



Immunosuppression induced in mice with specific antibody  
by Joan Bader MacDonald

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of  
MASTER OF SCIENCE in Microbiology  
Montana State University  
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Abstract:

Immunosuppression by antibody was studied with particular emphasis on the role of the macrophage. Suppression was achieved by injecting 64 HA units of anti-SRBC serum. This is close to the threshold amount needed to establish suppression. The hemolysin and hemagglutinin titers of mice injected with anti-SRBC serum were 5% and 3% respectively of the mean control titers at day 10 post vaccination. Mice passively immunized with anti-SRBC serum responded normally to  $5 \times 10^7$  human red blood cells but failed to respond to  $5 \times 10^7$  SRBC. Sixty-four HA units was found to be sufficient to establish suppression and maintain suppression for 25 days at which time a slight increase in the response occurred. Balb/c mice receiving anti-SRBC serum 5 days previously were responsive to a second dose of  $5 \times 10^7$  SRBC. Five days after receiving the second injection of sheep cells titers from experimental animals were 80% of the mean control titers. By day 25 post immunization there was no significant difference between the titers of experimental and control animals. Macrophages obtained from adult animals and incubated with anti-SRBC serum were not capable of inducing a response to SRBC from neonates. Macrophages which were incubated with normal mouse serum were effective in this regard.

Macrophages incubated with anti-SRBC serum were able to induce a response to guinea pig erythrocytes. Macrophages that were incubated with anti-SRBC serum, washed, and stained with fluorescein labelled rabbit anti-mousey globulin fluoresced brightly indicating a direct interaction between anti-SRBC serum and the macrophage.

It was concluded that immunosuppressive antibody exerts its effects on the macrophage and probably functions in vivo to bypass a second primary response once memory cells are established.

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by 1141

JOAN BADER MAC DONALD

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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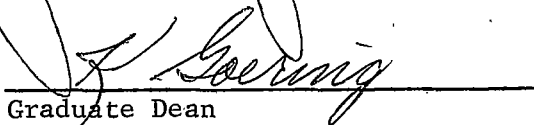
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March, 1970

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation to my advisor, Dr. John Jutila, for his patience, encouragement, and advice during the course of my studies.

I would also like to thank Mrs. Mary Ann Saunders for her assistance in typing the rough draft and Mrs. Darlene Harpster for typing the final manuscript.

I would also like to thank my husband, Paul MacDönaId, for his encouragement and support of my graduate work.

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## ABSTRACT

Immunosuppression by antibody was studied with particular emphasis on the role of the macrophage. Suppression was achieved by injecting 64 HA units of anti-SRBC serum. This is close to the threshold amount needed to establish suppression. The hemolysin and hemagglutinin titers of mice injected with anti-SRBC serum were 5% and 3% respectively of the mean control titers at day 10 post vaccination. Mice passively immunized with anti-SRBC serum responded normally to  $5 \times 10^7$  human red blood cells but failed to respond to  $5 \times 10^7$  SRBC. Sixty-four HA units was found to be sufficient to establish suppression and maintain suppression for 25 days at which time a slight increase in the response occurred. Balb/c mice receiving anti-SRBC serum 5 days previously were responsive to a second dose of  $5 \times 10^7$  SRBC. Five days after receiving the second injection of sheep cells titers from experimental animals were 80% of the mean control titers. By day 25 post immunization there was no significant difference between the titers of experimental and control animals. Macrophages obtained from adult animals and incubated with anti-SRBC serum were not capable of inducing a response to SRBC from neonates. Macrophages which were incubated with normal mouse serum were effective in this regard.

Macrophages incubated with anti-SRBC serum were able to induce a response to guinea pig erythrocytes. Macrophages that were incubated with anti-SRBC serum, washed, and stained with fluorescein labelled rabbit anti-mouse  $\gamma$  globulin fluoresced brightly indicating a direct interaction between anti-SRBC serum and the macrophage. It was concluded that immunosuppressive antibody exerts its effects on the macrophage and probably functions in vivo to bypass a second primary response once memory cells are established.

## INTRODUCTION

Inhibition of antibody synthesis by specific antibody has been studied extensively as a possible mechanism for regulating the concentration of specific antibody in the circulation. Theobald Smith (1909) first noticed that guinea pigs injected with diphtheria toxin-antitoxin mixtures containing excess antitoxin could not generate a response whereas guinea pigs injected with toxin-antitoxin mixtures at equivalence generated a strong response. Since then many authors have reported similar findings (Glenny and Sudmersen, 1921; Ramon and Zoeller, 1933; Barr et al., 1950).

Specificity is the most striking characteristic of this type of immunosuppression. Using the diphtheria toxin-antitoxin system, Uhr and Baumann (1961) suggested that suppression was specific after showing that non specific  $\gamma$  globulin did not suppress antitoxin formation. Rowley and Fitch (1964) demonstrated that rats suppressed with anti-sheep erythrocyte serum (SRBC), while not able to respond to SRBC, were fully responsive to purified Salmonella typhosa flagella. Likewise, rats receiving anti-S. typhosa did not respond to S. typhosa antigen but did respond to SRBC. Similarly, Axelrad and Rowley (1968) demonstrated that animals treated with anti-SRBC did not respond to SRBC but did elicit a response to horse red blood cells and Bordetella pertussis vaccine.

On the basis of this specificity it has been speculated that the mechanism for suppression is antigen elimination or masking of the antigenic site (Britton and Moller, 1968; Dixon, Jacot-Guillarmod, and McConahey, 1967; Uhr and Moller, 1968). Uhr and Finkelstein (1963) found that below a critical level of antigen the rate of antibody formation is antigen dependent. This lends support to the notion that the retention and level of antigen might act as a control mechanism.

Further support for the concept of antigen elimination was given by differences found in the suppressive capabilities of 19S and 7S antibodies. Moller and Wigzell (1965) determined that 7S antibody was 115 times more efficient at suppression than 19S antibody. Finkelstein and Uhr (1964) also observed that 19S antibody was a less effective suppressant than 7S antibody. This phenomenon might be explained by  $\delta$  M's short half life and  $\gamma$  G's greater affinity for antigen. This argument was supported when it was shown that high avidity antibody was an effective suppressant at concentrations much lower than those needed when using low avidity antibody (Walker and Siskind, 1967).

The use of antibody fragments has also supplied valuable information for if the mechanism of suppression is antigen elimination,  $F(ab')_2$  should be as effective as whole antibody. However, if

antibody is exerting its effect directly on cells, removing the Fc fragment should impair the antibody's suppressive abilities. The initial work with antibody fragments indicated the  $F(ab')_2$  fragment was as effective as whole antibody in achieving suppression (Cerrettoni, McConahey, and Dixon, 1969). However, St. C. Sinclair (1969) reported that whole antibody was from 100 - 1000 times more effective than  $F(ab')_2$ . This difference might be explained by the short half life of antibody fragments. Investigating this possibility, St. C. Sinclair, Less, and Elliott (1968) used complexes of  $F(ab')_2$  and its corresponding antigen which have longer half lives than unbound  $F(ab')_2$ . Their results again showed that whole antibody is far more effective than antibody fragments. These investigators feel that the conflicting data of other authors is due to the use of preparations contaminated with whole 7S antibody.

The amount of antibody necessary for suppression is also important. One may postulate that there must be enough antibody present to cover all or most antigenic determinants. Dixon and co-workers recently produced evidence for this concept using Keyhole Limpet Hemocyanin (KLH) as the antigen (Dixon, Jacot-Guillarmod, McConahey, 1967). However, using the classical diphtheria toxin-antitoxin system, Uhr and Baumann (1961) achieved suppression with fewer antibody molecules than the minimum number of known major

determinants on the antigen. Haughton and Nash (1969) did the first truly quantitative study by isotopically determining that  $3-4 \times 10^{14}$  antibody molecules were required to saturate all of the antigenic determinants on  $5 \times 10^8$  SRBC. However suppression was achieved with as little as  $8 \times 10^{11}$  antibody molecules.

Inconclusive evidence for antigen elimination led to studies on the effects of passive antibody on cells. Rowley and Fitch (1964) investigated the immunocompetence of passively immunized lymphocytes. They transferred  $5 \times 10^8$  passively immunized spleen cells into an x-irradiated host which was then challenged with antigen. In all cases titers in animals receiving passively immunized spleen cells were lower than those found in animals receiving normal spleen cells. However, this work could not be repeated by other investigators (Moller, 1964; Uhr and Moller, 1968) and it was postulated that Rowley and Fitch passively transferred antibody with their cells.

Greenburg and Uhr (1968) investigated the incorporation of  $H^3$  leucine in lymph node cells from immune animals and lymph node cells treated with antibody 48 hours after immunization. Immune cells not treated with antibody showed a linear increase in leucine incorporation from day 5 to day 7 while immune cells exposed to antibody showed a marked increase from day 3 to day 4 followed by a

decline to day 7. The significance of this altered protein synthesis has not been fully investigated.

Pierce (1969) studied the effect of passive antibodies on cells using a tissue culture system. Lymphoid cells and macrophages in tissue culture together produced antibody when exposed to antigen. If the culture was incubated with specific antibody before exposure to the antigen no antibody was produced. When lymphoid cells were incubated with antibody, washed, mixed with macrophages and exposed to the antigen, normal levels of antibody were produced. However, when macrophages were incubated with antibody, washed, mixed with lymphocytes and exposed to antigen, no antibody was produced.

This work was given further support by the studies of Ryder and Schwartz (1969). They injected SRBC into passively immunized mice where they were presumed to be coated with antibody. It was found that such cells were fully antigenic, whereas cells left in the original recipients did not stimulate a response. In other experiments they showed that SRBC fed macrophages from passively immunized mice failed to elicit a response whereas mice receiving SRBC laden macrophages from untreated mice responded.

The purpose of this study was to further examine the effects of passive antibody paying particular attention to the macrophage and the possible interactions between the macrophage and passively

administered antibody.

## MATERIALS AND METHODS

### Mice

Adult inbred Balb/c and randomly bred Swiss Manor mice 3 to 6 months of age were used throughout the study. The former were obtained in 1966 from the National Cancer Institute (Bethesda, Maryland) in the germ free state and were conventionalized six months later. The Swiss Manor mice were obtained in 1964 from Manor Farms (Straatsburg, New York). The mice are maintained by brother-sister matings. All mice were fed Purina Laboratory Chow and water ab libitum.

### Preparation of Antisera

Ten animals were given 3 intraperitoneal (i.p.) injections of 0.1 ml thrice-washed 10% SRBC at 7 day intervals. Five days after the final injection the animals were bled from the retro-orbital plexus and the blood was pooled. Serum was separated on a sucrose density gradient by ultracentrifugation at 37,000 RPM in a SW-39 rotor for 17 hours using a Beckman Model L-2 Ultracentrifuge (Beckman Instruments Inc., Palo Alto, California). A gradient consisting of 1.2 ml of 40%, 30%, 20% and .7 ml of 10% sucrose dissolved in .01 M phosphate buffered saline (PBS) was prepared in tubes. Ten fractions were collected dropwise from the bottom of the tubes and titered. Fractions containing high concentrations of 7S

antibody were pooled, dialyzed against PBS, and titered again. The antibody, diluted to contain 64 hemagglutination units per 0.1 ml, was stored frozen in 0.5 ml aliquots.

#### Determination of Antibody Titers

Hemagglutinin titers were determined by serial two-fold dilutions in PBS. To a 0.1 ml volume of diluted serum, 0.1 ml of 1% SRBC was added. The tubes were incubated in a 37°C water bath for 30 minutes. The tubes were centrifuged at 3,700 RPM for 1 minute, shaken, and the degree of agglutination scored as a 4+ (pellet rises in one clump), 3+ (pellet rises in several large clumps), 2+ (pellet rises in several small clumps and loose cells), 1+ (pellet rises in loose cells with a few persistent clumps), or negative (pellet rises in a cloud of loose cells). The last dilution giving a score of 1+ or more was taken as the titer.

The hemolysin titer was determined by adding .05 ml guinea pig complement (BBL) diluted 1:5 to the above agglutination system. Tubes were incubated for 30 minutes at 37°C and refrigerated overnight. The dilution of the last tube showing complete hemolysis was taken as the titer.

### Immunosuppression

The mice were injected i.p. with 64 hemagglutination (HA) units of 7S anti-SRBC antibody contained in 0.1 ml. Controls received 0.1 ml PBS i.p. One hour later all mice received  $5 \times 10^7$  SRBC. To determine the duration of suppression mice were bled and their sera titered on days 3, 5, 10, 17, 24, 32, and 42. In other experiments  $5 \times 10^7$  human red cells were injected to check for specificity. Controls received saline and human red blood cells. On days 5 and 10 the mice were bled from the retro-orbital sinus and their sera titered. To determine whether suppressed mice were capable of yielding a secondary response the mice were given a second dose of  $5 \times 10^7$  SRBC several days after immunosuppression. Animals were bled and their sera titered on days 7, 17, 25, and 30.

### Cell Transfer into Neonates

Mice from 1 to 3 months old were injected i.p. with 3 mls of thioglycollate (Difco). Three to seven days later the mice were sacrificed and their peritoneal cavities washed twice with 5 mls. Hanks Balanced Salt Solution (Microbiological Associates Inc., Bethesda, Md.) containing 1% fetal calf serum and  $10 \mu$  heparin/ml. Cell preparations were divided into experimental and control groups. The experimental group was incubated with 64 HA units anti-SRBC per

donor mouse for one hour at 37°C, while the control group was simultaneously incubated with an equal volume of normal mouse serum. Ten x 10<sup>6</sup> cells were injected i.p. into neonates according to the technique of Argyris (1968). Three days after the injection of peritoneal cells, 5 x 10<sup>7</sup> SRBC were injected i.p. into the mice. Four days later mice were sacrificed, their spleens removed and assayed using the plaque technique of Jerne and Nordin (1963).

#### Fluorescent Antibody

Fluorescein labelled rabbit anti-mouse  $\gamma$  globulin (Difco) was used to examine macrophages from passively immunized mice. The antisera was purified by passage through a column of DEAE-Cellulose according to the technique of Wood, Thompson, and Goldstein (1965). Fractions were assayed for fluorescent labelled antibody by measuring the absorption at 280 m $\mu$  and the %T at 485 m $\mu$  on a Coleman 124 Double Beam Spectrophotometer (Coleman Instruments Division, Maywood, Illinois).

Peritoneal exudates were induced in mice with 3 mls thioglycolate broth. Macrophages were removed as above and incubated at 37°C for 1 hour with 0.1 ml 1:100 rabbit anti-mouse  $\gamma$  globulin to bind any non-specific globulins that might be on the macrophages. The preparations were washed twice and incubated with 128 HA units

anti-SRBC or normal mouse serum (NMS). The macrophages were washed twice, smeared on clean slides, air dried, and fixed in 95% ethanol. Smears were stained with fluorescent rabbit anti-mouse  $\gamma$  globulin for two hours in a moist atmosphere, washed in PBS for two twenty minute intervals, and mounted with phosphate buffered glycerin. Slides were observed using a Leitz Labolux fluorescent microscope with BG-12 and BG-38 filters.

## RESULTS

### Degree of Immunosuppression Achieved Using Passive Antibody

Balb/c and Swiss Manor mice suppressed by anti-sheep erythrocyte (SRBC) serum were bled and their sera titered on days 3, 5, and 10 post vaccination. The results of hemolysis and hemagglutinin titrations are shown in Tables I and II.

The hemolysin response in Balb/c mice was completely suppressed at day 3, 11% of mean control titers at day 5, and 5% of mean control titers at day 10 post vaccination. Moreover, all of the control mice responded to immunization while only 57% of experimental animals responded.

Similar results were obtained for the hemagglutinin response. The anti-SRBC response of Balb/c mice treated with anti-SRBC antibody was totally suppressed at day 3, 1% of mean control titers at day 5, and 3% of mean control titers at day 10. Once again all of the control mice responded while only 33% of the experimental mice responded. The results obtained in Swiss Manor mice (Table II) were virtually identical to those described above and eliminate, in effect, the possibility of strain effect.

### Specificity of Immunosuppression

Balb/c and Swiss Manor mice given anti-SRBC responded normally to  $5 \times 10^7$  human red blood cells but failed to respond to  $5 \times 10^7$

Table I

The effect of passive immunization on the hemolysin and hemagglutinin response in Balb/c mice.

Treatment <sup>a</sup>	Days of Titration <sup>b</sup>	Antibody Titers <sup>c</sup>					
		Hemolysin			Hemagglutinin		
		No. responding <sup>d</sup>	Mean	Range	No. responding	Mean	Range
Anti-SRBC	3	0/9	0	0	0/9	0	0
	5	4/7	3.3	0-8	1/7	.14	0-2
	10	4/7	1.6	2-4	2/7	.3	0-2
Saline Control	3	3/3	3.3	2-4	0/3	0	0
	5	3/3	16	8-32	3/3	11.3	4-16
	10	2/2	20	8-32	2/2	10	4-16

<sup>a</sup> The mice received a single i.p. injection of Balb/c anti-SRBC 1 hr before receiving  $5 \times 10^7$  sheep erythrocytes, i.p.

<sup>b</sup> Day after immunization mice were bled for antibody titrations.

<sup>c</sup> Titers are expressed as reciprocals of serum dilutions.

<sup>d</sup> The numerator indicates the number of mice responding; the denominator the total number immunized.

Table II

The effect of passive immunization on the hemolysin and hemagglutinin response in Swiss Manor mice.

Treatment <sup>a</sup>	Days of Titration <sup>b</sup>	Antibody Titers <sup>c</sup>					
		No. responding <sup>d</sup>	Hemolysin		No. responding	Hemagglutinin	
			Mean	Range		Mean	Range
Anti-SRBC	5	5/11	1.1	0-4	4/11	1.1	0-4
	10	6/11	1.9	0-4	5/11	2.5	0-6
Saline Control	5	4/4	76	64-128	4/4	48	32-64
	10	4/4	68	64-128	4/4	80	64-128

<sup>a</sup> The mice received a single i.p. injection of Swiss Manor anti-SRBC 1 hr before receiving  $5 \times 10^7$  sheep erythrocytes, i.p.

<sup>b</sup> Day after immunization mice were bled for antibody titers.

<sup>c</sup> Titers are expressed as reciprocals of serum dilutions.

<sup>d</sup> The numerator indicates the number of mice responding; the denominator the total number immunized.

SRBC (Table III). Hence, the immunosuppressive effect of passively administered antibody was found to be antigen specific. The lack of detectable hemolysin titers to HRBC was presumed to be due to the anti-complementary effects of human erythrocytes (Hoffmann, 1969).

#### Duration of Suppression

To determine the duration of suppression following antiserum treatment, Balb/c mice were given a single dose of anti-SRBC and  $5 \times 10^7$  SRBC and then bled at intervals of 7 days. The results of serum titrations (Figure 1) show that a partial recovery of the hemagglutinin response occurred between days 20 and 25 post vaccination. By day 32, 85% of the experimental animals had developed a response. Mean hemolysin titers were never observed to exceed 1:4 whereas mean hemagglutinin titers reached 1:16, still well below mean control titers. One may postulate that when the level of passively administered antibody had dropped sufficiently due to natural decay, enough antigen still persisted to stimulate a moderate response.

#### Effect of Passive Antibody on a Second Injection of Antigen

Balb/c mice receiving anti-SRBC serum 5 days previously were injected i.p. with a second dose of  $5 \times 10^7$  SRBC to determine whether immune impairment could be interrupted. These data show that animals whose primary response was markedly suppressed by passive immunization

Table III

The effect of passively administered anti-SRBC on the hemolysin and hemagglutinin response to SRBC and human red blood cells (HRBC) in Balb/c and Swiss Manor mice.

Strain	Treat- ment <sup>a</sup>	Days of Titration <sup>b</sup>	Antibody Titers <sup>c</sup>					
			Hemolysin			Hemagglutinin		
			No. respond- ing <sup>d</sup>	Mean	Range	No. respond- ing	Mean	Range
Balb/c	Anti-SRBC + HRBC	5	0/8	0	0	8/8	18	8-32
		10	0/8	0	0	8/8	27	3-64
	Anti-SRBC + SRBC	5	1/2	1	0-2	2/2	3	2-4
		10	1/2	2	0-2	2/2	4	4-4
Swiss Manor	Anti-SRBC + HRBC	5	0/8	0	0	8/8	120.5	32-256
		10	0/8	0	0	8/8	116	32-256
	Anti-SRBC + SRBC	5	0/4	0	0	3/4	3	0-4
		10	2/4	2	0-2	4/4	3	2-4

16

<sup>a</sup> The mice received a single i.p. injection of anti-SRBC 1 hr before receiving  $5 \times 10^7$  SRBC or  $5 \times 10^7$  HRBC i.p.

<sup>b</sup> Day after immunization mice were bled for antibody titrations.

<sup>c</sup> Titers are expressed as reciprocals of serum dilutions.

<sup>d</sup> The numerator indicates the number of mice responding; the denominator the total number immunized.

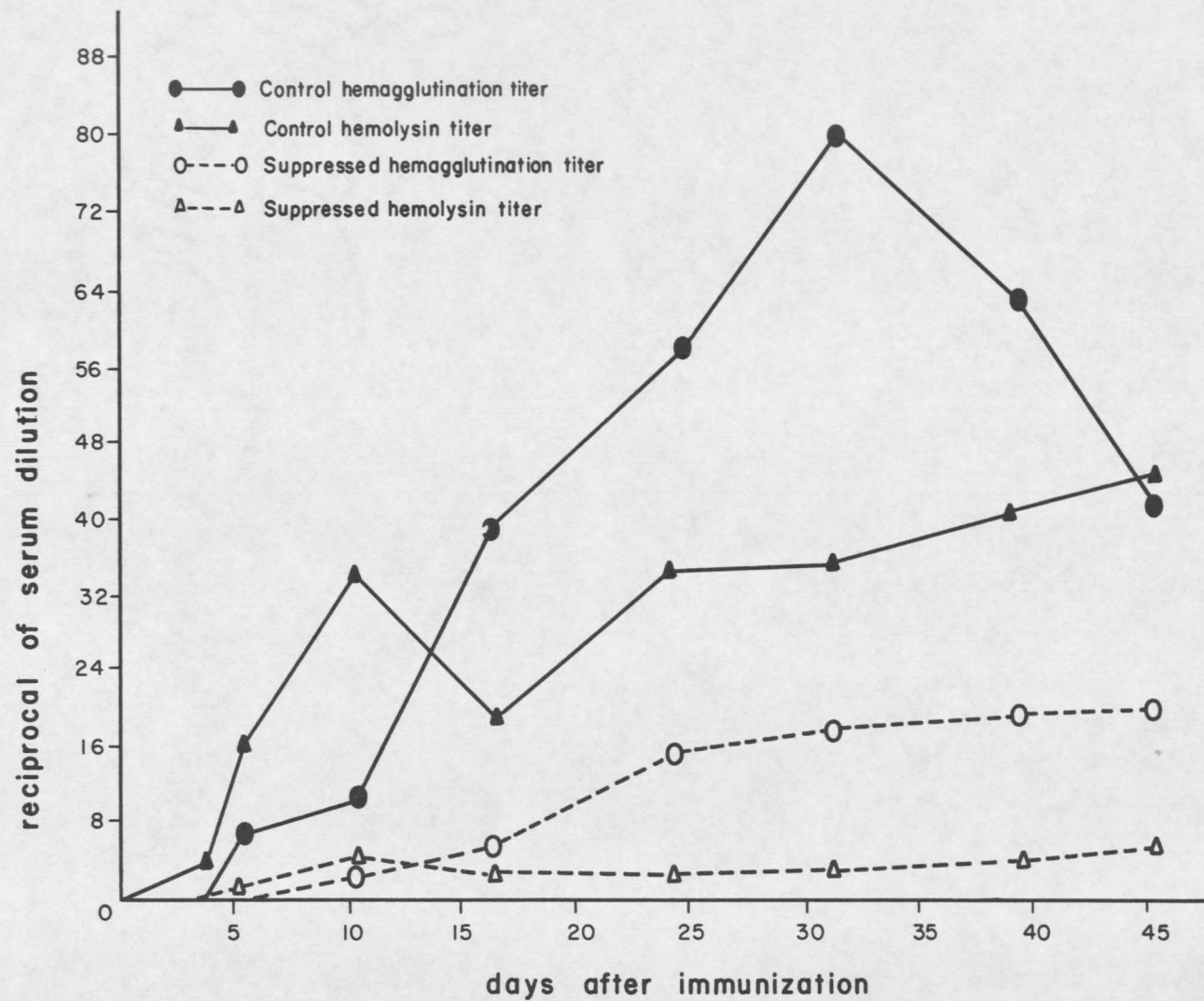


Figure 1. Duration of suppression of hemolysin and hemagglutination response in Balb/C mice.

were responsive to a second antigenic stimulus (Figure 2). On day 5, titers from experimental animals were 3% of mean control titers, when given a second injection of SRBC on day 5, the titers of experimental animals were 80% of mean control titers 5 days post-vaccination. By day 25, there was no significant difference between the titers of experimental and control mice.

#### Passive Transfer of Specific Immunosuppression

To determine the effect of passive immunization on the macrophage, peritoneal washings containing about 87% macrophages were treated with either anti-SRBC or normal mouse serum and injected into neonates which were subsequently challenged i.p. with  $5 \times 10^7$  SRBC. Four days later the spleens were assayed for the number of plaque forming cells using the direct Jerne plaque technique. The results (Table IV) indicate that macrophages incubated with anti-SRBC failed to evoke strong response in neonates as macrophages incubated with normal mouse serum. The average response in both Swiss Manor and Balb/c neonates receiving macrophages treated with anti-SRBC serum was 29% of the mean control response. The data from two control groups designed to establish the background count in neonatal spleens and the specificity of the suppression seen in neonates are not included in Table IV. To determine the background neonatal response,

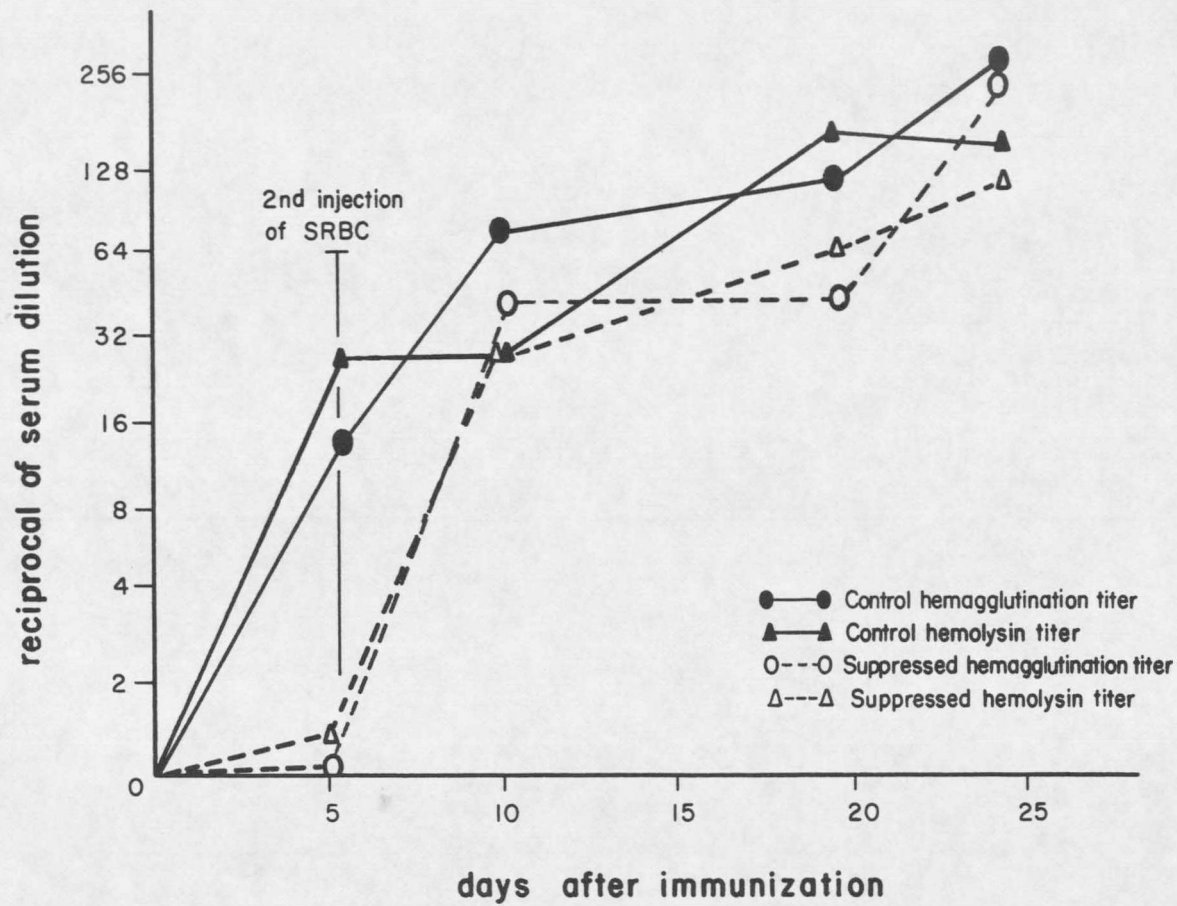


Figure 2 . Effect of passive immunization on a second injection of sheep erythrocytes in Balb/C mice.

Table IV

Effect of passive antibody on the ability of macrophages to transfer immunity to neonates.

Strain of Mice	No. of litters injected	No. of mice injected		Average No. of PFC per				Ratio E/C
				10 <sup>6</sup> Spleen Cells		Whole Spleen		
				E <sup>a</sup>	C <sup>b</sup>	E	C	
Swiss Manor	2	11	11	.45	3.22	5.52	30.3	.14
	1	2	2	7.73	15.5	200.8	383.3	.49
	1	3	3	1.42	4.93	30.4	119.1	.29
Balb/c	1	3	5	1.22	5.41	21.1	54.9	.38
	2	4	4	.77	4.57	7.47	46.6	.16

<sup>a</sup> Macrophages were incubated for 1 hour with anti-SRBC serum before transfer.

<sup>b</sup> Macrophages were incubated for 1 hour with normal mouse serum before transfer.

neonates were injected with 0.1 cc Hanks BSS on day 0 followed by  $5 \times 10^7$  SRBC three days later. The mean number of plaque forming cells (PFC) per  $10^6$  spleen cells was 1.13. The mean number of PFC per whole spleen was 17. These results are comparable to the results obtained with the transfer of passively immunized macrophages.

The second control was designed to demonstrate specificity in this system. Macrophages incubated with anti-SRBC serum were injected into neonates which were subsequently challenged with  $5 \times 10^7$  guinea pig erythrocytes. The mean number of PFC per  $10^6$  spleen cells was 10 while the mean number of PFC per whole spleen was 60. These responses were comparable to the responses evoked by macrophages treated with normal mouse serum.

#### Direct Interaction of Passive Antibody and the Macrophage.

Macrophages incubated with anti-SRBC serum or normal mouse serum, were stained with fluorescein-labelled rabbit anti-mouse IgG (FA-IgG) to detect a mouse IgG immunoglobulin on the macrophage. To insure that the FA-IgG was reacting with anti-SRBC antibodies macrophages were first incubated with non-labelled rabbit anti-mouse globulin to cover antibodies non-specifically absorbed from  $\delta$  peritoneal fluids prior to removal. The anti-SRBC serum reacting with "covered" macrophages was presumed to be the only immunoglobulin

detected by the FA system. Five hundred cells were counted from experimental and control preparations. Of the macrophages incubated with anti-SRBC 98% were brightly fluorescing whereas only 5% of the cells incubated with normal mouse serum were fluorescing.



Figure 3. Macrophages incubated with normal mouse serum, washed and stained with fluorescein labelled rabbit anti-mouse globulin.

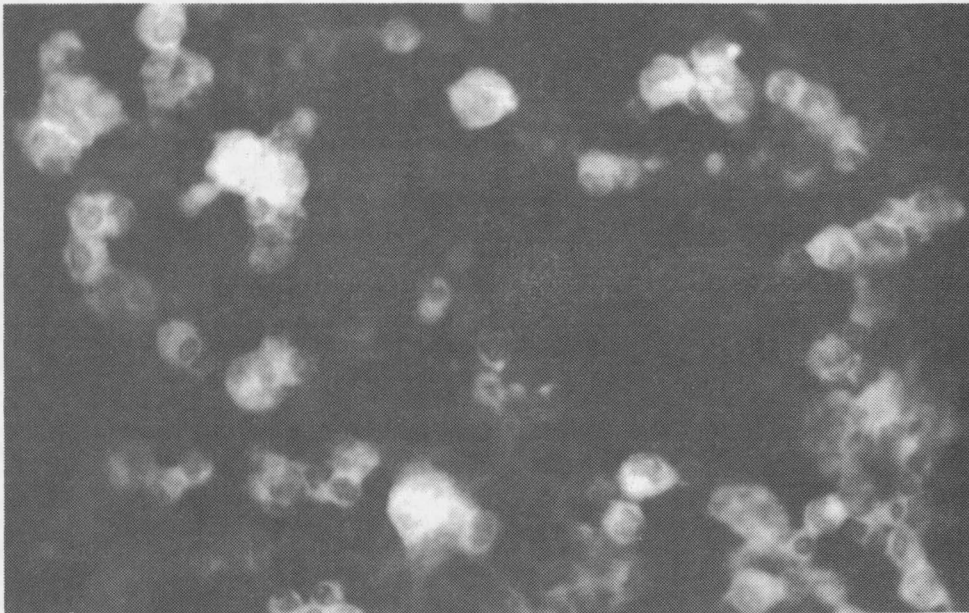


Figure 4. Macrophages incubated with mouse anti-SRBC serum, washed and stained with fluorescein labelled rabbit anti-mouse globulin.

## DISCUSSION

These studies show that passively transferred anti-SRBC serum strongly and specifically inhibited the development of an immune response in Balb/c and Swiss Manor mice. Similar findings have been reported using a wide variety of antigens such as diphtheria toxin (Uhr and Baumann, 1961), Salmonella typhosa flagella (Rowley and Fitch, 1964), and Bordetella pertussis vaccine (Axelrad and Rowley, 1968). Suppression was most effective until day 20 at which time some mice began to show partial recovery. Variations seen in the duration of suppression are probably due to differences in the titer and amounts of passively transferred antibody and time of challenge. In our system, 64 HA units were initially administered which is far less than the amounts used by the investigators cited above.

Balb/c mice receiving anti-SRBC serum five days previously and injected with a second dose of  $5 \times 10^7$  SRBC responded vigorously. The response evoked by a second dose of antigen appeared to be a typical primary response (Figure 2). Similar results have been reported by St. C. Sinclair (1969). The primary response is probably mediated by either new macrophages or macrophages rendered functional due to membrane turnover.

Other investigators found that a second injection of antigen led to a typical secondary response implying that the accumulation of memory cells is not affected by passive immunization. Ryder and

Schwartz (1969) immunized 6 week old mice that had been passively immunized with 8192 HA units at birth. The mice failed to respond, but when given a second injection of antigen at 12 weeks of age the mice produced a typical secondary response. It is conceivable that during the 6 week interval between immunizations functional macrophages arose which were stimulated and induced a feeble primary response leading to the accumulation of memory cells.

Macrophages incubated with normal mouse serum and injected into a neonate which was subsequently immunized rendered the neonate immunologically competent. The responses elicited by the neonates were comparable to those reported by Argyris (1969). On the other hand, macrophages incubated with anti-SRBC serum and injected into a neonate which was subsequently immunized did not evoke a significant response. The number of PFC found in the spleens of these animals was comparable to the number of PFC found in the spleens of neonates which were not injected with macrophages. These results indicate that immunosuppression exists at the macrophage level and support the in vitro findings of Pierce (1969) as well as the in vivo studies done by Ryder and Schwartz (1969). They also provide a unique correlation between in vitro exposure of macrophages to antibody and the functioning of these macrophages in vivo. The use of neonates as the recipients of the macrophages provides an advantage.

The consistent and very slight response of neonates when injected with antigen provides a background which can serve to indicate whether the passive transfer of free antibodies with the cell preparation might have occurred. Thus, if neonates receiving passively immunized macrophages had responded more weakly than neonates that received no macrophages one might assume that free antibody was being transferred, that is, free antibody should suppress the development of background PFC's. Since results from the two groups of mice were similar we can assume that no free antibody was passively transferred.

Fluorescein labelled rabbit anti-mouse  $\gamma$  globulin detected an interaction between the macrophage and anti-SRBC serum. Since fluorescence was fairly uniform throughout the cell, it was difficult to determine the site of localization of the antibody. If antibody was localizing only on the surface of the cell one might expect a halo effect as found by White, Jenkins, and Wilkinson (1963) who investigated the binding of skin sensitizing antibody to mast cells. If the antibody is binding to the macrophage membrane the effect of the antibody is probably mechanical. One could speculate that either the RNA-immunogen complex is being "captured" by the antibody as it leaves the macrophage or the direct macrophage-lymphocyte interaction seemingly necessary for antibody production is sterically prevented.

On the other hand, if antibody is being incorporated into the macrophage the effect is most probably a physiological one.

Antigen processing might be prevented altogether or antigen processing might be altered in such a way that unique information is passed to the lymphocyte. The latter possibility is supported by the work of Greenburg and Uhr (1968) showing that a unique synthetic event seems to be occurring in lymphocytes from suppressed animals.

The role antibody plays in the regulation of the in vivo response is still obscure. Several investigators feel antibody is operative in the prevention of hyperimmunization (Finkelstein and Uhr, 1964; Axelrad and Rowley, 1968; Moller and Wigzell, 1965). However, there are several aspects of suppression by antibody that do not lend support to this hypothesis. Hyperimmunization contributes to a secondary response mediated principally by memory cells. Memory cells do not require antigen processing by the macrophages and moreover, once memory cells have accumulated they are not suppressed by passive antibody (Ryder and Schwartz, 1969). In addition, the secondary response generally yields an antibody of high affinity and greater life span, properties which have considerable survival value to the animal. Thus, it could be concluded that a more likely function of suppression by antibody would be to insure that once the more efficient memory cells have accumulated antigen processing by the

macrophage, an energy consuming and now unnecessary step, would not occur.

## SUMMARY

Immunosuppression was induced in Balb/c and Swiss Manor mice with 64 HA units of hyperimmune, isogeneic anti-SRBC serum. The degree of immunosuppression was estimated using serum titrations and the Jerne Plaque technique. Hemolysin and hemagglutinin titers in suppressed mice were 5% and 3% respectively of mean control titers 10 days post immunization. Suppression was still effective at 45 days post immunization. Passive transfer of macrophages into neonates was used to determine the effect of antibody on the macrophage. Macrophages incubated with anti-SRBC serum failed to induce a response to SRBC in neonates whereas macrophages incubated with normal mouse serum were effective in this regard. Macrophages incubated with anti-SRBC serum and washed several times were shown to directly interact with the antibody by the use of immunofluorescence. It was postulated that immunosuppressive antibody exerts its effect on the macrophage.

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