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The Immune Response Suppressed by Specific Antibody

D. A. ROWLEY, F. W. FITCH, M. A. AXELRAD* AND C. W. PIERCE†

Department of Pathology, University of Chicago, Chicago, Illinois, U.S.A.

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Summary. Homologous or heterologous anti-sheep erythrocyte serum given passively to normal rats markedly suppressed their spleen plaque-forming cell and serum antibody response to sheep erythrocytes. Passive immunization against bovine γ-globulin prevented 'sensitization' by a first injection of the antigen. The suppressive effect of passive antibody was prevented or partially prevented by adjuvants, *B. pertussis* vaccine, *S. typhi* endotoxin or Freund's complete adjuvant. Passive antibody or adjuvants had relatively little effect on the primary response when given more than 24 hours after antigen or on the secondary response when given with antigen.

The kinetics of the early spleen plaque-forming cell response were measured using sheep erythrocytes as antigen, homologous anti-sheep erythrocyte serum for passive immunization and *B. pertussis* vaccine as adjuvant. With a constant antigen dose, larger amounts of passive antibody caused increased suppression. Suppression apparently resulted from a decrease in the number of cells initially responding; the rate of proliferation of cells that did respond was not affected by passive antibody. If the amount of passive antibody was kept constant, an increase in antigen dose or addition of adjuvant to the antigen increased the rate of proliferation of the cells that did respond; an effect sufficient to completely mask suppression produced by smaller amounts of passive antibody. These findings can be accounted for by assuming that passive antibody and higher antigen doses or adjuvant affect different interactions required for the antibody response. Thus, the magnitude of the antibody response is dependent not only on the amounts of antigen and passively given antibody but also on the amount and activity of any intentionally or unintentionally introduced factor having adjuvant activity.

INTRODUCTION

The primary antibody response to some antigens is profoundly suppressed by passive immunization with specific antibody to the antigen (Uhr and Möller, 1968). Advantage is taken of this phenomenon to prevent Rh sensitization of human females (Freda, Gorman and Pollack, 1966). In recent studies using rats, passive antibody together with antigen profoundly and specifically suppressed induction of delayed type hypersensitivity (Axelrad and Rowley, 1968) or renal allograft rejection (Stuart, Saitoh and Fitch, 1968). Thus, an understanding of the mechanism of antibody suppression of the immune response is not only of general biological interest but may be important eventually for controlling other immunological reactions encountered in clinical medicine.

* Present address: Department of Pathology, Queen's University, Kingston, Ontario, Canada.
† Present address: National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.

Presumably, antibody interacts with antigen to reduce the antigenic stimulus. The fact that equivalent responses can result from either a combination of high antigen doses and passive antibody or with low antigen doses alone supports this contention. The present findings suggest, however, that the mechanism of suppression is more complex. Whereas lower antigen doses cause slower rates of division of responding cells, passive antibody appears to decrease the number of cells initially responding. Adjuvants may mask the effect of antibody suppression by increasing the rate of division of cells that do respond, a phenomenon of considerable importance since antigen or antibody preparations may contain material with adjuvant activity.

MATERIALS AND METHODS

Young male Sprague–Dawley rats were used. Intravenous injections were made in the lateral tail vein; intracutaneous injections were made on the shaved flank.

Antigens, adjuvants and antisera

The antigens were washed sheep erythrocytes or bovine γ-globulin (BGG), (Pentex, Kankakee, Illinois). Washed whole erythrocytes were prepared from sterile sheep's blood stored in Alsever's solution. A single sample of blood was used in each experiment for immunizing animals and titrating immune responses. The sheep erythrocytes were washed four times in large volumes of freshly prepared, pyrogen-free saline and used immediately. Doses of sheep erythrocytes were standardized by cell counts. The BGG was purified by the batch DEAE-cellulose procedure of Stanworth (1960); the pooled eluates, dialysed against large volumes of distilled water, were lyophilized and stored at 4°. The adjuvants were *Bordetella pertussis* vaccine (Lilly, strain 37790, Lot T-59 187 was kindly supplied through Dr H. Campbell, Jr, Biological Development, Eli Lilly and Co., Indianapolis, Indiana), *Salmonella typhi* endotoxin (Difco Laboratories) or Freund's complete adjuvant (Difco Laboratories).

Pooled antisera were: (1) a late primary response rat anti-sheep erythrocyte serum with an agglutinin titre of 1280 for total and 160 for '7S' antibody (antibody not inactivated by 2-mercaptoethanol); (2) a rabbit anti-sheep erythrocyte serum with an agglutinin titre of 10,240 for total and 2560 for '7S' antibody, and (3) a concentrated early primary response rat anti-BGG serum with a passive haemagglutinin titre of 10,240 for total antibody, and no detectable '7S' antibody. The anti-erythrocyte sera were prepared from rapidly chilled blood; aliquots of the freshly collected sera were immediately frozen and stored at $-30°$ until used. The rat anti-BGG serum was obtained 4 days after a single intravenous injection of 10 ml of BGG mixed with 100 μg *S. typhi* endotoxin. The antibody was concentrated by batch DEAE-cellulose chromatography followed by an elution procedure (Stanworth, 1960; Levy and Sober, 1960).

Normal rats each passively immunized with 1·0 ml of the undiluted rabbit antisera had mean titres against sheep erythrocytes of 350 at 4 days; normal rats injected with 1·0 ml of the undiluted rat anti-sheep erythrocyte serum had mean titres of 35 at 4 days.

Sheep erythrocyte–antibody complexes were prepared by incubating the antigen, 10^{10} erythrocytes/ml, with an equal volume of heat inactivated rat anti-sheep erythrocyte serum for 20 minutes at 37°. The complexes, dispersed and freed of non-complexed antibody by washing in large volumes of saline, were reconstituted to contain 4×10^9 sheep erythrocytes/ml.

Spleen plaque-forming cells and antibody titres

The methods for measuring antibody titres for sheep erythrocytes and numbers of plaque-forming cells in spleens producing haemolysins for sheep erythrocytes have been described (Rowley, Fitch, Mosier, Solliday, Coppleson and Brown, 1968). Antibody against BGG was titrated by the tanned cell passive haemagglutinin procedure of Stavitsky (1954). Sera were also titrated for antibody inactivated by 2-mercaptoethanol; for convenience, antibody not inactivated by 2-mercaptoethanol is referred to as '7S' antibody.

The calculated total number of plaque-forming cells per spleen was transformed by taking the logarithm of the number to the base 10. Where means are recorded untransformed, they represent the antilog of the mean of the logarithmically transformed data. All statistical calculations were performed on the logarithmically transformed data. An antibody titre was taken as the reciprocal of the highest serum dilution producing 1+ agglutination of erythrocytes. For convenience in comparing the plaque-forming cells and antibody responses, titres were also transformed by taking the logarithm of the number to the base 10. Where mean titres are recorded untransformed, they represent the antilog of the mean of the logarithmically transformed data.

Cell doubling and cell cycle times

Methods for estimating cell doubling and cell cycle times have been presented in detail (Rowley *et al.*, 1968). Doubling time was calculated from the increase in spleen plaque-forming cells occurring 1–4 days after immunization; it is the time required for the population of cells to double. Cell cycle time was estimated using mitotic blocking agents. Rats were injected intravenously with colchicine or Velban 2, 3 or 4 days after immunization. The animals were killed 2·5–7 hours after drug injection. The number of plaque-forming cells in spleens of these animals was compared with the number in similarly immunized but non drug-treated controls. Drug treatment caused a reduction in number of spleen plaque-forming cells, the extent of reduction being directly proportional to the duration of drug treatment. The drug effects were consistent with assumptions that: (1) plaque-forming cells arrested in mitosis do not release sufficient antibody to be detected; (2) mitotic blocking agents by arresting plaque-forming cells in metaphase prevent not only detection of these cells but also the increase in numbers of cells which would have resulted from cell division; and (3) mitotic blocking agents do not affect release of antibody by cells in interphase. Cell cycle times based on the extent of reduction of plaque-forming cells per unit time of drug treatment were estimated using a mathematical model appropriate for exponentially increasing populations of cells. In the present experiments Colchicine, 0·5 mg/ml, injectable, was purchased from Eli Lilly and Co. The drug dose was 1·5 mg/kg.

EXPERIMENTAL RESULTS

SUPPRESSION OF IMMUNE RESPONSE BY PASSIVELY ADMINISTERED ANTIBODY

Adjuvant enhancement of the immune response suppressed by antibody

The effects of different adjuvants on several immunological responses were tested:

Twenty-five rats were each injected with 10^9 sheep erythrocytes and twenty-five were each injected with 10^9 sheep erythrocytes mixed with 0·15 ml of *B. pertussis* vaccine. Thirty minutes before active immunization five rats in each group were passively immunized with 1·0 ml of rabbit anti-sheep erythrocyte serum diluted 1 : 4, 1 : 20, 1 : 100 or 1 : 500. Five remaining

TABLE 1

ADJUVANT ENHANCEMENT OF THE IMMUNE RESPONSE SUPPRESSED BY HETEROLOGOUS ANTIBODY

Immunization*		Plaque-forming cells per spleen†	Antibody titre†
Passive	Active		
+ (1 : 4)	SRBC+*B. pertussis*	468 (2·67±0·20)	79 (1·90±0·00)
+ (1 : 4)	SRBC	36 (1·56±0·15)	68 (1·83±0·08)
+ (1 : 20)	SRBC+*B. pertussis*	2,455 (3·39±0·17)	20 (1·30±0·00)
+ (1 : 20)	SRBC	363 (2·56±0·17)	17 (1·23±0·08)
+ (1 : 100)	SRBC+*B. pertussis*	91,210 (4·96±0·20)	53 (1·72±0·20)
+ (1 : 100)	SRBC	4,787 (3·68±0·16)	10 (1·00±0·00)
+ (1 : 500)	SRBC+*B. pertussis*	794,400 (5·90±0·01)	742 (2·87±0·15)
+ (1 : 500)	SRBC	52,490 (4·72±0·17)	69 (1·84±0·24)
None	SRBC+*B. pertussis*	2,188,000 (6·34±0·11)	4,266 (3·63±0·16)
None	SRBC	134,900 (5·13±0·15)	646 (2·81±0·10)

* Passively immunized rats received 1·0 ml of the indicated dilution of rabbit anti-sheep erythrocyte serum 30 minutes before active immunization with 10^9 sheep erythrocytes or 10^9 sheep erythrocytes mixed with 0·15 ml of *B. pertussis* vaccine.

† The number of plaque-forming cells or antibody titre is the antilog of the mean for five rats killed 4 days after immunization; the log of the mean ± the standard error of the mean is given in parentheses.

animals in each group served as controls. Responses, measured 4 days after immunization, are presented in Table 1. Suppression was approximately ten-fold less with each five-fold dilution of antiserum. All dilutions of antiserum contained sufficient antibody to produce a marked suppression of an active serum antibody response. Adjuvant had the effect of enhancing responses by seven- to nineteen-fold; enhancement for rats passively immunized with antiserum diluted 1 : 500 was sufficient to result in appreciable titres of actively produced antibody.

S. typhi endotoxin produced enhancement similar to that of *B. pertussis* vaccine.

Ten rats were each injected with 10^9 sheep erythrocytes and ten rats were each injected with 10^9 sheep erythrocytes mixed with 100 μg of *S. typhi* endotoxin. Thirty minutes before active immunization, five rats in each group were passively immunized with 1·0 ml of rat anti-sheep erythrocyte serum. At 4 days spleens of rats receiving only antigen contained 91,240 (4·96±0·17) plaque-forming cells; passive immunization reduced the response to 562 (2·75±0·27) plaque-forming cells. The adjuvant enhanced responses to 1,381,000 (6·14±0·13) for rats receiving antigen and to 20,420 (4·31±0·18) for the passively immunized rats.

A third adjuvant, Freund's complete adjuvant, also prevented suppression produced by antibody.

Five rats were each injected intracutaneously in three equal depots on the flank with a total of 10^9 sheep erythrocyte–antibody complexes suspended in 0·5 ml of saline; five rats were similarly injected with 10^9 sheep erythrocyte–antibody complexes suspended in 0·25 ml of

TABLE 2

THE EFFECT OF FREUND'S COMPLETE ADJUVANT ON THE ANTIBODY RESPONSE TO ANTIGEN–ANTIBODY COMPLEXES

Immunization*	Antibody titre†
SRBC–AB complexes+FCA	372 (2·57±0·11)
SRBC–AB complexes	<10 –
SRBC+FCA	646 (2·81±0·14)
SRBC	159 (2·20±0·10)

* Rats were injected intracutaneously with 10^9 sheep erythrocytes or 10^9 sheep erythrocyte–antibody complexes suspended in saline or adjuvant.

† The antibody titre is the antilog of the mean for five rats bled 9 days after immunization; the log of the mean ± the standard error of the mean is given in parentheses.

saline and mixed with an equal volume of Freund's complete adjuvant. Controls included five rats injected similarly with 10^9 sheep erythrocytes in 0·5 ml of saline and five rats injected with 10^9 sheep erythrocytes in 0·25 ml of saline and mixed with an equal volume of Freund's complete adjuvant. The results, recorded in Table 2, show that animals receiving complexes alone had no detectable antibody response (at 9 days or any time later) but animals receiving the complexes mixed with the adjuvant had higher titres than controls receiving antigen alone.

The phenomenon of adjuvant enhancement of responses suppressed by antibody was also demonstrable using a soluble antigen.

Twelve rats were injected intravenously with 1·0 μg of BGG; twelve rats in a second group were injected intravenously with 1·0 μg of BGG mixed with 100 μg of *S. typhi* endotoxin. Six rats in each group had been passively immunized with 1·0 ml of homologous anti-BGG serum 30 minutes before active immunization. The rats were bled to detect the level of antibody 4, 8, 12 and 28 days later. After the bleeding on the 28th day, all animals were injected intravenously with 1·0 μg of BGG. The animals were bled 4, 6 and 8 days after the second immunization. Peak antibody responses, occurring 4 days after the first and 6 days after the second immunization, are recorded in Table 3. Rats initially immunized with BGG alone, whether passively immunized or not, had no detectable circulating antibody 4 or more days after the first immunization. Rats initially immunized with BGG mixed with endotoxin, whether passively immunized or not, had

TABLE 3

THE EFFECT OF *S. typhi* ENDOTOXIN ON ANTIBODY SUPPRESSION OF THE RESPONSE TO BOVINE γ-GLOBULIN

First immunization*		Primary response antibody titre†	Second immunization‡	Secondary response antibody titre‡
Passive	Active		Active	
+	BGG+*S. typhi* endotoxin	363 (2·56±0·14)	BGG	2884 (3·46±0·05)
+	BGG	<10 –	BGG	<10 –
None	BGG+*S. typhi* endotoxin	324 (2·51±0·16)	BGG	2571 (3·41±0·11)
None	BGG	<10 –	BGG	1289 (3·11±0·11)

* The passively immunized rats received 2·0 ml of rat anti-BGG serum 30 minutes before active immunization. Rats were actively immunized by intravenous injection of 1·0 μg of bovine γ-globulin (BGG) or with 1·0 μg of BGG mixed with 100 μg of *S. typhi* endotoxin.

† The antibody titre is the antilog of the mean for six rats bled 4 days after the first immunization or 6 days after the second immunization; the log of the mean ±the standard error of the mean is given in parentheses.

‡ All rats were injected intravenously with 1·0 μg of BGG 28 days after the first immunization; none of the animals had measurable antibody to BGG at this time.

moderately high titres of antibody 4 days after the first immunization. None of the animals had detectable titres of anti-BGG antibody 28 days after the first immunization. Rats immunized initially with BGG alone had high antibody titres following the second immunization indicating that the first antigen injection had 'sensitized' for a secondary response. In contrast, rats passively immunized prior to the first immunization with BGG alone had no detectable antibody response 4, 6 or 8 days after the second immunization with BGG. Thus, passive immunization apparently prevented 'sensitization' by the first injection of antigen alone. Passive immunization did not suppress the primary response when endotoxin was given with the antigen, and these animals had high secondary antibody responses to antigen alone.

Adjuvant enhancement of responses suppressed by antibody might occur because adjuvant caused a rapid loss of passively given antibody. However, antibody titres 1–4 days after passive immunization alone were unaffected by adjuvant injections. Adjuvant enhancement might be suppressed by serum from animals receiving adjuvant alone. However, no such effect was observed in repeated attempts using 'early' or 'late' serum obtained from rats injected with adjuvants.

The failure of antibody to suppress and adjuvant to enhance once immunization had occurred

No detectable increase in spleen plaque-forming cells occurs for 24 hours following a first injection of sheep erythrocytes. Suppression by antibody or enhancement by adjuvant was almost as great when the agents were given 24 hours after antigen as when they were given with antigen. However, after 24 hours, when spleen plaque-forming cells are increasing exponentially, the agents have much less effect on responses.

Thirty-five rats were each injected with 10^9 sheep erythrocytes. Two days after antigen injection spleens of five rats contained 840 ($2{\cdot}92\pm0{\cdot}10$) plaque-forming cells. Ten of the remaining rats served as controls, ten were each injected with 2·0 ml of rat anti-sheep erythrocyte serum and ten were each injected with 0·15 ml of *B. pertussis* vaccine. At 3 and 4 days spleens of control rats contained 18,900 ($4{\cdot}28\pm0{\cdot}05$) and 47,100 ($4{\cdot}67\pm0{\cdot}10$) plaque-forming cells; spleens of passively immunized rats contained 25,500 ($4{\cdot}41\pm0{\cdot}17$) and 34,000 ($4{\cdot}53\pm0{\cdot}10$) and spleens of the adjuvant treated rats contained 16,700 ($4{\cdot}22\pm0{\cdot}15$) and 67,000 ($4{\cdot}82\pm0{\cdot}13$) plaque-forming cells.

The secondary response measured many weeks after a first antigen injection differs from the primary response in that spleen plaque-forming cells begin to increase exponentially within a few hours after antigen injection, and the peak response occurs 1 day earlier, i.e. on the 3rd rather than the 4th day. Amounts of antibody or adjuvant which profoundly affect the primary response have only slight or no effect on the secondary response even when the agents are given with antigen as shown in the following experiments.

In the first experiment, fifteen rats injected with 10^9 sheep erythrocytes 5 weeks previously were divided into three groups of five animals each: controls which received no additional injections; rats in a second group which were injected with 10^9 sheep erythrocytes, and rats in a third group which were injected with 2·0 ml of rat anti-sheep erythrocyte serum 30 minutes before injection with 10^9 sheep erythrocytes. Responses were measured at 3 days. Spleens of the controls contained 1700 ($3{\cdot}23\pm0{\cdot}12$), spleens of rats receiving a second antigen injection contained 135,300 ($5{\cdot}13\pm0{\cdot}14$), and spleens of rats passively immunized before the second antigen injection contained 86,000 ($4{\cdot}93\pm0{\cdot}27$) plaque-forming cells. Mean serum antibody responses were equal for the second and third groups.

In the second experiment, twenty-two rats injected with 10^9 sheep erythrocytes 5 weeks previously were divided into one control group of six animals and four test groups of four animals each. The six controls received no additional injections. One group was injected with 10^9 sheep erythrocytes and a second group with the same amount of antigen mixed with 0·15 ml *B. pertussis* vaccine. A third group was injected with 10^6 sheep erythrocytes and the fourth group with the same amount of antigen mixed with 0·15 ml *B. pertussis* vaccine. Responses were measured at 3 days. Spleens of controls contained 1400 ($3{\cdot}15\pm0{\cdot}11$) plaque-forming cells. For the high antigen dose, spleens contained 222,400 ($5{\cdot}35\pm0{\cdot}10$) or with adjuvant 497,200 ($5{\cdot}70\pm0{\cdot}15$) plaque-forming cells. For the low antigen dose spleens contained 14,000 ($4{\cdot}15\pm0{\cdot}21$) or with adjuvant 17,600 ($4{\cdot}25\pm0{\cdot}33$) plaque-forming cells.

THE MECHANISM OF SUPPRESSION BY PASSIVE ANTIBODY

The preliminary experiments reported in the previous section gave no clue to the mechanism whereby antibody suppressed and adjuvant enhanced responses. In the following experiments the kinetics of the early spleen plaque-forming cell responses were measured using sheep erythrocytes as antigens, homologous anti-sheep erythrocyte serum for passive immunizations and *B. pertussis* vaccine as adjuvant. These experiments indicate that antibody decreases the number of cells initially responding to antigen while increasing the antigen dose or adding an adjuvant to the antigen increases the rate of division of those cells that do respond.

Kinetics of the plaque-forming cell response suppressed by antibody

The effect of various dilutions of rat anti-sheep erythrocyte serum on the early primary response was measured.

> One hundred and sixty rats were divided into four equal groups. Rats in one group received 1·0 ml of saline; rats in the second group received 1·0 ml of the serum diluted 1 : 5; rats in the third group received 1·0 ml of the serum diluted 1 : 50, and rats in the fourth group received 1·0 ml of the serum diluted 1 : 500. Thirty minutes after injections all animals were actively immunized with 10^9 sheep erythrocytes. Ten rats in each group were killed 1, 2, 3 and 4 days later.

Spleens of all animals killed at 1 day and spleens of rats passively immunized with serum diluted 1 : 5 and killed at 2 days contained twenty-five to 100 plaque-forming cells, about the same number as found in the spleens of normal rats. Responses for the remaining groups are plotted in Fig. 1. Between days 2 and 3 plaque-forming cell doubling times were 5·6, 5·5 and 5·5 hours for rats receiving no antisera or serum diluted 1 : 500 or 1 : 50; between days 2 and 4 doubling times were 7·0, 7·8 and 7·9 hours, respectively, for these groups.

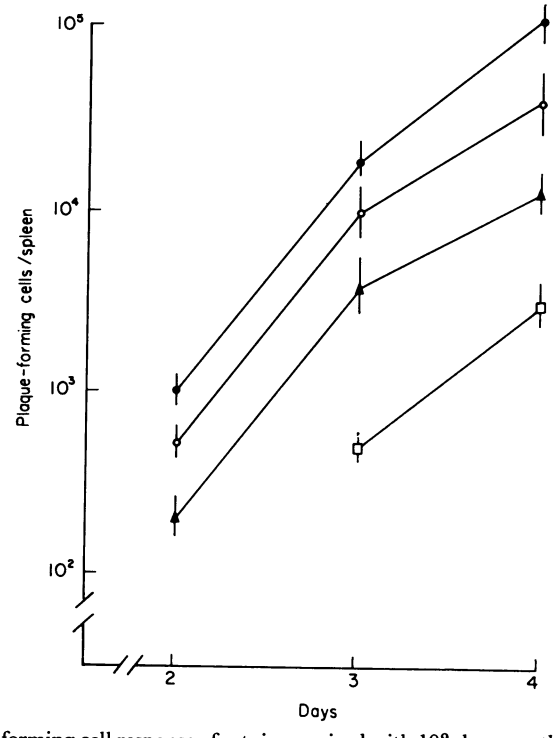

FIG. 1. Spleen plaque-forming cell response of rats immunized with 10^9 sheep erythrocytes. Groups were injected 30 minutes before active immunization with either saline (●) or rat anti-sheep erythrocyte serum diluted 1 : 5 (□), 1 : 50 (▲) or 1 : 500 (○). Each point represents the mean for ten rats ± the standard error of the mean plotted as a vertical bar.

Doubling times could not be estimated between days 2 and 3 for rats suppressed with antiserum diluted 1 : 5; between days 3 and 4 the doubling time was 9·2 hours for this group. (The doubling time for the non-passively immunized rats was 9·6 hours between days 3 and 4.) In the following experiment cell cycle times were estimated using a method which had the advantage that the rate of division of plaque-forming cells could be estimated at 3 days independent of responses at 2 days.

Sixteen rats in one group were injected with 1·0 ml of saline; sixteen rats in a second group were injected with 1·0 ml of rat anti-sheep erythrocyte serum diluted 1 : 20. Thirty minutes later all rats were injected with 10^9 sheep erythrocytes. On the 3rd day, 5 hours before being killed, eight rats in each group were injected with 1·0 ml of saline and eight remaining rats in each group were injected with colchicine, 1·5 mg/kg.

TABLE 4

PLAQUE-FORMING CELL CYCLE TIMES FOR PASSIVELY AND ACTIVELY IMMUNIZED RATS

Immunization*		Colchicine treatment†	Plaque-forming cells/spleen‡	Cell cycle time (hours)
Passive	Active			
+ (1 : 20)	SRBC	None	1,951 (3·11±0·16)	
+ (1 : 20)	SRBC	−5 hours	278 (2·37±0·10)	6·6
None	SRBC	None	51,850 (4·61±0·12)	
None	SRBC	−5 hours	11,062 (3·98±0·09)	7·2

* Passively immunized rats received 1·0 ml of a 1 : 20 dilution of rat anti-sheep erythrocyte serum 30 minutes before active immunization with 10^9 sheep erythrocytes.

† Rats treated with colchicine 1·5 mg/kg on the 3rd day after immunization 5 hours before being killed.

‡ The number of plaque-forming cells is the antilog of the mean for eight rats; the log of the mean ± the standard error of the mean is given in parentheses.

The results are recorded in Table 4. Colchicine caused equivalent reduction in spleen plaque-forming cells in control and passively immunized rats. Cell cycle times estimated from the drug effect are nearly equal. In three other experiments of similar design, cycle times for plaque-forming cells of passively immunized rats were equal to or shorter than for rats receiving the same antigen dose.

The following experiment compares the response of animals receiving a low dose of antigen with that of animals receiving a combination of passive immunization and a high dose of antigen.

Fifteen rats were each injected with 5×10^7 sheep erythrocytes. Fifteen rats in a second group were passively immunized with 1·0 ml of rat anti-sheep erythrocyte serum diluted 1 : 20 30 minutes before injection of 10^9 sheep erythrocytes. Five rats in each group were killed at 2, 3 and 4 days.

Responses were linear for both groups. For rats receiving the low antigen dose, mean responses were 4,885 (3·59±0·16) and 114,950 (4·99±0·13) plaque-forming cells on the 2nd and 4th days with a mean cell doubling time of 10·3 hours. For passsively immunized rats receiving a high dose of antigen, mean responses were 638 (2·74±0·12) and 44,280 (4·61±0·09) plaque-forming cells on the 2nd and 4th days with a mean cell doubling time of 7·7 hours. Apparently, lower responses produced by decreasing the antigen dose result from slower proliferation of responding cells, whereas passive antibody decreases the number of cells initially responding without affecting the rate of proliferation of cells that do respond.

Adjuvant enhancement of the plaque-forming cell response suppressed by antibody

Without passive antibody, adjuvant enhanced responses by increasing the rate of division of plaque-forming cells (Rowley *et al.*, 1968). A series of experiments, the following being an example, demonstrated that adjuvant also enhanced antibody suppressed responses by the same mechanism.

Sixty-four rats were passively immunized with 1·0 ml of rat anti-sheep erythrocyte serum diluted 1: 20. Thirty minutes later thirty-two were injected with 10^9 sheep erythrocytes and thirty-two with 10^9 sheep erythrocytes mixed with 0·15 ml of *B. pertussis* vaccine. Eight rats in each group were killed 2, 3 and 4 days later.

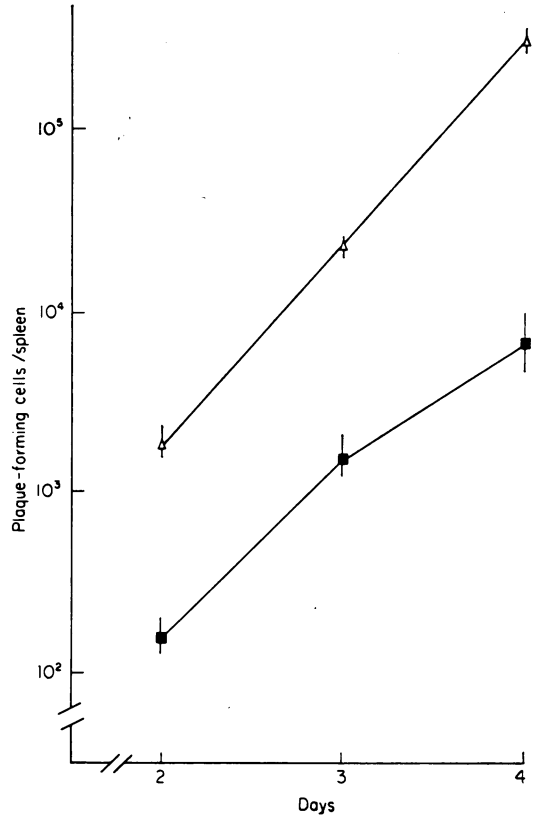

FIG. 2. The effect of an adjuvant. *B. pertussis* vaccine, on the spleen plaque-forming cell response of rats passively immunized with rat anti-sheep erythrocyte serum diluted 1 : 20 and actively immunized with 10^9 sheep erythrocytes. Each point represents the mean for eight rats ± the standard error of the mean plotted as a vertical bar. △, Antiserum diluted 1 : 20 and adjuvant; ■, antiserum diluted 1 : 20 and saline.

One day after injections the mean number of spleen plaque-forming cells for all groups was less than 100. Responses after the 1st day are plotted in Fig. 2. Without adjuvant, passive antibody caused marked suppression. With adjuvant the response was higher by the 4th day than would have been expected with antigen alone (compare with Fig. 1). Cell doubling times between the 2nd and 4th days were 6·6 hours for the adjuvant treated and 8·8 hours for the non-adjuvant treated rats.

DISCUSSION

The exponential increase in number of cells releasing specific antibody during the early primary immune response results from division of antibody-forming cells (Dutton and Mishell, 1967; Rowley *et al.*, 1968; Szenberg and Cunningham, 1968). The magnitude of

response is a function of the rate of division of antibody-forming cells; higher antigen doses or adjuvant increase the rate of division of antibody-forming cells without causing appreciable recruitment of additional responding cells or a measurable increase in the rate of antibody synthesis by individual cells (Rowley *et al.*, 1968).

In the present experiments, passive antibody appeared to decrease the initial number of responding cells without affecting the rate of proliferation of cells that did respond. In passively immunized rats, higher antigen doses or adjuvant increased the rate of proliferation of antibody-forming cells without increasing the initial number of responding cells, an effect sufficient to completely mask suppression produced by smaller amounts of passive antibody.

These findings can be accounted for by assuming that passive antibody and higher antigen doses or adjuvants affect different interactions required for the antibody response. Recent evidence of Mitchell and Miller (1968) indicates that at least two cell types are involved in the plaque-forming cell response of mice to sheep erythrocytes, one type, a thymus derived or dependent cell and the other, a bone marrow derived cell which proliferates and synthesizes specific antibody. Mosier and Coppleson (1968) find that the interaction of at least two and possibly three cell types is required for the induction of the primary immune response *in vitro*; the cell types are exclusive of any large population of macrophages which may phagocytose and 'process' antigen in some manner.

If two or more cell types are required, then the magnitude of the response should be a function of the number of interacting cells stimulated by antigen and the rate of division of the cell type synthesizing antibody. Passive antibody may interfere with the interaction between antigen and antigen recognition cells while adjuvants or higher antigen doses may increase the rate of division of antibody-forming cells by increasing the rate of interaction between cell types required for antibody synthesis. Since neither passive antibody nor adjuvant has much effect on responses once immunization occurs, it seems unlikely that these agents have any direct effect on antibody-forming cells.

In the present experiments, the separate effects of antibody suppression and adjuvant enhancement could be readily distinguished, since the antigen had little or no adjuvant activity, i.e. sheep erythrocytes in the doses used have no measurable effect on the simultaneous response to non-cross-reacting antigens. Some antigens, however, may contain intrinsic adjuvants. For example, simultaneous immunization of animals with *S. typhi* vaccine or the flagellar antigen derived from *S. typhi* cultures enhances the antibody response to sheep erythrocytes. Also, the antibody response of rats to a whole typhoid vaccine was not enhanced by *B. pertussis* vaccine (Rowley, Chutkow and Attig, 1959), presumably because typhoid vaccine contained sufficient intrinsic adjuvant to produce maximum enhancement. Thus, it can be expected that passive antibody will be less effective in suppressing the primary responses to some antigens because the antigens have intrinsic adjuvant activity.

Material with adjuvant activity may be introduced inadvertently. For example, we have prepared several purified fractions of anti-sheep erythrocyte globulin with high antibody activity to sheep erythrocytes *in vitro*, which enhanced the response of rats to sheep erythrocytes. These preparations also enhanced markedly the response of rats to swine erythrocytes, a reaction not produced with other anti-sheep erythrocyte sera or antibody fractions. A bacterial contaminant was identified in one preparation; an endotoxin was presumably introduced inadvertently at some point during the fractionation procedure of the other preparation. Contradictory reports about the relative suppressive effects of

different antibody fractions may be the result of adjuvant contamination of the preparations used. In any event, the ability to regulate an immune response, either to produce enhancement with an adjuvant or suppression with an antibody preparation, is dependent not only on the amounts of antigen and/or antibody used but also on the amount and activity of any intentionally or unintentionally introduced factor having adjuvant activity.

The present study is not concerned directly with delayed type hypersensitivity (cell mediated reactions), but it is of interest that passive antibody used with intravenous antigen specifically and profoundly suppresses development of hypersensitivity to the antigen (Axelrad, 1968; Axelrad *et al.*, 1968) or markedly prolongs survival of renal allografts (Stuart *et al.*, 1968). Hypersensitivity to the antigen was induced with Freund's adjuvant. Passive antibody alone only partially prevented induction of hypersensitivity, possibly as a result of the same action of the adjuvant which prevents passive antibody from completely suppressing the antibody response. Passive antibody alone only partially prevents graft rejection, possibly because the graft has an adjuvant effect on the recipient. These findings suggest that an understanding of the action of passive antibody and adjuvants may be essential for developing a rational procedure for specifically suppressing cell-mediated immune reactions.

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