Immunological Interaction of Erythrocyte Isoantigens: Effects of Passive Antibody* (33646)

L. W. SCHIERMAN,[1] E. LECKBAND,[1,2] AND R. A. MCBRIDE[1,3]
(Introduced by S. Schiller)

Department of Surgery, The Mount Sinai School of Medicine, New York, New York 10029

Erythrocyte isoantigens determined by the *B* blood-group locus in chickens have adjuvant activity with regard to the development of immunity toward less antigenic isoantigens. This adjuvant action occurs only if the antigens are present on the same erythrocyte and the recipients are not rendered immunologically tolerant of the B antigens (1). The immunological relationship of the A and B system isoantigens is closely analogous to a hapten-carrier system since very few immature birds respond immunologically following inoculation with A antigens alone. That the H-2 antigens of mice may also have adjuvant properties for weak isoantigens of other systems is indicated by the earlier results of Berrian and McKhann (2).

It has been well documented that antibody given passively or complexed with antigen (in antibody excess) may result in suppression of specific antibody synthesis to the antigen (3). The purpose of the present study was to determine the effects of coating, prior to inoculation, erythrocytes possessing foreign A and B system antigens with anti-B antibodies.

Materials and Methods. The animals used in this study were from a partially inbred line of white leghorns whose blood group genotypes have been identified for five independently inherited systems. Immunizations carried out over several generations indicate that this line is genetically homogeneous for other blood-group systems. Donors and recipients were matched for all but the A and B systems. Different combinations of donor–recipient *B*-locus genotypes were used in two experiments. In the first experiment ten 3-month-old birds of A^1/A^1, B^1/B genotype received five weekly intravenous inoculations of erythrocytes from a donor whose genotype was A^1/A^2, B^1/B^3. Thus, two serologically distinct foreign antigens, i.e., A_2 and B_3, were present on the donor erythrocytes. For each inoculation, five of the recipient birds received 2 ml of a 20% suspension of erythrocytes in saline and five others received a similar volume of erythrocytes which had been incubated for 1–2 hr with anti-B_3 antiserum. Three vol of antiserum to 1 vol of packed erythrocytes were used. This antiserum represented a pool of three bleedings from a single bird which had been hyperimmunized. Molecular weight of the antibodies used for coating the erythrocytes was not determined; however, a previous study showed that hyperimmune anti-B antibodies are predominantly of the 7S class (4).

Because the genotype of the bird which had produced the B_3 antibodies was B^1/B^2 it is possible that not all the foreign antigenic factors comprising the B_3 antigen were coated. Possibly, factors which B_3 possesses in common with B_2 remained uncoated. For this reason, three groups of recipient birds were used in a second experiment. Fifteen 2-month-old birds of the A^1/A^1, B^1/B^1 genotype received a series of four injections of erythrocytes from a single donor whose genotype was A^1/A^2, B^1/B^2. Five recipients received uncoated erythrocytes, six received erythrocytes coated with anti-B_2 antibodies produced by a B^1/B^3 bird and four others received erythrocytes coated with anti-B_2 an-

* Supported by grants from the National Science Foundation (GB-3854) and the United States Public Health Service (AM-10036).

[1] Present Address: Department of Pathology, New York Medical College, Flower and Fifth Avenue Hospitals, New York, New York 10029.

[2] Recipient of Postdoctoral Fellowship CA-24,-381-03 from USPHS.

[3] Recipient of Career Scientist Award of The Health Research Council of the City of New York. Contract number I-520.

TABLE I. Outline of Experiments.

Expt.	Genotype		Group	No. of birds	Antibody coating erythrocytes	
	Recipients	Donors			Specificity	Genotype of antibody producer
1	$A^1/A^1, B^1/B^1$	$A^1/A^2, B^1/B^3$	A	5	Anti-B_3	B^1/B^2
			B	5	Uncoated	—
2	$A^1/A^1, B^1/B^1$	$A^1/A^2, B^1/B^2$	A	6	Anti-B_2	B^1/B^3
			B	4	Anti-B_2	B^1/B^1
			C	5	Uncoated	—

tibodies produced by a B^1/B^1 bird. Theoretically, antibodies produced by the latter bird should coat all factors of the B_2 antigen which are foreign to B^1/B^1 birds. Again, only hyperimmune antisera from pooled bleedings of single recipients were used. The experiments are outlined in Table I.

In both experiments, the volume and titer of the anti-B antisera were sufficient to cause strong agglutination of the inoculated erythrocytes. Presumably, free antibodies were present in the inocula since the agglutinated erythrocytes were injected without removing excess antibodies that may have been present. Recipient birds were, with one exception, bled at weekly intervals (prior to each immunization and 1 week following the last) and the titer of anti-A and anti-B hemagglutinins determined in separate tests with erythrocytes possessing only one of the foreign antigens. One drop of a 2% erythrocyte suspension was added to 0.2 ml of twofold dilutions of sera in 0.85% saline and, after incubation, the degree of agglutination scored macroscopically.

Results. The patterns of A2 and B3 antibody production by Expt. 1 recipients, as shown in Fig. 1, were markedly different for the birds inoculated with anti-B_3 coated erythrocytes as compared to the birds inoculated with uncoated erythrocytes. While the production of anti-B_3 was suppressed by the passive B3 antibody, the production of anti-A_2 antibody was enhanced. Similar patterns of A2 and B2 antibody production were obtained with the birds used in Expt. 2 (Fig. 2). Essentially no differences were observed between the two groups of birds that received erythrocytes coated with different types of B2 antibody; i.e., whether the anti-B_2 was produced by a B^1/B^3 or B^1/B^1 recipient. The results obtained from these two groups were therefore pooled for the comparisons made between birds receiving coated and uncoated erythrocytes. The mean hemagglutinin titers of the sera from birds in both experiments, bled 1 week following the last inoculation, are presented in Table II. It is clear that the birds that received erythrocytes coated with anti-B antibodies were, at this time, producing significantly more anti-A antibodies than

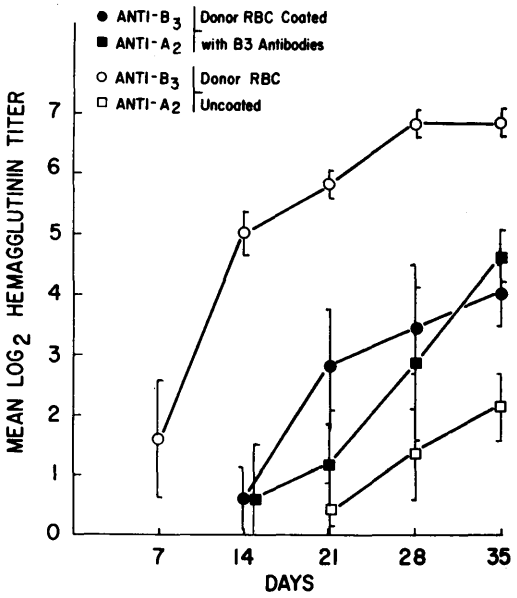

FIG. 1. Hemagglutinin titers of A2 and B3 antibodies in birds of A^1/A^1, B^1/B^1 genotype inoculated with erythrocytes possessing foreign A_2 and B_3 isoantigens. Each point represents the mean titer determined 7 days following and immediately preceding weekly immunization; vertical bars indicate standard error of the mean.

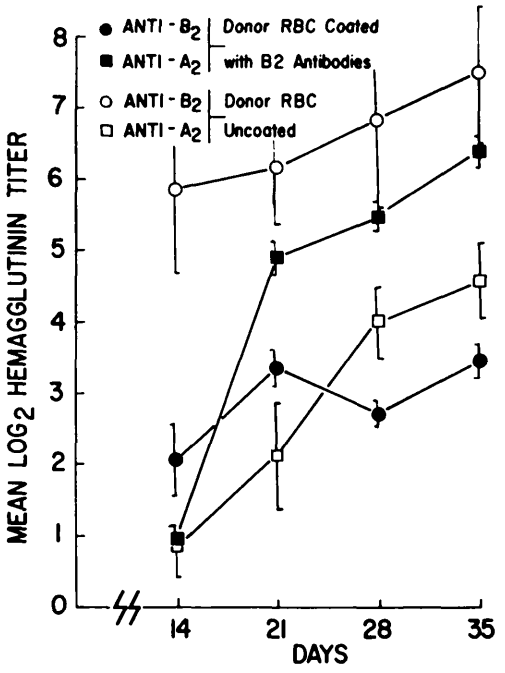

FIG. 2. Hemagglutinin titers of A2 and B2 antibodies in birds of A^1/A^1, B^1/B^1 genotype inoculated with erythrocytes possessing foreign A_2 and B_2 isoantigens. Each point represents the mean titers determined 7 days following and immediately preceding weekly immunization; vertical bars indicate standard error of the mean.

were the controls (t test on $\log_{10}$ transformed data: $p<.05$ and $<.01$ for Expts. 1 and 2, respectively).

Discussion. The results of both experiments indicate that coating erythrocytes with anti-B antibody suppresses production of anti-B antibodies by the recipients. The actual degree of suppression cannot be determined because of the passive transfer of antibodies with each immunization. However, the apparent plateau in the response curves (Figs. 1 and 2) suggests that suppression was incomplete and some active synthesis occurred. Regardless of the degree of suppression, the demonstration of an enhanced response to A isoantigens does not support the hypothesis that passive antibody mediated immune suppression is due to antigen elimination.

TABLE II. Effects on Antibody Production of Passively Coating Erythrocytes Possessing Foreign A and B Isoantigens with Anti-B Antibodies.

RBC treatment	Antibodies produced	Hemagglutinin titer* Expt. 1	Expt. 2
Anti-B coated	A	24.3 (8– 64)	78.7 (64–256)
	B	16.0 (8–128)	10.5 (4– 16)
Uncoated	A	4.6 (0– 8)	24.3 (8– 64)
	B	111.4 (64–128)	188.8 (16–512)

* Geometric mean and (range) of hemagglutinin titers determined 1 week following the last immunization.

When comparing the patterns of antibody response to A and B isoantigens it is apparent that, with birds receiving uncoated erythrocytes, considerable anti-B antibody production occurs prior to detectable anti-A antibody production. Significant anti-A antibody synthesis occurs only after the anti-B titer approaches a maximum. This finding, together with the observation that suppression of anti-B antibody synthesis is accompanied by an enhanced anti-A response, suggests the possibility that B antigens compete with A antigens during early stages of immunization. Coating B antigens with specific antibody might diminish competition with A antigens. An alternate interpretation is that B antigens act as an adjuvant for A antigens most effectively when coated with actively synthesized or passively administered antibody. In this case the degree of adjuvant activity may depend on the quantity and quality (class and avidity) of the anti-B antibody. The absence or deficiency (low avidity?) of anti-B antibody in birds tolerant of B antigens may account for the loss of the adjuvant property in this situation (1). Results obtained from inoculating chickens which are immunologically tolerant of B antigens with anti-B coated erythrocytes possessing foreign A and B isoantigens may aid interpretation of the present findings.

The results obtained in this study are in agreement with those of Pearlman (5) in which passive antibody to sheep erythrocytes, while suppressing antibody formation of the erythrocytes, appeared to enhance the response to heterologous determinants conjugated to the erythrocytes. The results differ

from those of Brody *et al.* (6) in which passive antibody to one hapten suppressed antibody formation to the specific hapten but had no effect on antibody production to a second haptenic determinant coupled to the same carrier molecule. They also contrast with a study in which anti-Forssman antibody, given passively to rabbits, suppressed antibody formation to both Forssman and isophile antigens of sheep erythrocytes (7). Possibly, these discrepancies can be accounted for on the basis of differences in degree of suppression of antibody synthesis to the specific antigen or differences in immunization procedures.

Clinical studies, in which anti-Rh antibodies are passively administered to prevent maternal Rh sensitization (8), are relevant. It may be important to determine whether this procedure might enhance immunological responses to isoantigens of other systems present on fetal erythrocytes.

Summary. The effects of passively coating, with specific isoantibodies, erythrocyte isoantigens determined by the *B* blood-group locus in chickens on the immune response to A blood-group system isoantigens were investigated. Antibody synthesis to the B isoantigens was suppressed while the immunological response to A isoantigens, present on the same erythrocytes, was significantly enhanced. Since B isoantigens act as an adjuvant to A isoantigens, these results suggest that passive anti-B antibodies diminish competition between B and A antigens or, alternatively, enhance the adjuvant activity of the B antigens.

We gratefully acknowledge the able technical assistance of Mr. A. Murgo and Mr. V. Pinkney.

1. Schierman, L. W. and McBride, R. A., Science **156**, 658 (1967).

2. Berrian, J. H. and McKhann, C. F., Ann. N. Y. Acad. Sci. **87**, 106 (1960).

3. Uhr, J. W. and Möller, G., *in* "Advances in Immunology" (F. J. Dixon and H. G. Kunkel, eds.), Vol. 8, p. 81, Academic Press, New York (1968).

4. Keily, S. D., Schierman, L. W., and Nordskog, A. W., Proc. Soc. Exptl. Biol. Med. **121**, 137 (1966).

5. Pearlman, D. S., J. Exptl. Med. **126**, 127 (1967).

6. Brody, N. I., Walker, J. G., and Siskind, G. W., J. Exptl. Med. **126**, 81 (1967).

7. Greenbury, C. L. and Moore, D. H., Nature **219**, 526 (1968).

8. Freda, V. J., Gorman, J. G., and Pollack, W., Science **151**, 828 (1966).

Received Sept. 27, 1968. P.S.E.B.M., 1969, Vol. 130.