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Regulation of the Immune Response

II. FURTHER STUDIES ON DIFFERENCES IN ABILITY OF $F(ab')_2$ AND 7S ANTIBODIES TO INHIBIT AN ANTIBODY RESPONSE

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Summary. The ability of $F(ab')_2$ antibody preparations to suppress an immune response is much less than that of intact 7S antibody. The activity possessed by $F(ab')_2$ preparations withstood repurification procedures, hence contamination with intact 7S antibody is unlikely. Daily or thrice daily injections of antibody did not make equal the suppressive activities of $F(ab')_2$ and intact antibody, indicating that rapid excretion of $F(ab')_2$ antibody is not the sole factor involved in the difference in immunosuppressive potency between intact 7S and $F(ab')_2$ antibody. Some possibilities for distinct differences in the mechanism of the immuno-suppressive action of $F(ab')_2$ and 7S antibodies are raised and discussed.

INTRODUCTION

Previous publications from this laboratory have indicated that there is a large difference between $F(ab')_2$ and intact 7S antibodies in their ability to inhibit the anti-sheep erythrocyte haemolysin response (Sinclair, Lees and Elliott, 1968; Sinclair, 1969). $F(ab')_2$ antibody preparations were shown to possess one-hundredth to one-thousandth the activity of whole antibody in suppressing long-lasting IgG immunological priming when antibody preparations were given in a single injection (Sinclair, 1969). Since the difference between the two antibody preparations was so large, the possibility of contamination of $F(ab')_2$ with small amounts of intact 7S antibody (0·1–1·0 per cent contamination) remained a distinct possibility. In order to investigate this possibility, the $F(ab')_2$ preparations were reisolated on sucrose gradients and the immunosuppressive activity of the $F(ab')_2$ which was isolated once was compared with an $F(ab')_2$ preparation which underwent the isolation procedures twice. The activities of the two $F(ab')_2$ preparations were comparable, indicating that $F(ab')_2$ antibody does have some innate activity in regulating the immune response.

$F(ab')_2$ antibody has been shown to be rapidly excreted (Spiegelberg and Weigle, 1965, 1966). The possibility that rapid excretion of the $F(ab')_2$ antibody could explain its small effect in regulating the immune response was investigated by compensating for excretion with daily injections of antibody. The results established that the defectiveness of $F(ab')_2$ antibody was still apparent, and that under-compensation for a simple excretion factor was not the sole explanation for it.

MATERIALS AND METHODS

Mice

Inbred male and female Swiss mice originally obtained from Carworth Farms, New City, New Jersey, U.S.A., 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 per cent suspension of sheep erythrocytes (2×10^8 cells) (Grand Island Biological Co., Grand Island, New York, U.S.A.) 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. Following five ammonium sulphate precipitations (40 per cent saturation), digestion was carried out in a 0·07 M sodium acetate and 0·05 M sodium chloride buffer at pH 4·0 and 37° for 18 hours, in the presence of 1 mg of pepsin (2650 units/mg, twice crystallized and lyophilized, obtained from Sigma Chemical Company, St Louis, Missouri, U.S.A.) per 100 mg of protein (Nisonoff, Wissler, Lipman and Woernley, 1960). Following pepsin digestion or exposure to acetate buffer, antibody samples were dialysed against phosphate buffered saline (PBS) (0·8 per cent NaCl, 0·1 M phosphate, pH 7·8). Pepsin digestion decreased the haemolysin titres by up to 5 $\log_2$ units without altering the haemagglutinin titres. The 5S $F(ab')_2$ and 7S (intact and undigested) anti-sheep erythrocyte antibodies were separated on 5–20 per cent 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 $9/16\times3\frac{3}{4}$ in. nitrocellulose tubes. The gradients were centrifuged at 40,000 rev/min for 40 hours at 5° in the Beckman L2-65B ultracentrifuge. The separation of 5S and 7S antibodies can be seen in Fig. 1. Haemolysin 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 the same titre in each experiment. In the reisolation experiment, varying amounts of intact 7S or one of the two 5S $F(ab')_2$ antibody preparations were incubated with sheep erythrocytes at various concentrations and the erythrocyte/antibody mixtures were injected intraperitoneally into inbred Swiss mice, so that each animal received either 10^7, 10^6 or 5×10^5 sheep erythrocytes. A period of 56 days was left between this first dose of antigen and re-immunization.

Reimmunization

Whether or not the priming dose of antigen actually did prime was assessed by re-immunizing with 2×10^8 sheep erythrocytes and observing the 7S haemolysin response following this second injection to see if it was in the range of a primary or secondary response.

Measurement of serum haemolysin and haemagglutinin activity

Blood was obtained from the retro-orbital sinus, and diluted 2 : 1 with 0·9 per cent saline to avoid gel formation in the serum. The serum was collected following centrifugation, and endogenous complement inactivated by incubating the serum at 56° for 30 minutes. Serum samples were serially diluted 1 : 1 with 0·9 per cent saline in Microtiter plates (Cooke Engineering Co., Arlington, Virginia, U.S.A.) (Sever, 1962). A standard amount of guinea-pig complement (Grand Island Biological Co., Grand Island, New

York, U.S.A.) was added, and the serum and complement incubated for 30 minutes at 37°. The preincubation of serum and complement lowered the incidence of titrations which were negative at low dilutions but became positive at higher dilutions. Washed sheep erythrocytes (0·05 ml of a 0·5 per cent suspension) were added and the complete mixture incubated for 2 hours at 37° and then read, the end point being roughly half haemolysis on visual inspection. The plates were stored overnight at room temperature and read again the following morning. Titres were usually 1 $\log_2$ unit higher on the second reading. The titres are expressed as the $\log_2$ of dilution. Haemagglutinin titres 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'. In all experiments involving determinations of serum antibody activity, there are six animals at each point.

Ultracentrifugation

Pooled serum, layered on a 10–40 per cent linear sucrose gradient made up to 0·9 per cent with respect to NaCl concentration, was centrifuged in Beckman Model L, L2-50 or L2-65B ultracentrifuge at 35,000 rev/min for 15 hours at 5°. The pooled serum samples were centrifuged in the Type 50 titanium fixed-angle rotor, fitted with adaptors to accommodate $\frac{1}{2} \times 2\frac{1}{2}$ in. polyallomer tubes, or in the Type 40·3 aluminium fixed-angle rotor. The separation of 19S and 7S haemolysins in these 6·5 ml polyallomer tubes in fixed-angle rotors was slightly better than that obtained in the SW50 swinging-bucket rotor (Sinclair and Elliott, 1968). Twenty-four fractions were collected by puncturing the centrifuge tube at the bottom with a No. 18 lumbar puncture needle and each fraction was tested for haemolysin activity.

Calculations

The calculations of total 19S and 7S haemolysin activity were carried out as previously described (Sinclair, 1967).

Haemolytic plaque-forming cell assays

Spleen cell suspensions for assays were prepared by gentle homogenization with a loose fitting tissue crusher which gave similar results to teasing with forceps, but was not such a time consuming process. The cell suspension was prepared in a Medium 199 (Grand Island Biological Co., Grand Island, New York, U.S.A.) and diluted so that 10^6 or 10^7 spleen cells could be plated. The actual number of haemolytic plaque-forming cells (PFC) was determined on duplicate and triplicate plates at the two cell dose levels according to the method described by Jerne *et al.* (Jerne, Nordin and Henry, 1963). The number of antibody-forming cells is expressed as the number of PFC/10^6 spleen cells.

RESULTS

COMPARISON OF REGULATORY ABILITY OF INTACT ANTIBODY AND $F(ab')_2$ ANTIBODY WHICH HAS BEEN ISOLATED EITHER ONCE OR TWICE

Fig. 1 shows the sucrose gradient profile of the pepsin digest and the 5S peak isolated from this digest. The 5S peak of the pepsin digest served as the $F(ab')_2$ which was isolated once, and the 5S peak from the recentrifugation of the original 5S material served as the

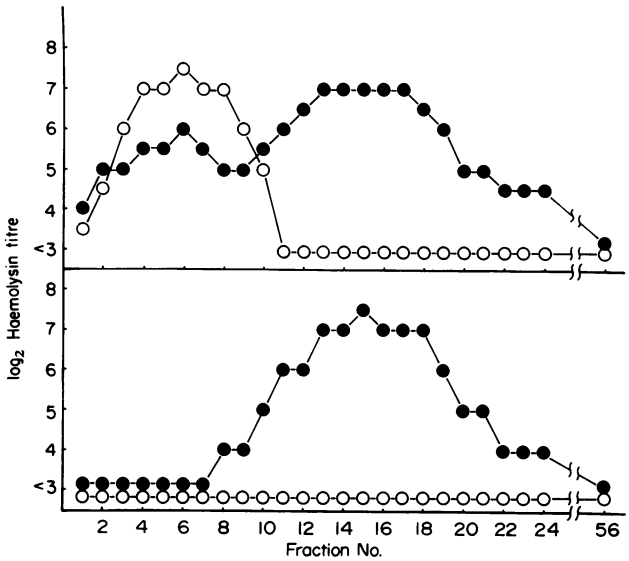

FIG. 1. Sucrose gradient profiles of the pepsin digest of IgG antibody (upper graph) and of the 5S agglutinin peak from the first centrifugation (lower graph). Closed circles (●) indicate haemagglutinin activity and open circles (○) indicate haemolysin activity. Fractions are numbered from the bottom of the tube. Fraction Nos. 12–21 (upper graph) were dialysed against PBS and centrifuged in a sucrose gradient to give a single 5S peak (lower graph).

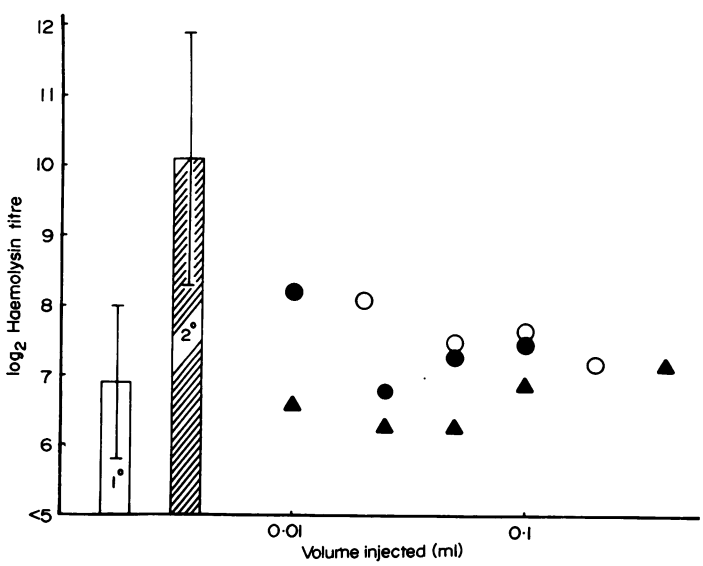

FIG. 2. Effect of varying amounts of $F(ab')_2$ antibody which has been isolated once (●) or twice (○) or intact 7S (▲) antibody given with the priming dose of 5×10^5 erythrocytes on the 7S haemolysin titre 7 days after a second injection of 2×10^8 sheep erythrocytes. The open bar indicates the control primary response, and the hatched bar indicates the titre of control primed animals (secondary response). The limits of three standard deviations of the (reported) mean of the controls are indicated by the vertical lines. Each point represents the geometric average of determinations in six mice.

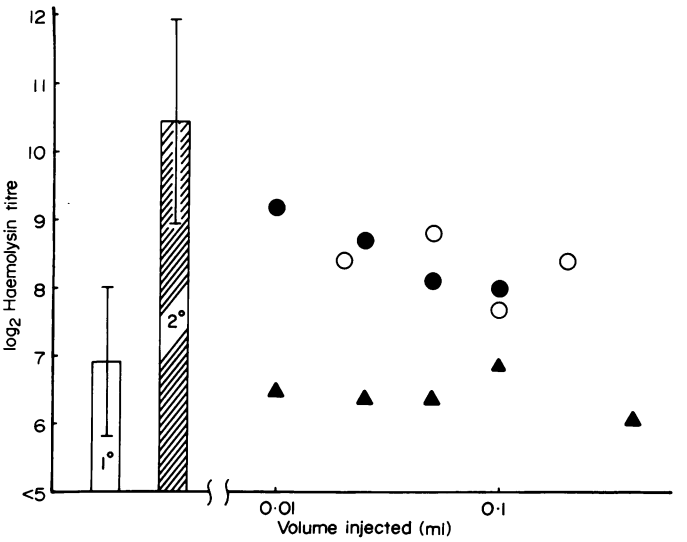

FIG. 3. Effect of varying amounts of $F(ab')_2$ antibody which has been isolated once (●) or twice (○) or intact 7S (▲) antibody given with the priming dose of 10^6 sheep erythrocytes on the 7S haemolysin titre 7 days after a second injection of 2×10^8 sheep erythrocytes. Other details as in Fig. 2.

$F(ab')_2$ which was isolated twice. No 7S antibody could be detected on the second sucrose gradient, indicating that the separation of 5S from 7S was good enough to lower the concentration of the 7S below threshold levels. Figs. 2–4 show the effect of the two $F(ab')_2$ antibody preparations and intact 7S antibody on long-lasting IgG immunological priming when 5×10^5, 10^6 or 10^7 sheep erythrocytes per mouse were given as the priming dose. The dose range of $F(ab')_2$ antibody studied was at the high end of the scale where the transition from priming to lack of priming was observed in the previous study (Sinclair, 1969).

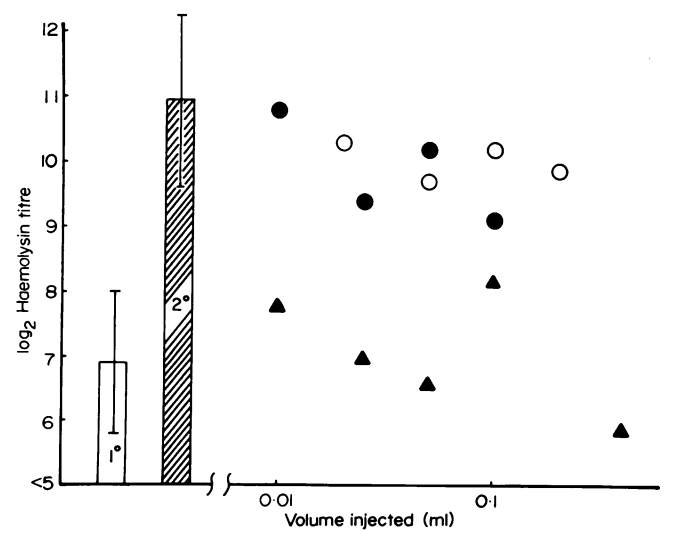

FIG. 4. Effect of varying amounts of $F(ab')_2$ antibody which has been isolated once (●) or twice (○) or intact 7S (▲) antibody given with the priming dose of 10^7 sheep erythrocytes on the 7S haemolysin titre 7 days after a second injection of 2×10^8 sheep erythrocytes. Other details as in Fig. 2.

All antibody preparations were adjusted to a haemagglutinin titre of 7. With a priming dose of 5×10^5 sheep erythrocytes per mouse, the $F(ab')_2$ antibody preparations prevented priming at all antibody dose levels (Fig. 2). With 10^6 sheep erythrocytes per mouse as the priming dose and a low dose of either $F(ab')_2$ antibody preparation, the levels of 7S antibody approached the secondary response range and were approximately 2 $\log_2$ units higher than the 7S-treated group and the control primaries (Fig. 3). These values are transitional between the primary and secondary responses. At a priming dose of 10^7 sheep erythrocytes per mouse, the $F(ab')_2$ gave little evidence of preventing priming whereas the 7S antibody was effective (Fig. 4). In these experiments which cover the transitional range between obtaining and not obtaining priming, there is no evidence that reisolation lessened the activity of the $F(ab')_2$ antibody containing preparation, indicating that contamination with 7S is probably not the reason for the activity of $F(ab')_2$ antibody preparations in suppressing the immune response.

COMPARISON OF $F(ab')_2$ AND INTACT 7S ANTIBODY IN ABILITY TO INHIBIT EARLY IgG PRIMING

More rapid excretion of $F(ab')_2$, compared to intact antibody, could have been the sole explanation for its low capacity in regulating the immune response. To compensate for or lessen the possibility of rapid excretion, antibody was administered daily, and the presence of priming was assessed at 0, 1 and 2 days after priming rather than 56 days in the study reported in the above section or 60 days in a previous study (Sinclair, 1969). In this experiment, 10^6 sheep erythrocytes per mouse were injected intraperitoneally and the animals were given a second injection of 2×10^8 erythrocytes per mouse on the same day or 1 day or 2 days later. The level of 7S haemolysin 7 days after the reimmunization was used to

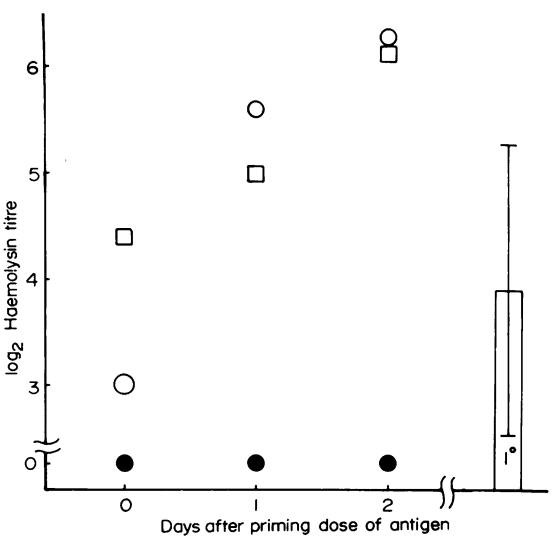

FIG. 5. Effect of $F(ab')_2$ (○) and intact 7S (●) antibody, given daily for 3 days, on the 7S haemolysin titre 7 days after a second injection of 2×10^8. The time interval between the priming dose of 10^6 and the second injection of antigen was varied between 0 and 2 days. The antibody was given 2 and 1 days prior to and on the day of second injection of antigen (therefore, the time-relationship between antibody injections and bleeding for the serum 7S haemolysin titre remained constant). The open bar indicates the 7S haemolysin titre attained in control primary responses, and the vertical line indicates the limits of three standard deviations of the (reported) mean. The onset of priming in mice which were given saline instead of antibody is indicated by the open squares (□). Six mice are included in each point.

assess the presence of priming (Fig. 5). There was an increase in amount of 7S antibody as the time interval between the priming dose and rechallenge was lengthened, and the 7S antibody levels exceeded that of the primary response after 2 days. $F(ab')_2$ and 7S antibody preparations were adjusted to a haemagglutinin titre of 6, and 0·1 ml of antibody was injected 2 and 1 days before and on the day of rechallenge, so that each group received the same amount of antibody. As seen in Fig. 5, the $F(ab')_2$ antibody did not prevent early IgG priming, even when it was given as a daily dose. The 7S antibody prevented any response to the second dose of antigen, so that it cannot be stated whether or not priming occurred, but this dose level of 7S antibody is far in excess of that needed to inhibit long-term IgG priming (Sinclair, 1969). Therefore, even when excretion effects are minimized by daily injections of antibody and by early detection of priming, there is still an observable difference between the $F(ab')_2$ and intact 7S antibody in their effects on priming.

EFFECT OF MULTIPLE DOSES OF ANTIBODY ON THE PRIMARY HAEMOLYSIN RESPONSE

In order to determine whether or not $F(ab')_2$ could inhibit primary antibody responses if given in sufficient amounts, animals were injected with 0·1 ml of $F(ab')_2$ or intact 7S antibody preparations (having haemagglutinin titre of 6) containing 10^7 sheep erythrocytes per 0·1 ml. $F(ab')_2$ or intact 7S antibody (0·1 ml) was also injected 1, 2, 3, 4 and 8 days after immunization. The $F(ab')_2$ antibody had been reisolated on sucrose gradients. Fig. 6 shows that both $F(ab')_2$ and 7S antibodies inhibited the primary haemolysin response (all the haemolysin activity in the 7S-treated group could be attributed to the 7S haemolysin injected). Therefore, with sufficiently vigorous treatment, purified $F(ab')_2$ antibody preparations can be shown to inhibit the primary haemolysin response. A second injection of

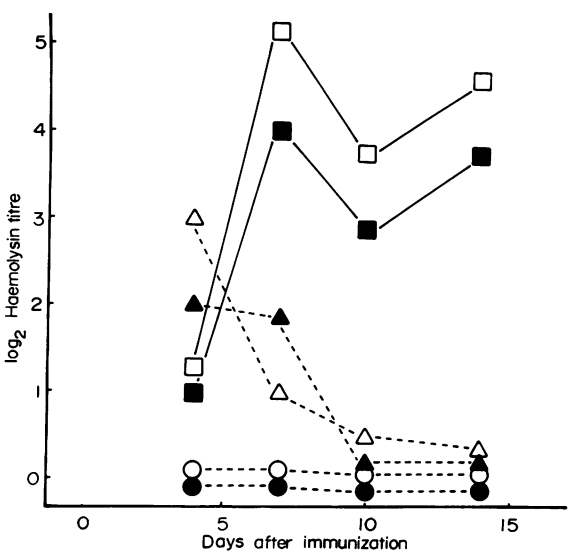

FIG. 6. Effect of $F(ab')_2$ (●) and intact 7S (▲) antibody on the primary total haemolysin response to 10^7 sheep erythrocytes injected intraperitoneally. The open circles (○) and triangles (△) indicate the haemolysin titre of animals which received only $F(ab')_2$ and 7S antibody respectively. The control primary haemolysin responses to 10^7 sheep erythrocytes with (■) and without (□) injections of saline are shown. Antibody or saline injections were given 0, 1, 2, 3, 4 and 8 days after immunization with sheep erythrocytes. Six mice are included in each experimental point and two in those points when antigen was not administered.

TABLE 1

EFFECT OF INTACT (7S) AND $F(ab')_2$ ANTIBODY ON PRIMING WITH 10^7 SHEEP ERYTHROCYTES

Experimental groups*	$\log_2$ Haemolysin titres†	
	19S	7S
Antigen treated		
$F(ab')_2$ (1) + sheep RBC	9·4	11·5
$F(ab')_2$ (2) + sheep RBC	8·4	11·7
Intact 7S + sheep RBC	9·5	7·2
Saline + sheep RBC	10·5	13·0
Sheep RBC	9·7	11·4
Antibody controls		
$F(ab')_2$ (1)	8·9	7·1
$F(ab')_2$ (2)	9·3	6·8
Intact 7S	9·4	6·0

* Initial groups injected with antigen [10^7 sheep erythrocytes (RBC)] and/or antibody preparations. $F(ab')_2$ (1) antibody was purified once and $F(ab')_2$ (2) underwent the purification procedures twice.

† Titres of sera obtained 7 days after challenge with 2×10^8 sheep erythrocytes 3 months after initial treatment with antigen (10^7 sheep erythrocytes) and/or antibody preparations.

antigen revealed that 7S antibody prevented priming whereas the $F(ab')_2$ antibody did not (Table 1).

This initial study did not lend itself to the establishment of the quantitative differences between $F(ab')_2$ and intact antibody in suppressing the primary haemolysin response. Two multiple dose (of antibody) experiments, using varying quantities of antibody and a higher dose of antigen (2×10^8) were set up and the number of PFC in the spleen 4 days after immunization was determined. This allowed a much wider range of the immune

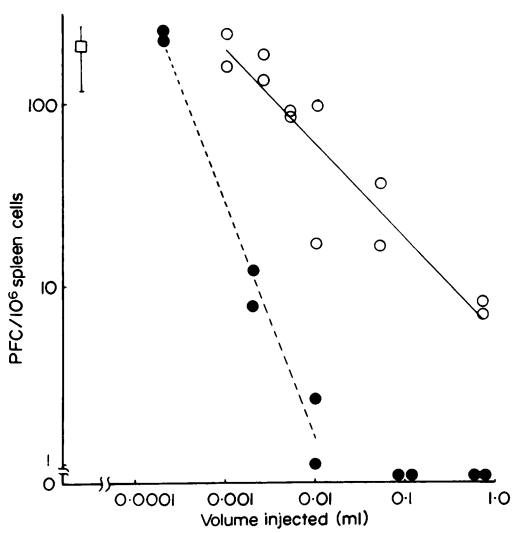

FIG. 7. Effect of $F(ab')_2$ (○) and intact 7S (●) antibody on the number of plaque-forming cells (PFC) per 10^6 spleen cells. Both the dose of antibody and the PFC are plotted on logarithmic scales. The level of the control primary response is indicated by the open square (□). The vertical bar indicates the limits of three standard deviations of the (reported) means. The lines indicate the lines of best fit as calculated by the least squares method. Each experimental point indicates the concentration of PFC in the spleen of one animal as determined on triplicate plating.

response to be observed (100- to 1000-fold in the PFC assay versus an eight-fold range in the serum haemolysin assay), with no contribution of injected antibody in the measurement of the antibody response. In the first experiment (Fig. 7), the $F(ab')_2$ and intact 7S antibody preparations were adjusted to a haemagglutinin titre of 8. Varying amounts of $F(ab')_2$ were injected daily from days 0–3 after immunization. The amount of $F(ab')_2$ antibody injected on each day was kept constant within each experimental group. Varying amounts of 7S antibody were also injected daily, but the amounts given on days 1, 2 and 3 were reduced to one-fifth of the initial amount of 7S antibody given on day 0. This was done to compensate for the fact that the half-life of intact 7S antibody is approximately 4–5 days (Fahey and Sell, 1965), while that of $F(ab')_2$ is likely to be less than 1 day (Spiegelberg and Weigle, 1965, 1966). The amounts of antibody recorded on the x-axis in Fig. 7 represents the initial dose of antibodies given on day 0, therefore some attempt to compensate for the more rapid elimination of $F(ab')_2$ over intact 7S has been made in constructing the graph. In Fig. 7, the amount of antibody initially injected is plotted against the PFC/10^6 spleen cells on a log–log plot. Two observations can be made. Firstly, the linear relationship between the log of amounts of antibody injected and the log of the PFC observed is quite good, the correlation coefficient squared being 0·85 for the $F(ab')_2$ and 0·99 for the intact 7S antibody, whereas the results did not fall on a straight line when plotted on arithmetic or semi-log scales. Secondly, the most obvious difference between the two lines is the differing slopes. $F(ab')_2$ antibody induced a fall off in PFC with a slope of $-0{\cdot}5$, whereas intact 7S antibody gave a slope of $-1{\cdot}3$. There is 1 chance in a 1000 ($P = 0{\cdot}001$) that the slope for the 7S line includes a value of $-0{\cdot}5$. There is one chance in a 1000 that the slope for $F(ab')_2$ is as steep as $-0{\cdot}83$, so that the P-value to include $-1{\cdot}3$ would be very much smaller than 0·001.

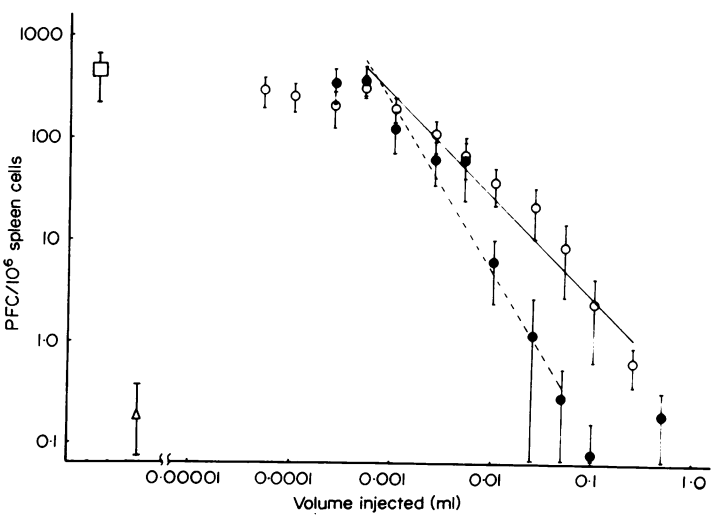

FIG. 8. Effect of $F(ab')_2$ (○) and intact 7S (●) antibody on the number of plaque-forming cells (PFC) per 10^6 spleen cells. Both the dose of antibody and the PFC are plotted on logarithmic scales. The level of the control primary response is indicated by the open square, and the background level of PFC (no antigen administered) by the open triangle. The vertical bars indicate the limits of three standard deviations of the (reported) means. The lines indicate the lines of best fit as calculated by the least squares method. Portions of the PFC response which were consistently within either control or background levels were excluded, and only the portion where there was an observable dose-response relationship was included in the construction of the line. Each value given is the average of six mice, and each mouse was tested in triplicate.

In another experiment, antibody preparations (haemagglutinin titres of 11) were administered three times a day (Fig. 8). The amount of $F(ab')_2$ administered at each injection was the same within one dose level. With 7S antibody, the amount administered on first injection was five times as concentrated as that given on subsequent injections. Assays for the number of PFC in the spleen and the concentration of antibody in circulation at 4 days after administration of antigen were performed. The slopes of the lines in this experiment are $-1{\cdot}60$ for intact 7S antibody and $-0{\cdot}98$ for $F(ab')_2$ antibody. The correlation coefficient squared is 0·92 for the $F(ab')_2$ line and 0·90 for the 7S line. The slopes are significantly different ($P<0{\cdot}001$). The abscissa in this graph (Fig. 8) indicates the amount of antibody given at the initial injection, and some preliminary data indicate that, under the injection schedule used, this corresponded approximately to the serum titre attained by the passively administered antibody. Therefore, compensation for differences in rates of excretion between the two antibody preparations was reasonably complete in this experiment. Fig. 9 presents the data on a semi-logarithmic plot, showing more clearly that there is a ten-fold difference in amount of antibody needed to reduce the number of PFC/10^6 spleen cells to 1·0. If this were a survival curve analysis of antigen-reactive cells, it would give little evidence of a sensitive and a resistant cell population, but more or less of a continuum from highly sensitive to highly resistant (indicative, presumably, of a continuum in the binding-affinities of antigen-receptors on the antigen-reactive cells).

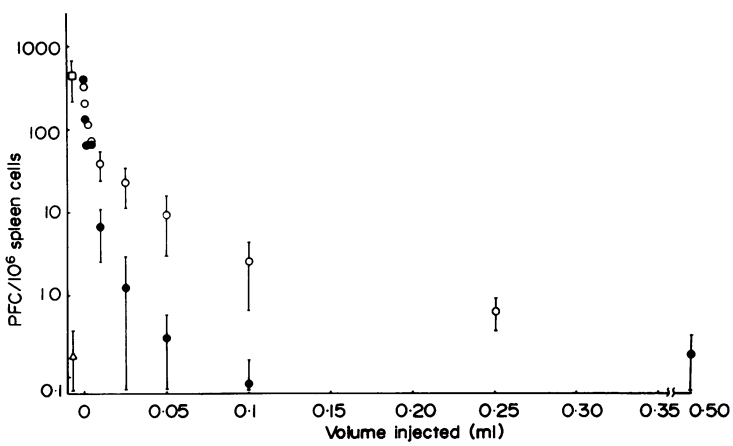

FIG. 9. Effect of $F(ab')_2$ (○) and intact 7S (●) antibody on the number of plaque-forming cells (PFC) per 10^6 spleen cells. The dose of antibody is plotted on an arithmetic scale, while the PFC are plotted on a logarithmic scale (semilogarithmic). The control values are indicated as in Fig. 8. The vertical bars indicate the limits of three standard deviations of the (reported) means. Each value given is the average of six mice, and each mouse was tested in triplicate.

DISCUSSION

Pepsin-digested $F(ab')_2$ antibody is much less effective in inhibiting the immune response than is intact 7S antibody. The activity of the $F(ab')_2$ preparations in inhibiting the response is not likely to be due to contamination with whole 7S because repurification on sucrose gradients did not lessen the activity. The difference in slope of the line when the logarithm of the PFC/10^6 spleen cells was plotted against the logarithm of antibody given (Figs. 7 and 8) also indicates that the suppressive activity of $F(ab')_2$ preparations is not due to contaminating 7S antibody. If it were, then the line should be shifted to the right without a change in the slope.

Daily or thrice daily replacement of antibody did not obliterate the difference between $F(ab')_2$ and 7S antibody, suggesting that excretion is not the sole factor involved in the difference. In the PFC experiments, the difference in excretion rates between $F(ab')_2$ and intact 7S antibody was compensated for by replacing all the $F(ab')_2$ antibody at each injection, while replacing only one-fifth the amount of intact 7S antibody given in the first injection. Regardless of the replacement procedure, the slopes of the two lines differed significantly ($P<0{\cdot}001$). If the compensation for the excretion had been inadequate, the $F(ab')_2$ line would be moved to the right (in a simple model for excretion) but the slope would not have been altered. A more complicated explanation based on an excretion hypothesis would be that the half-life is decreased with increasing dose of $F(ab')_2$ antibody at a much larger rate than that reported for intact 7S antibody (Fahey and Sell, 1965). Difference in the rates of increase in excretion of $F(ab')_2$ and intact 7S antibody with increasing doses of antibody would have to be such that, when 7S antibody lowered the PFC to 1·0, the $F(ab')_2$ excretion rate would have increased approximately ten to 100 times faster than that of intact 7S antibody. This prediction on the basis of an excretion hypothesis for the difference between $F(ab')_2$ and intact 7S antibody is open to experimental analysis, but seems rather unlikely.

At first sight, the results reported here are at variance with those reported by others (Tao and Uhr, 1966; Rowley and Fitch, 1968; Greenbury and Moore, 1968). We first considered the possibility that other laboratories were working with $F(ab')_2$ preparations which were contaminated by 7S antibody (Sinclair *et al.*, 1968; Sinclair, 1969) but, as stated above, we now believe that $F(ab')_2$ antibody does have an innate ability to suppress the antibody response which is independent of the activity possessed by intact 7S antibody. The reason why we have observed the difference between $F(ab')_2$ and 7S while others have not may relate to the different experimental models used or because we have varied the dose of both antibodies over a large range instead of dealing only with large amounts of antibodies when both antibodies would lead to suppressed immune responses. The question asked by other investigators was, would the $F(ab')_2$ antibody suppress the immune response, whereas this laboratory addressed itself to the question, do the $F(ab')_2$ and 7S antibodies differ in their abilities to suppress immune responses. An affirmative answer can now be given to both questions. It should be stressed that an affirmative answer to the first question does not allow one to conclude that there is no difference in mechanism of action between $F(ab')_2$ and intact antibodies in suppressing immune responses, but an affirmative answer to the second question suggests that differences in mechanism do exist.

What function may be served by the Fc-fragment making it important in the regulation of the immune response by antibody? Excretion has been ruled out as the sole factor although it probably plays some role. On combination of antibody with antigen, the Fc-portion may play a role in the destructive phagocytosis of the antigen by aiding in the attachment of antibody–antigen complexes to macrophages by a cytophilic mechanism (Berken and Benacerraf, 1966) and by activation of the degradative processes in macrophages (Greenbury and Moore, 1968). The Fc-fragment may also act as a negative feedback signal to the antigen-sensitive cells following combination with and transport by the antigen to the antigen-sensitive cells. If this were true, the administration of more antigen would allow the combination of more antibody with antigen and this would turn on more negative Fc-signals, resulting in a greater suppression at higher antigen dosage. This is, in fact, the case in one system; with exposure to a constant amount of antibody, the absolute level of the immune response to higher antigen doses may be lower than that attained with

lower doses of antigen (Möller, 1969). Such an observation cannot be accounted for by the simple masking of antigenic determinants or by a consideration of binding-affinities of receptor sites on antigen-sensitive cells (Benacerraf and Gell, 1959; Uhr and Baumann, 1961; Möller, 1963; Dixon, Jacot-Guillarmod and McConahey, 1967; Uhr and Möller, 1968), but favours the idea that a part of the immune response system can be altered (either reversibly or irreversibly). This alteration of a cellular process involved in the immune response could be attained through a signaling system which involves the Fc-portion on antibodies which are attached to antigen.

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