2$ and 7S antibody for antigenic determinants could occur. $F(ab')_2$ showed competition with both 7S antibody which was not exposed to

pepsin and 7S antibody which had survived pepsin digestion. Slight enhancement of hemolysis at low $F(ab')_2$ antibody concentrations has been observed in this and subsequent experiments. To insure that the 5S preparation did not contain 7S antibody which would be detectable if separated from the bulk of the $F(ab')_2$, the 5S antibody preparation was recentrifuged in sucrose gradients

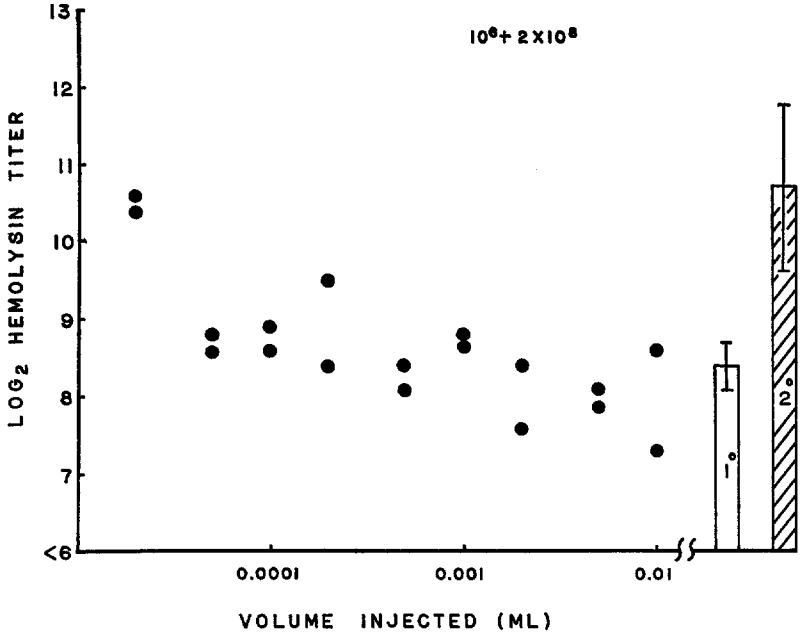

FIG. 3. Effect of varying amounts of 7S antibody given with the priming dose of 10^6 sheep erythrocytes on the 7 day total hemolysin titers after a second injection of 2×10^8 sheep erythrocytes. The open bar indicates the titer of control, unprimed animals (both injected and not injected with antibody at the time of the first dose of priming antigen in the experimental animals) and the hatched bar indicates the titer of control primed animals. The limits of three standard deviations of the mean of the controls (standard errors) are indicated by the vertical lines.

after dialysis against PBS. No evidence for detectable 7S was found (Fig. 2), and one can assume that the level of 7S contamination was less than 1%, but since the threshold of the assay system is high, one cannot conclude that 7S antibody was absent altogether.

Establishment of Criteria for Distinguishing between Primary and Secondary Responses.—Table II gives the total, 19S and 7S hemolysin titers after injection of 2×10^8 sheep erythrocytes into nonprimed mice and mice primed with 10^6 sheep erythrocytes 60 days previously. Total hemolysin titers at 7 and 11 days after immunization were statistically different between primary and sec-

ondary responses ($P < 0.001$), but not the total hemolysin titers at 4 days after injection of 2×10^8 sheep erythrocytes. The 19S hemolysin titers did not show evidence of enhancement (and were generally lower in the control secondary responses), indicating that the conditions for demonstrating 19S memory were not adequate because either the time interval between priming and second anti-

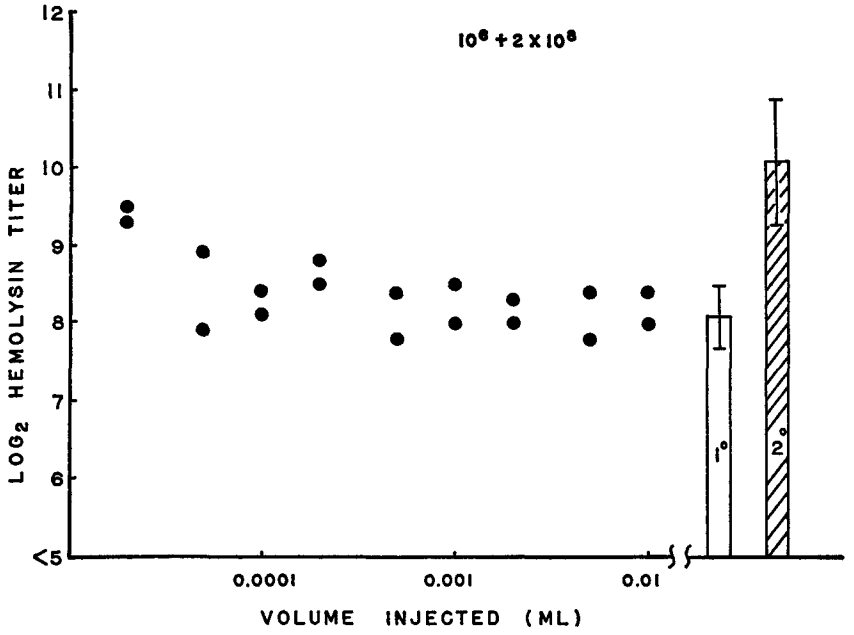

FIG. 4. Effect of varying amounts of 7S antibody given with the priming dose of 10^6 sheep erythrocytes on the total hemolysin titers 11 days after a second injection of 2×10^8 sheep erythrocytes. The open bar indicates the lysin titer of the primary response controls and the closed bar that of the secondary response control. The limits of three standard errors of control titers are indicated by the vertical lines.

gen injection was too long (60 days) or the 19S hemolysin titers earlier in the response at 2 or 3 days should have been determined. The level of 7S antibody at 4 and 7 days after immunization with 2×10^8 sheep erythrocytes showed clear statistical differences between primary and secondary responses ($P < 0.001$). In the following presentation of results, only those conditions which gave a clear difference between primary and secondary responses will be considered.

Effect of 7S and $F(ab')_2$ Antibody on Priming with an Antigen Dose of 10^6 Sheep Erythrocytes per Mouse.—When 10^6 sheep erythrocytes were used as the priming dose, 7S antibody given with the priming dose has a marked effect on priming. Figs. 3 and 4 show the effect of 7S on priming when the total hemolysin

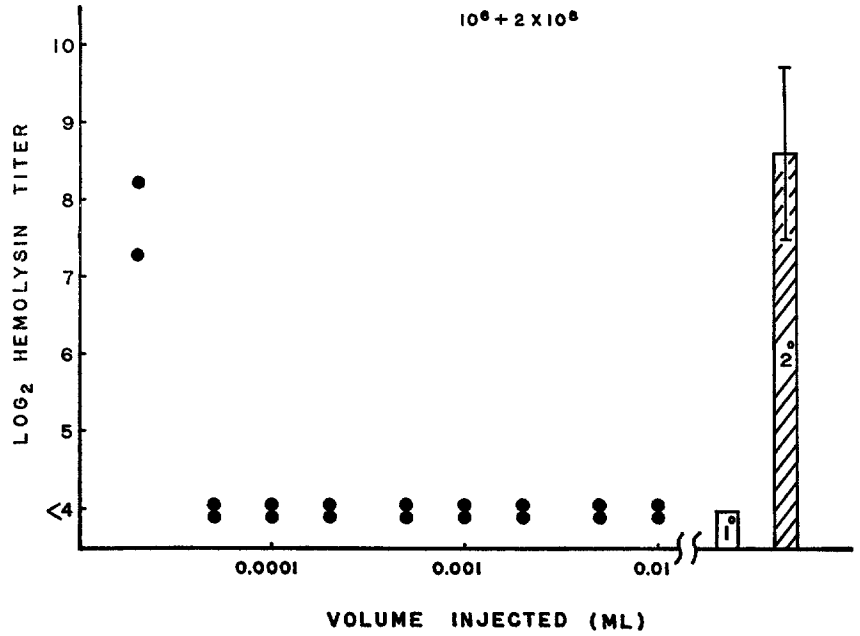

FIG. 5. Effect of 7S antibody on the 7S hemolysin titer 4 days after second injection of antigen. Priming dose was 10^6 sheep erythrocytes per mouse.

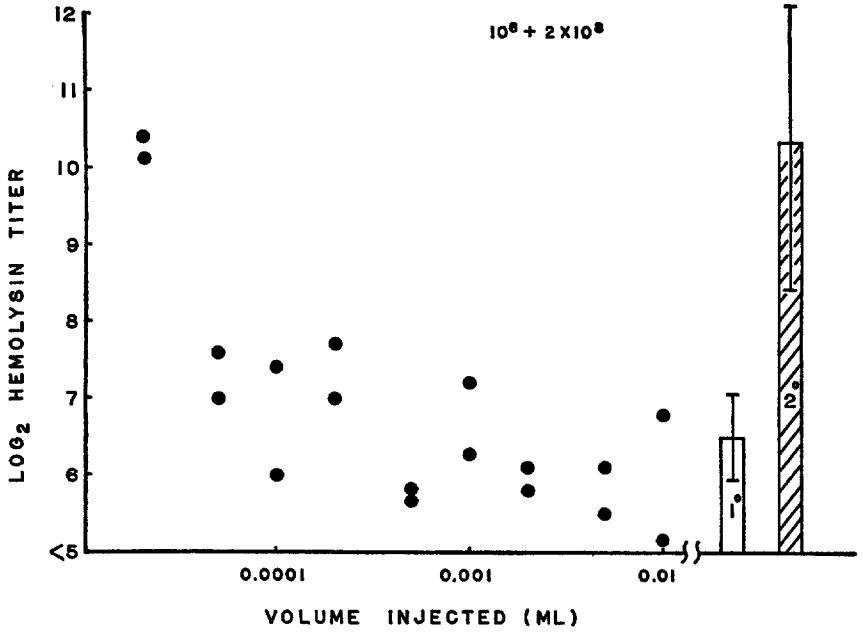

FIG. 6. Effect of 7S antibody on the 7S hemolysin titer 7 days after second injection of antigen. Priming dose was 10^6 sheep erythrocytes per mouse.

titers at 7 and 11 days after second antigen injection were used for distinguishing between primary and secondary responses. When 7S hemolysin at 4 and 7 days after second immunization was measured, the strong inhibitory effect of 7S on priming with 10^6 sheep erythrocytes was also demonstrated (Figs. 5 and 6). With these methods for assessing for the presence or absence of priming, a dose of 0.00005 ml of 7S antibody-containing solution was sufficient to prevent

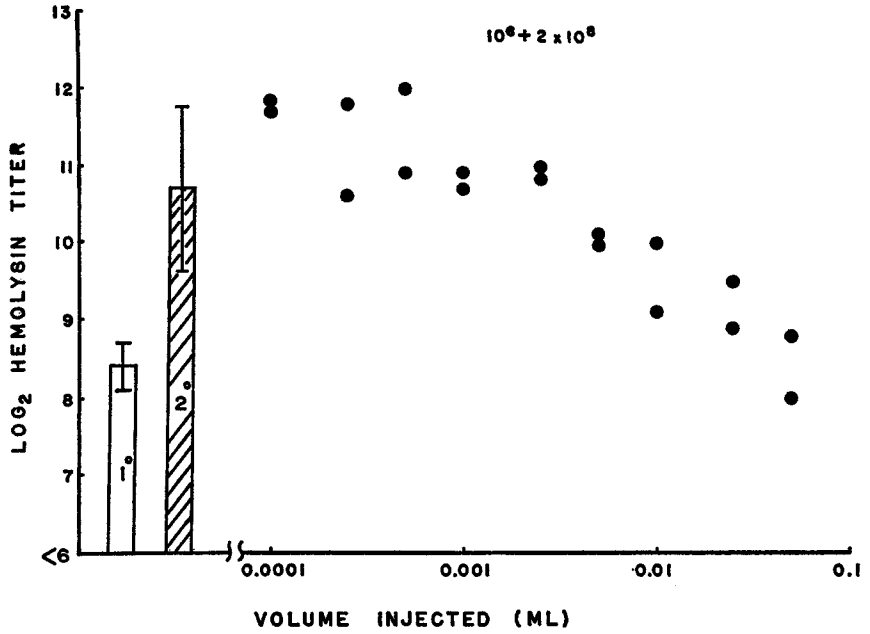

FIG. 7. Effect of $F(ab')_2$ antibody on the total hemolysin titer 7 days after second injection of antigen. Priming dose was 10^6 sheep erythrocytes per mouse.

priming with 10^6 sheep erythrocytes and to allow only a primary response when animals were reinjected 60 days later with 2×10^8 sheep erythrocytes. The response allowed by the lowest dose of 7S antibody (0.00002 ml) was significantly higher ($0.001 < P < 0.005$) than the responses in mice treated with larger amounts of 7S antibody. A straight line fitted the data better when the response at 0.00002 ml of 7S antibody was not included, and this response was in the range of the control secondary, which indicates that 0.00002 ml of 7S antibody allowed priming to occur.

In comparison with the 7S antibody preparation having the same agglutinin activity, the $F(ab')_2$ antibody preparation was much less effective in inhibiting priming. Figs. 7 and 8 show the effect of $F(ab')_2$ on priming when the total hemolysin titers at 7 and 11 days after second immunization were used for

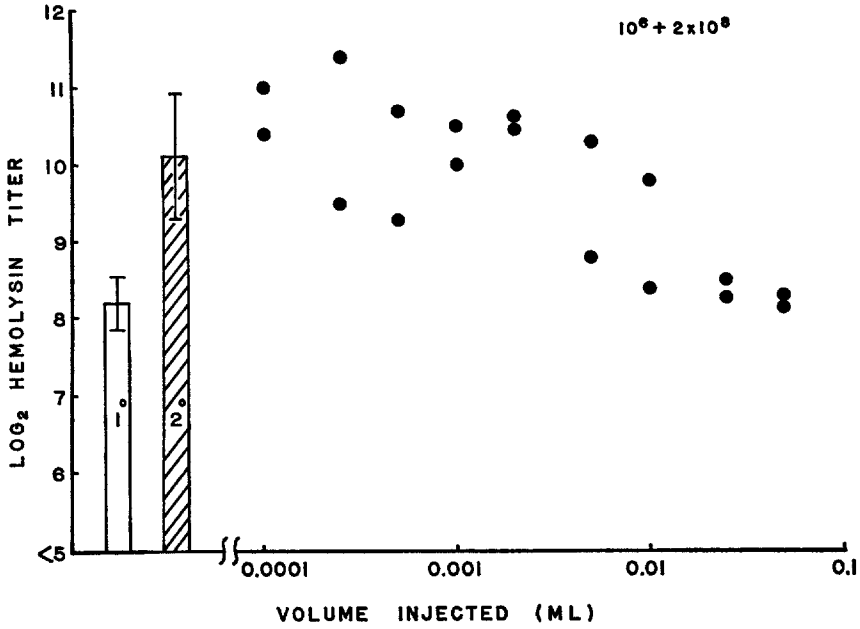

FIG. 8. Effect of $F(ab')_2$ antibody on the total hemolysin titer 11 days after second injection of antigen. Priming dose was 10^6 sheep erythrocytes per mouse.

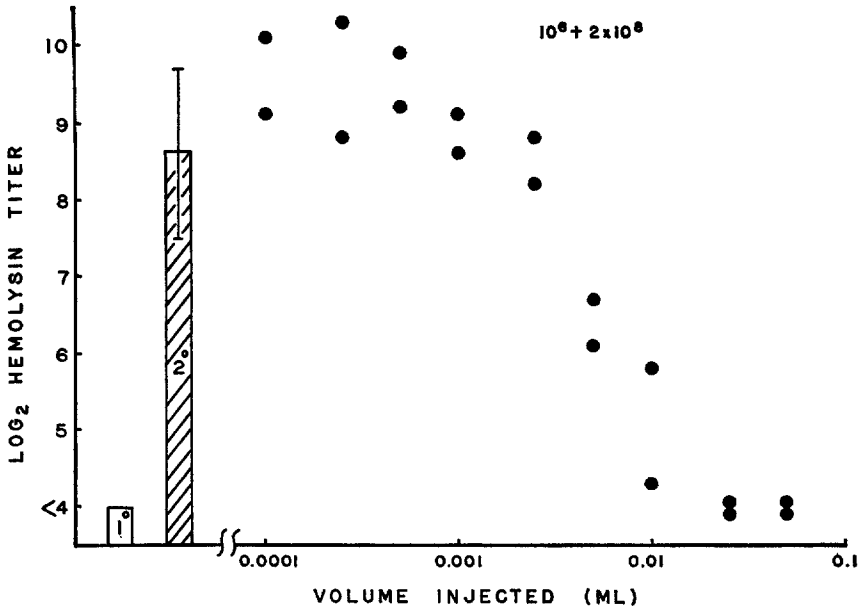

FIG. 9. Effect of $F(ab')_2$ antibody on the 7S hemolysin titers 4 days after second injection of antigen. Priming dose was 10^6 sheep erythrocytes per mouse.

distinguishing between the two responses. When the 7S hemolysin titers at 4 and 7 days after second immunization were determined, the relatively weak inhibitory effect of $F(ab')_2$ on priming with 10^6 sheep erythrocytes was demonstrated also (Figs. 9 and 10). With these methods for assessing for the presence or absence of priming, a dose of 0.005 to 0.05 ml of $F(ab')_2$ antibody-containing solution was needed to prevent priming and to allow only a primary response

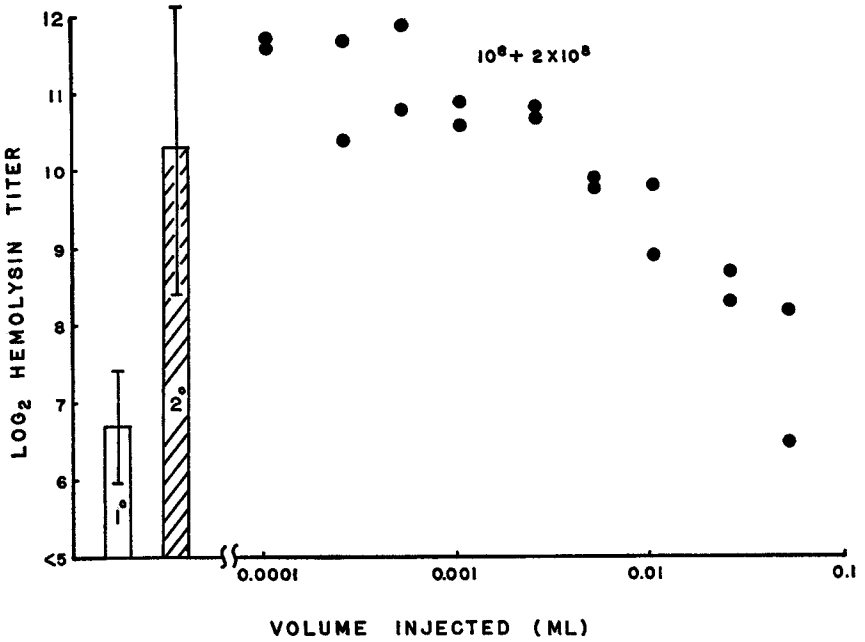

FIG. 10. Effect of $F(ab')_2$ antibody on the 7S hemolysin titers 7 days after second injection of antigen. Priming dose was 10^6 sheep erythrocytes per mouse.

when mice were given the second injection of antigen. Therefore, intact 7S antibody contained approximately 100-fold to 1000-fold the activity of $F(ab')_2$ in suppressing the establishment of immunological memory. With low doses of the $F(ab')_2$ preparation (0.0001–0.001 ml) and 10^6 sheep erythrocytes, there was a slight ($P = 0.1$) increase in the responses over the secondary control values (4 days after immunization measuring 7S hemolysin, Fig. 9).

Effect of 7S and $F(ab')_2$ Antibody on Priming with an Antigen Dose of 5×10^5 Sheep Erythrocytes per Mouse.—When 5×10^5 sheep erythrocytes were used for the priming dose of antigen, only the levels of 7S hemolysin distinguished between primary and secondary responses ($P < 0.001$) when mice were reinjected 60 days later with 2×10^8 sheep erythrocytes (Table III). Figs. 11 and 12 give the 4 and 7 day hemolysin titers of animals receiving intact

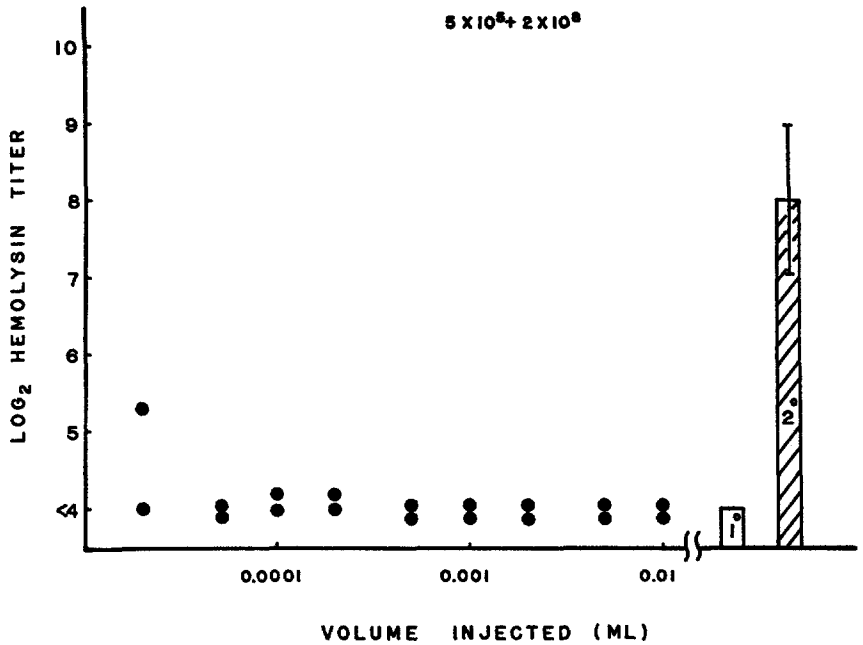

FIG. 11. Effect of 7S antibody on the 7S hemolysin titer 4 days after second injection of antigen. Priming dose was 5×10^5 sheep erythrocytes per mouse.

TABLE III

Hemolysin Titers after Injection of 2×10^8 Sheep Erythrocytes into Nonprimed Mice and Mice Primed with 5×10^5 Sheep Erythrocytes

Days after immunization with 2×10^8 sheep erythrocytes	Nonprimed	Primed
	total hemolysin titers	
4	11.3 ± 0.12* (72)‡	10.9 ± 0.23 (16)
7	8.0 ± 0.06 (72)	8.7 ± 0.24 (16)
	19S hemolysin titers	
4	11.3 ± 0.12 (72)	10.5 ± 0.3 (4)
7	7.4 ± 0.05 (13)	5.4 ± 0.36 (4)
	7S hemolysin titers	
4	0	8.0 ± 0.32 (4)
7	6.3 ± 0.11 (18)	8.5 ± 0.36 (4)

* Arithmetic mean ± standard error of the (reported) mean.

‡ Number of independent determinations, see Table II.

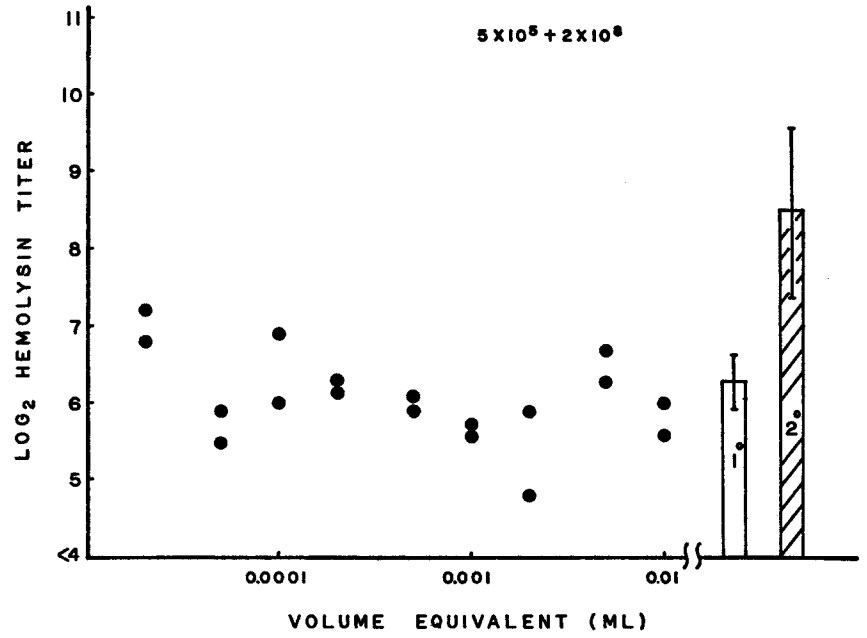

FIG. 12. Effect of 7S antibody on the 7S hemolysin titer 7 days after second injection of antigen. Priming dose was 5×10^5 sheep erythrocytes per mouse.

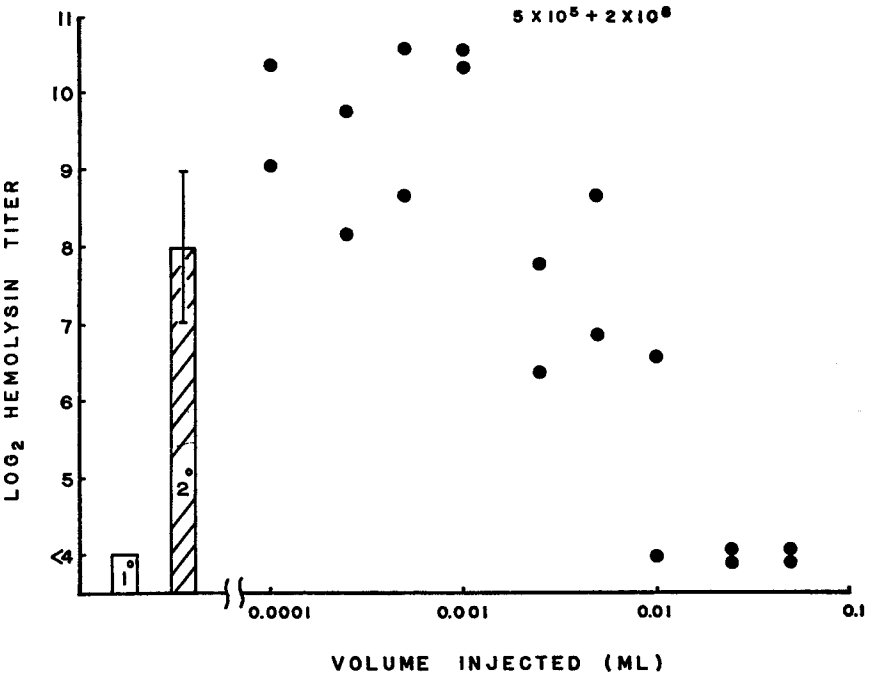

FIG. 13. Effect of $F(ab')_2$ antibody on the 7S hemolysin titer 4 days after second injection of antigen. Priming dose was 5×10^5 sheep erythrocytes per mouse.

7S antibody with the priming dose of antigen, and Figs. 13 and 14 give the 4 and 7 day 7S hemolysin titers of animals which had received $F(ab')_2$ with the priming dose (5×10^5 sheep erythrocytes). When intact 7S antibody was studied, a dose low enough to give an undoubted secondary response was not determined, but it is below 0.00002 ml and at this dose level nonprimary-type responses were seen. With $F(ab')_2$ the antibody dose at which there was a shift

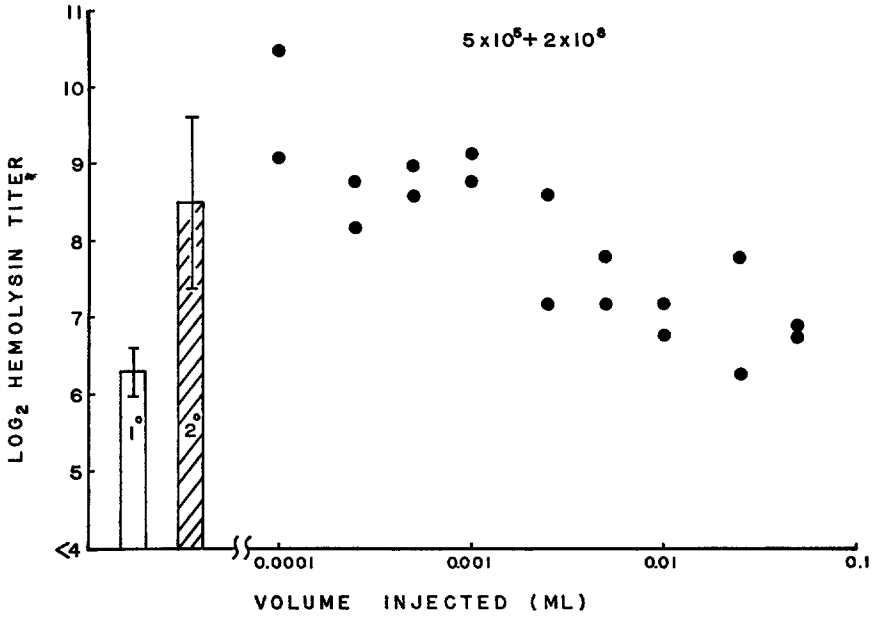

FIG. 14. Effect of $F(ab')_2$ antibody on the 7S hemolysin titers 7 days after second injection of antigen. Priming dose was 5×10^5 sheep erythrocytes per mouse.

from secondary to primary type immune responses is in the range of 0.005 to 0.02 ml. Therefore, the difference in potency of 7S and $F(ab')_2$ antibody in inhibiting immunological priming with a dose of 5×10^5 sheep erythrocytes is at least 100-fold to 1000-fold. With low doses of the $F(ab')_2$ preparation (0.0001–0.001 ml) and 5×10^5 sheep erythrocytes, the early 7S response was four times that of the secondary response control (Fig. 13), and this difference was significant ($P = 0.005$). When the 7S responses at 4 days after immunization of animals receiving priming doses of both 10^6 and 5×10^5 sheep erythrocytes with low doses of $F(ab')_2$ preparations (0.0001–0.001 ml) were combined, the difference between these responses and control secondary responses was statistically significant ($P = 0.001$–0.002).

DISCUSSION

With priming doses of 10^6 and 5×10^5 sheep erythrocytes per mouse, the 7S preparation had 100 to 1000 times the activity of the $F(ab')_2$ in preventing the establishment of immunological memory. It should be pointed out that a contamination of one part 7S antibody in 100 to 1000 parts $F(ab')_2$ in the $F(ab')_2$ preparation was not excluded so that no claim for any activity of $F(ab')_2$ antibody in inhibiting priming can be made. An experiment in which the $F(ab')_2$ preparation was recentrifuged and a comparison made between $F(ab')_2$ which was isolated once and $F(ab')_2$ which underwent the isolation procedures twice has been set up, and may help to decide whether the residual activity in the $F(ab')_2$ preparation, reported here, is due to some contamination with undigested 7S antibody or is attributable to the $F(ab')_2$ antibody itself. Whatever the outcome, it is obvious that $F(ab')_2$ antibody is grossly deficient in preventing priming.

There are a number of reasons why the large reduction in suppressive activity on pepsin digestion can be attributed to the loss of the Fc portion of the immunoglobulin molecule. The sedimentation coefficient of the digested antibody was decreased from 7S to 5S. The antibody remained bivalent and the antigen-binding capacity, as measured by the agglutinin titer, was not appreciably affected by the digestion procedure. The ability of the 5S $F(ab')_2$ to inhibit the expression of hemolysis by normal 7S antibody and 7S antibody which had survived pepsin digestion was similar, indicating that no obvious selection for antibodies with certain antigen-binding specificities had occurred during digestion. The agglutinin activities of 7S and $F(ab')_2$ antibody preparations were adjusted to the same titer, so that the comparison was made between two preparations with comparable antibody activities. Preferential degradation of certain subclasses of 7S immunoglobulins has not been excluded.

The Fc portion may affect the behavior of the antibody prior to combining with antigen by altering the rate of excretion or localization of antibody. The $F(ab')_2$ antibody is known to be excreted rapidly, but is protected from rapid excretion when combined with antigen (17, 18). In the present studies, antibody preparations were incubated for 1 hr at 37°C in vitro to insure that most of the antigenic determinants had combined with antibody, but the amount of antibody at high concentrations was well in excess of the amount which could actually be absorbed by the low concentration of erythrocytes (unpublished observations), so that rapid excretion could be a factor in the lack of suppressive activity of $F(ab')_2$ antibody. The intact 7S antibody may localize in such a way that it contacts antigen which is most relevant to the immune response (that is, antigen which is in a position to initiate an immune response), whereas $F(ab')_2$ antibody does not localize in immunologically relevant sites and inter-

acts with any antigen, whether or not the antigen is in a position to initiate the immune response (29). Low doses of $F(ab')_2$ antibody preparations show signs of enhancing priming, and this may indicate that complete exclusion from the immune response mechanism (by elimination or localization) cannot be the whole explanation since this should lead to no change whatsoever.

It would be expected that the 7S antibody-antigen complexes would be removed from the circulation more rapidly than the $F(ab')_2$ antibody-antigen complexes (18) and this is worthy of further investigation. With the erythrocyte system, it appears that rapid phagocytosis of the antibody-antigen complexes occurs and may be a major factor responsible for the depressed immune responses (19). The $F(ab')_2$ antibody-antigen complexes may be incapable of increased rates of elimination, that is, phagocytosis and antigen catabolism may depend on the presence of the Fc portion of the immunoglobulin molecule. Therefore, the regulation of the immune response by antibody may include both the combination of antibody with antigen leading to the masking of antigenic determinants, but also the rapid elimination of antigen, at least in some antigen systems.

The question of possible direct interaction of antibody with the cell or cells which are stimulated by antigen (antigen-sensitive cells) has been investigated with conflicting results (4, 20). These experiments have involved the incubation of antibody-containing preparations with lymphoid cells, washing the lymphoid cells, transferring them to hosts which were incapable of giving immune responses, and investigating whether or not the exposure of lymphoid cells to antibody inactivated them. In these investigations (4, 20), the presence or absence of antigen at the time of incubation of lymphoid cells with antibody-containing sera was not established and may have influenced the results. It is possible that antigen is needed to allow an interaction between the antibody and the antigen-sensitive cell. This role of antigen could be two-fold. First, the antibody may have to be carried to or concentrated on the antigen-sensitive cells by antigen. Second, the interaction between antibody and antigen-sensitive cells may take place only when antibody is complexed to antigen. The recently demonstrated effect of antigen plus antibody in suppressing certain immune responses (7, 21, 22) and the unresponsiveness produced by antigen-antibody complexes (25) lend support to this line of argument. From the results presented here, it may be speculated that, if interaction between antibody and the antigen-sensitive cell does occur, the antibody alters the antigen-sensitive cell by a mechanism involving the Fc portion of the antibody molecule. It is of interest that release from antigen competition could be demonstrated only when the two antigenic groups were located on different molecules of carrier (26) and may indicate that suppression by antibody complexed to a different but neighboring antigenic determinants could prevent a response against the exposed determinant (2). The dependency of the action of anti-lymphocyte serum on

the presence of the Fc fragment (23, 24) may also indicate that the Fc portion, after combination of the anti-lymphocyte antibody with antigenic determinants, is able to inactivate the antigen-sensitive cell population directly.

The data presented here indicate that antibody regulation of the immune response involves more than the simple masking of antigenic determinants, and that the Fc portion of the immunoglobulin molecule plays an important role in the mediation of this regulation. The results are different from those reported by other investigators (19, 27, 28). The reasons for this difference are not clear (12), but it is suggested that $F(ab')_2$ inhibition of 7S activity and the need for physical separation of 5S from 7S antibody after pepsin digestion indicate that occult contamination of 5S $F(ab')_2$ by 7S antibody may account for the results reported by other laboratories. Whatever the ultimate explanation of the reduction in ability of the $F(ab')_2$ antibody and the apparent importance of the Fc portion may be in the regulation of the immune response, it is clear that this phenomenon is by no means minor in terms of degree (and possibly in terms of mechanism also), so that this report may help to stimulate new interest in the biological properties associated with the Fc portion of the immunoglobulins.

SUMMARY

The ability of 7S and $F(ab')_2$ antibody fragments to suppress priming with low doses of antigen was compared. The 7S preparation was approximately 100-1000 times more potent than the $F(ab')_2$ preparation when the agglutinin titers of the two preparations were the same. The presence of any ability to suppress priming in the $F(ab')_2$ preparation may reflect an inherent capacity of the $F(ab')_2$ antibody or contamination with small amounts of 7S antibody.

The difference between 7S and $F(ab')_2$ antibody in ability to suppress priming is attributed to the lack of the Fc portion on the $F(ab')_2$ antibody. The Fc portion may be needed to prevent rapid excretion of antibody from the body, to induce rapid phagocytosis of antigen-antibody complexes with consequent breakdown and elimination of antigen, or to inactivate or suppress the antigen-sensitive cells from reacting to antigenic determinants. More detailed studies will permit a better assessment of the importance of these three possible regulatory roles of the Fc portion of the immunoglobulin in the immune response.

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BIBLIOGRAPHY

1. Uhr, J. W., and G. Moller. 1968. Regulatory effect of antibody on the immune response. *Advan. Immunol.* **8**:81.
2. Uhr, J. W., and J. B. Baumann. 1961. Antibody formation. I. The suppression

of antibody formation by passively administered antibody. *J. Exp. Med.* **113:** 935.

3. Neiders, M. E., D. A. Rowley, and F. W. Fitch. 1962. The sustained suppression of hemolysin response in passively immunized rats. *J. Immunol.* **88:**718.
4. Rowley, D. A., and F. W. Fitch. 1964. Homeostasis of antibody formation in the adult rat. *J. Exp. Med.* **120:**987.
5. Wigzell, H. 1966. Antibody synthesis at the cellular level. Antibody-induced suppression of 7S antibody synthesis. *J. Exp. Med.* **124:**953.
6. Henry, C., and N. K. Jerne. 1968. The depressive effect of 7S antibody and the enhancing effect of 19S antibody in the regulation of the primary immune response. *In* Gamma Globulins. J. Killander, editor. Almqvist and Wiksell, Stockholm. 421.
7. Axelrad, M. A. 1968. Suppression of delayed hypersensitivity by antigen and antibody. Is a common precursor cell responsible for both delayed hypersensitivity and antibody formation? *Immunology.* **15:**159.
8. Terres, G., and W. Wolins. 1959. Enhanced sensitization in mice by simultaneous injection of antigen and specific rabbit antiserum. *Proc. Soc. Exp. Biol. Med.* **102:**632.
9. Stoner, R. D., and G. Terres. 1960. Enhanced antitoxin responses in irradiated mice elicited by complexes of tetanus toxoid and specific antibody. *J. Immunol.* **91:**761.
10. Benacerraf, B., and P. G. H. Gell. 1959. Studies on hypersensitivity. I. Delayed and Arthus-type skin reactivity to protein conjugates in guinea pigs. *Immunology.* **2:**53.
11. Moller, G. 1963. Studies on the mechanism of immunological enhancement of tumor homografts. I. Specificity of immunological enhancement. *J. Nat. Cancer Inst.* **30:**1153.
12. Sinclair, N. R. StC., R. K., Lees, and E. V. Elliott. 1968. Role of the Fc fragment in the regulation of the primary immune response. *Nature.* **220:**1048.
13. Nisonoff, A., F. C. Wissler, L. N. Lipman, and D. L. Woernley. 1960. Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulfide bonds. *Arch. Biochem. Biophys.* **89:**230.
14. Sever, J. L. 1962. Application of a microtechnique to viral serological investigations. *J. Immunol.* **88:**320.
15. Sinclair, N. R. StC., and E. V. Elliott. 1968. Neonatal thymectomy and the decrease in antigen-sensitivity of the primary response and immunological 'memory' systems. *Immunology.* **15:**325.
16. Sinclair, N. R. StC. 1967. Effects of neonatal thymectomy on the haemolysin response to sheep erythrocytes in Swiss albino mice. A time-course study of total, 19S and 7S antibody. *Immunology.* **12:**549.
17. Spiegelberg, H. L., and W. O. Weigle. 1965. The catabolism of homologous and heterologous 7S gamma globulin fragments. *J. Exp. Med.* **121:**323.
18. Spiegelberg, H. L., and W. O. Weigle. 1966. The in vivo formation and fate of antigen-antibody complexes formed by fragments and polypeptide chains of rabbit γG-antibodies. *J. Exp. Med.* **123:**999.
19. Greenbury, C. L., and D. H. Moore. 1968. Non-specific antibody-induced suppression of the immune response. *Nature.* **219:**526.

20. Moller, G. 1964. Antibody-induced depression of the immune response: A study of the mechanism in various immunological systems. *Transplantation.* **2**:405.
21. Axelrad, M., and D. A. Rowley. 1968. Hypersensitivity: specific immunological suppression of the delayed type. *Science.* **160**:1465.
22. Stuart, F. P., T. Saitoh, and F. W. Fitch. 1968. Rejection of renal allografts: specific immunologic suppression. *Science.* **160**:1463.
23. James, K. 1967. Some factors influencing the ability of anti-lymphocytic antibody to suppress humoral antibody formation. *Clin. Exp. Immunol.* **2**:685.
24. Naysmith, J. D., and K. James. 1968. Effect of $F(ab')_2$ from rabbit anti-mouse lymphocyte IgG on the graft versus host reaction in F_1 hybrid mice. *Nature.* **217**:260.
25. Boyns, A. R., and J. Hardwicke. 1968. Immunological responsiveness following the continuous circulation of soluble antigen-antibody complexes. *Immunology.* **15**:263.
26. Brody, N. I., J. G. Walker, and G. W. Siskind. 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.
27. Tao, T. W., and J. W. Uhr. 1966. Capacity of pepsin-digested antibody to inhibit antibody formation. *Nature.* **212**:208.
28. Rowley, D. A., and F. W. Fitch. 1968. Clonal selection and inhibition of the primary antibody response by antibody. *In* Regulation of the Antibody Response. B. Cinader, editor. Charles C Thomas, Springfield, Ill. 127.
29. Dixon, F. J., H. Jacot-Guillarmod, and P. J. McConahey. 1967. The effect of passively administered antibody on antibody synthesis. *J. Exp. Med.* **125**:1119.