PASSIVE ANTIBODY AND THE IMMUNE RESPONSE

Factors Which Determine Enhancement and Suppression*

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It is well documented that passively administered antibody may suppress (1–15) or enhance (10–18) a specific immune response. The possibility that antibody-mediated suppression involves the direct interaction of antigen with the combining sites of antibody rests largely on demonstrations that suppression is immunologically specific (reviewed in ref. 19). Other findings, however, have been interpreted as indicating that suppression by antibody is mediated primarily through a direct effect on antibody-producing cells (20). Interpretations of the mechanisms of antibody-mediated enhancement of the immune response range from effects attributable to nonspecific alterations in the physical state of antigen (19) to specific carrier effects, whereby antibody to some determinants on a multideterminant antigen results in an increase in determinants that function as carriers (15). The latter interpretation was proposed to explain our observations that passive antibody to foreign B blood group isoantigens in fowl suppresses the specific anti-B response while enhancing the immune response to haptenic A isoantigens present on the same immunizing erythrocytes (14). According to this view, determinants coated with antibody are not capable of inducing antibody production to themselves but acquire the capacity to serve as carriers for noncoated determinants. Thus, we do not distinguish between two opposing effects of antibody (i.e. suppression or enhancement) but propose that antibody coating a determinant blocks the immune response to that determinant and the net effect is determined, at least in part, by the quantity of antibodies produced to noncoated determinants.

The present studies were undertaken to elucidate further the effects of passive antibody on the specificity of the immune response to multideterminant isoantigens. One interpretation of the results is that antibody-mediated enhancement and suppression are effects which depend on the structural relationships of coated and noncoated determinants. These structural relationships are inferred by the finding that enhancement and suppression are dependent on the phenotype of the immunizing erythrocyte. On the basis of these findings an interpretation is offered for the discovery that antibody formation and major histocompatibility antigens may be controlled by dominant genes at the same locus (21).

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Materials and Methods

Animals.—Partially inbred White Leghorn chickens (line G) were used as donors of erythrocytes (RBC).[1] Recipients, which were from a closely related subline (line G-B1), were homozygous at the *A* and *B* blood group loci (genotype A^1/A^1, B^1/B^1). Donors possessed foreign B and A or only foreign B isoantigens and were matched with the recipients with respect to isoantigens determined by other blood group loci. Breeding of the chickens is under our direction.

Production of Anti-B Antibody for In Vitro Coating of Donor RBC.—Hyperimmune anti-B_2 antibody was produced in a recipient of B^1/B^3 genotype by multiple weekly inoculations of washed RBC from a single donor. The specificity of the antibody was verified by testing RBC from birds of known blood group genotypes. The anti-B_2 antibody produced by this recipient did not cross-react with B_3 antigens. However, anti-B_2 antibody produced by a B^1/B^1 recipient does cross-react with B_3, presumably due to common B_2 and B_3 determinants. By use of a sensitive quantitative hemolytic assay employing rabbit antibody specific for chicken IgG and IgM,[2] we found that the anti-B_2 antibody used for coating donor RBC was predominantly of the IgG class and contained only trace amounts of IgM.

Isoimmunization With Coated and Noncoated RBC.—To coat donor RBC with anti-B_2 antibody, antiserum from pooled bleedings was added to an equal volume of washed and packed RBC and the mixture incubated at room temperature for 1 hr. Effects of the concentration of anti-B_2 antibody used for coating were determined by adding undiluted or fivefold dilutions of antiserum to the RBC. The titer of the antiserum used for coating was such that only large clumps of cells were present after equal volumes of donor RBC and undiluted serum were mixed. Before inoculation of the coated RBC, unbound antibodies were removed by washing and centrifugation. Recipients, 6–8 wk of age, were given weekly intravenous inoculations of 0.75 ml of a 25% suspension of RBC. They were bled (2.5 ml) for serum before each inoculation and 1 wk after the last. Each recipient group consisted of 7–10 animals.

Serological Procedures.—The titers of anti-A and/or anti-B hemagglutinins were determined in separate tests with RBC possessing only the relevant antigens, i.e., A_2, B_2, or B_3. Sera were also titrated against donor RBC to determine the combined effects of the antibodies produced (the "net immune response"). One drop of a 2% RBC suspension was added to 0.2 ml of twofold dilutions of serum in 0.15 M saline and, after incubation, the degree of agglutination scored macroscopically. Titers, which represent the last dilution showing detectable agglutination, were log-transformed for analysis. Where indicated, the specificity of the anti-B antibody was determined by absorption with appropriate RBC.

RESULTS

Effect of Passive Anti-B_2 Antibody on the Response to RBC Possessing B_2 and B_3 Antigens.—Two groups of chickens were given four weekly inoculations of RBC from a donor of B^2/B^3 genotype. Group 1 received noncoated RBC and group 2 received RBC coated with anti-B_2 antibody. Titrating nonabsorbed sera from members of each group against donor RBC revealed that coating caused a net enhancement of the immune response. Serum from the third and fourth bleedings was pooled for each bird to obtain sufficient amounts for ab-

[1] *Abbreviations used in this paper:* NRA, nonresponder antibody; RBC, erythrocytes.

[2] Bacon, L. D., L. W. Schierman, and R. A. McBride. Isoimmune hemolytic plaque-forming cells. I. The kinetics of appearance of 19S and 7S isoantibody releasing cells during a primary and secondary immune response. Manuscript in preparation.

sorption studies. The results are summarized in Table I. Nonabsorbed sera obtained from group 2 recipients were capable of agglutinating RBC possessing only B_2 antigens. Thus, the immune response to all determinants of the B_2 antigen was not abolished by passive anti-B_2 antibody. The crucial question we wished to answer was: To which B_2 determinants (i.e. coated or noncoated) was the immune response directed? This was answered by separately absorbing the sera with B_2 and B_3 antigens. In contrast to the results of absorption of group 1 sera, absorption of group 2 sera with B_3 antigens removed all reactivity against B_2. This indicates that the anti-B_2 hemagglutinins produced by group 2 birds were specific for noncoated determinants, i.e., those which B_2 shares with B_3.

The titer of the antibodies produced against B_3 determinants not shared with B_2 was determined after absorption with B_2 antigens. There was a significantly

TABLE I

Effect of Coating RBC Possessing B_2 and B_3 Antigens with Anti-B_2 Antibody

Group	Number of birds	Donor RBC treatment	Recipient serum absorbed with	Hemagglutinin titers* in tests with RBC possessing only	
				B_2	B_3
1	7	Noncoated	Nonabsorbed	210.0 (16–512)	512.0 (64–1024)
			B_2	0	256.0 (32–512)‡
			B_3	70.7 (0–256)	0
2	7	Coated§	Nonabsorbed	70.7 (32–256)	1024.0 (512–2048)
			B_2	0	760.9 (512–1024)‡
			B_3	0	0

* Geometric mean and (range) of antisera pooled from third and fourth bleedings.

‡ Differences in mean titers statistically significant. $P < 0.02$.

§ The anti-B antibody was prepared in a bird of B^1/B^3 genotype and coated only B_2 determinants not shared with B_3.

enhanced immune response to unique B_3 determinants among birds which received coated RBC (Table I). Since the titers of anti-B_2 hemagglutinins contained in nonabsorbed sera were lower in group 2 than in group 1, it appears that coating the RBC did not enhance the response to B_3 determinants shared with B_2.

Effect of Passively Coating Some B Determinants on the Immune Response to Other Determinants Controlled by the Same Gene.—The effect of coating unique B_2 determinants on the response to determinants shared with B_3 was further investigated. Two groups of recipients were inoculated with RBC possessing only foreign B_2 antigens (donor genotype B^2/B^2). Group 1 received noncoated RBC while group 2 received RBC which had been coated with anti-B_2 antibody. The anti-B_2 antiserum used for coating the RBC was the same as the one used in the previous study. That the immune response among group 2 recipients to

both coated and noncoated determinants was significantly suppressed can be seen from the results in Fig. 1. The anti-B_2 antibodies produced by recipients of noncoated RBC cross-reacted extensively with RBC possessing only B_3. This finding suggests that the shared determinants are potent immunogens. We as-

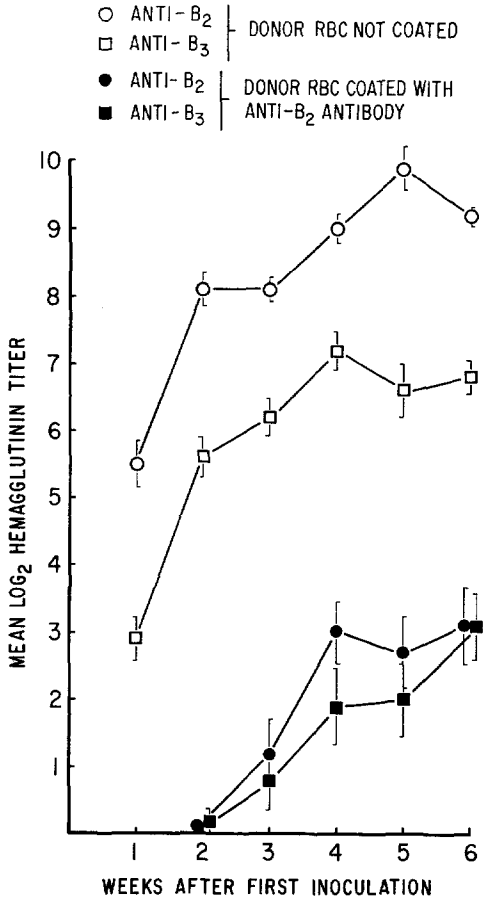

FIG. 1. The immune response of birds of B^1/B^1 genotype inoculated with RBC possessing foreign B_2 antigens. One group of birds received RBC coated in vitro with anti-B_2 antibody. Controls received noncoated RBC. Sera, obtained before each weekly inoculation, were titrated against RBC possessing only B_2 and only B_3 antigens. Each point represents the mean response of 10 birds and the vertical bars indicate the standard errors of the means.

sume that among the recipients of coated RBC, the anti-B_3 hemagglutinin titer reflects the degree of immune response to B_2 determinants not coated with antibody. Absorption studies support this interpretation since all of the antibodies produced by recipients of coated RBC were removed by absorption with B_3 antigens.

It is apparent that the net effect of passive anti-B_2 antibody on the immune response depends on the antigenic determinants not coated with antibody. Since in these studies noncoated determinants were products of the same genetic locus, it was of interest to examine the effects of the same antiserum, known to suppress the immune response to noncoated B_2 determinants, on the immune response to noncoated determinants controlled by another blood group locus. Two groups of recipients were inoculated with RBC possessing foreign A_2 and B_2 antigens (donor genotype = A^2/A^2, B^2/B^2). Group 1 received noncoated RBC whereas group 2 received RBC incubated, before inoculation, with the anti-B_2 antiserum. The findings are presented in Table II. The same passive anti-B_2 antibody that significantly suppressed the immune response to noncoated B_2 determinants significantly enhanced the response to noncoated A_2

TABLE II

Effect of Coating Some B_2 Determinants with Antibody on the Immune Response to RBC Possessing Foreign B_2 and A_2 Antigens

Group	Number of birds	Donor RBC treatment	Hemagglutinin titers* in tests with RBC possessing only		
			B_2	B_3‡	A_2
1	9	Noncoated	237.0 (128–256)§	69.1 (64–128)§	64.6 (16–256)§
2	9	Coated‖	2.0 (0–8)§	1.9 (0–8)§	375.8 (256–1024)§

* Geometric mean and (range) after three weekly inoculations.

‡ The hemagglutinin titers determined in tests with RBC possessing B_3 antigens is assumed to measure the response to noncoated B_2 determinants.

§ Differences in mean titers between groups 1 and 2 statistically significant. $P < 0.001$.

‖ The anti-B_2 antibody was prepared in a bird of B^1/B^3 genotype and coated only B_2 determinants not shared with B_3.

determinants. These results, in conjunction with our previous findings (14, 15), rule out the possibility that the anti-B_2 antiserum used in the present studies possessed exclusive properties.

Relationship of Suppression and Enhancement of the Immune Response to the Quantity of Passive Antibody.—Since suppression or enhancement of the immune response may be a function of antigen-antibody ratio, we wished to determine the relationship of specific suppression and enhancement to the quantity of passive antibody used to coat donor RBC. Four groups of recipients received weekly inoculations of RBC possessing foreign A_2 and B_2 antigens (donor genotype = A^2/A^2, B^2/B^2). One group received noncoated RBC and the remaining three groups received RBC incubated, before inoculation, with either undiluted, 5-fold, or 25-fold dilutions of the anti-B_2 antiserum. Significant differences between the groups, with respect to the mean titers of anti-A_2 and anti-B_2 antibodies, were evident after two inoculations. The relationship of the dilution of anti-B_2 antiserum used for coating to the degree of anti-B_2 suppression and

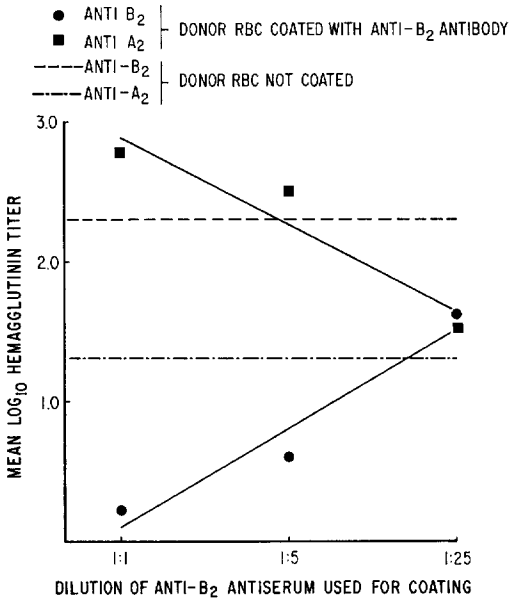

FIG. 2. The immune response of birds of B^1/B^1 genotype inoculated with RBC possessing foreign B_2 and A_2 antigens. Before inoculation, donor RBC were incubated with anti-B_2 antiserum which was either undiluted or diluted 5-fold or 25-fold. Control birds received RBC not coated with antibody. Sera from each of the four groups of recipients were titrated against RBC possessing only B_2 and only A_2 antigens 1 wk after the second weekly inoculation. Each group contained 10 birds. Regression lines were fitted by the least squares method.

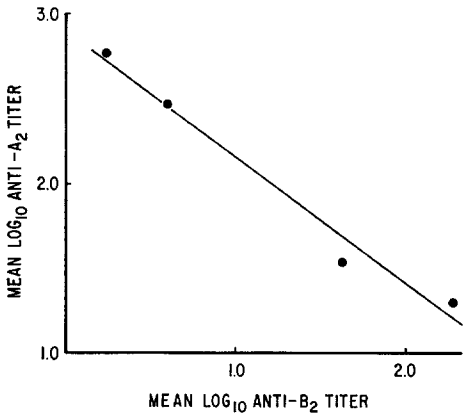

FIG. 3. The regression of anti-A antibody titer on the anti-B antibody titer calculated from the data presented in Fig. 2. The slope of the regression line, fitted by the least squares method, is highly significant ($P < 0.02$) and the titers of anti-A and anti-B antibodies have a high degree of negative correlation ($r = -0.988$).

anti-A_2 enhancement is depicted in Fig. 2. With maximal coating of B_2 determinants, achieved with undiluted anti-B_2 antiserum, there was nearly complete suppression of anti-B_2 synthesis. The degree of anti-A_2 enhancement was greatest under these same conditions. Both effects were diminished as the antiserum used for coating was diluted. Analysis of variance indicated a linear relationship between the dilution of anti-B_2 antiserum and the titers of anti-B_2 and anti-A_2 antibodies ($P < 0.01$ in both cases).

The slope of the regression line drawn in Fig. 3 is also highly significant and reflects a linear relationship between the log titers of anti-A and anti-B antibodies. The degree of enhancement is inversely proportional to the degree of suppression since there is a statistically significant negative correlation between the anti-A and anti-B titers ($r = -0.998$; $P < 0.02$).

DISCUSSION

The experiments described in this study were designed to determine the effects of passive antibody on the magnitude and specificity of the immune response to isoantigens. Three types of isoantigenic determinants were studied: (*a*) those determined by the same gene, (*b*) those determined by two allelic genes, and (*c*) those determined by genes at different loci. The passive antibody, which was administered in combination with the RBC isoantigens, was identical in all experiments, i.e., it was obtained from a single hyperimmunized bird. Since the recipients of the passively coated RBC were of the same genotype, we view the observed differences in response as dependent on the particular combination of coated and noncoated donor RBC determinants. Thus, in this system, overall suppression or enhancement of the immune response is not determined by the class of passive antibody.

Antigens controlled by the *B* blood group locus are multideterminant, with specificities shared by different antigens. By analogy with the biologically similar *H-2* system of mice, it is likely that cross-reactive specificities controlled by different *B* alleles are serologically identical (22). Thus, antibodies produced by recipients of B^1/B^1 genotype after immunization with B_2 antigens agglutinate RBC possessing B_3 antigens (Fig. 1).[3] However, the anti-B_2 antiserum which we used for coating donor RBC was produced by a bird of B^1/B^3 genotype and therefore contained antibodies specific only for those B_2 determinants not shared with B_3.

Coating donor RBC with anti-B_2 antibody results in suppression of the immune response to coated determinants and may either enhance (Table I) or

[3] Although specific B antigenic factors have not been identified, arbitrary symbols can be assigned. Accordingly, an anti-B_2 antiserum may be considered to contain a population of antibodies specific for determinants *a*, *b*, *c*, and *d*. If B_3 antigens possess determinants *c*, *d*, *e*, and *f*, RBC possessing B_3 would be agglutinated by anti-B_2 antiserum on the basis of shared specificities *c* and *d*.

suppress (Fig. 1) the immune response to noncoated determinants. Enhancement occurs where donor RBC possess B_3 determinants not shared with B_2, i.e., specificities determined by the B^3 gene. On the other hand, coating the same B_2 specificities results in suppression of the immune response to noncoated determinants if they are products of the same gene. At least two possibilities may account for suppression of the response to noncoated determinants. First, there may be steric hindrance brought about by antibody coating determinants when the noncoated determinants are on the same molecule or on separate but structurally closely related molecules (23, 24). Second, since RBC from B^2/B^2 homozygotes presumably have twice as many determinants available for coating by anti-B_2 as do RBC from B^2/B^3 heterozygotes, the additional quantity of absorbed antibody may be sufficient to result in net suppression. The lack of information on the biochemistry of the B antigens of fowl precludes firm conclusions as to the validity of the first possibility. It is known that in mice, different H-2 specificities determined by the same gene may be present on the same molecule (25) and that hybrid molecules exist in heterozygotes (26). In this regard, an in vitro phenomenon interpretable as steric hindrance mediated by antibody has been observed in the *H-2* system (23). The results of the experiment presented in Table II provide evidence against the second possibility. The same anti-B_2 antiserum used to coat RBC from B^2/B^2 homozygotes significantly enhances the response to A_2 determinants while suppressing the response to noncoated B_2 determinants present on the same RBC (hemagglutinin titer in tests with RBC possessing only B_3). An additional argument against the second possibility is inferred by the data presented in Figs. 2 and 3. With respect to B_2 and A_2 antigens, the degree of enhancement of the anti-A_2 response is greater as the opportunity for coating B_2 determinants is increased. This relationship is in accord with a previous prediction that a maximal anti-A response would occur when all foreign B determinants are coated (14). A linear relationship between the degree of enhancement and suppression and quantity of passive antibody has recently been reported by others (13). We view the degree of anti-A enhancement as a function of the number of B determinants converted to carrier by passive B antibody. Since the degree of anti-B suppression is also determined by the concentration of passive B antibody (Fig. 2), a negative correlation and linear relationship between the anti-A and anti-B titers exists at a particular phase of the immune response (Fig. 3). It is likely that the inverse relationship between the titers of anti-A and anti-B antibodies is a reflection of the total number of cells making isoantibodies and that this number is nearly constant among the four groups of recipients. On the basis of these considerations we assume that enhancement of the immune response to noncoated B determinants increases as the number of coated determinants increases, provided the coated and noncoated specificities are determined by alleles. We therefore believe that the failure to observe enhancement of the im-

mune response to noncoated B_2 determinants (Fig. 1) is because of steric hindrance, related to structural relationships, rather than effects attributable to the absorption of a greater quantity of anti-B_2 antibody by RBC from B^2/B^2 homozygotes.

The effects of passive antibody on the net immune response may differ even though the antibody is the same in each situation. By appropriate absorptions and tests with single antigens we were able to determine that the immune response to coated B_2 determinants was suppressed and that the net enhancement observed was because of an augmented immune response to noncoated determinants. The suggestion has been made, with regard to the two-determinant theory of antibody formation, that antibody coated determinants serve as carriers for noncoated determinants (15, 27). The present results suggest an additional requirement for immunogenicity, i.e., a certain spatial relationship between coated and noncoated determinants.

It is possible that this spatial relationship property has a bearing on the well-known fact that ABO incompatibility protects against Rh isoimmunization (28, 29). Perhaps the A, B, and Rh antigenic sites have a spatial relationship such that maternal isoantibody combining with A and/or B suppresses, by means of steric hindrance, the immune response to Rh. That significant steric effects because of antibody may exist between antigens determined by different genetic systems of mice (products of the *TL* system and the "D" end of the *H-2* system) has been shown in in vitro studies (24). Other studies, in addition to the present findings, suggest that in certain situations antibody to one or more determinants may suppress the immune response to other determinants which are physically closely associated (30, 31).

The demonstration that antibody may suppress the immune response to noncoated determinants, as well as block the response to coated determinants, may have relevance to genetic responder and nonresponder animals. The immune response to a variety of antigens behaves as a dominantly inherited trait apparently controlled by genes at the major histocompatibility locus (21, 32). As a possible explanation of this phenomenon we propose that nonresponders possess an antibody which reacts with determinants of the antigen in such a manner that the immune response to other determinants is inhibited. This nonresponder antibody (NRA) might arise (*a*) as a result of autoimmunization with antigens determined by histocompatibility genes which have undergone somatic mutation or (*b*) from prior exposure to environmental antigens possessing the determinants. According to this interpretation responders would not have this antibody because it has specificity for a self antigen, i.e., the major histocompatibility antigen present on their cells. Although NRA may be present in the circulation, it is plausible that it corresponds to cell-bound receptors which may function in carrier recognition and enhancement of anti-hapten immune responses (33–37). It is of interest in this regard that carrier-sensitized cells

have been shown to enhance anti-hapten immune responses in situations where passive antibody was ineffective (33–35, 37, 38). It remains to be seen whether "helper cells," like serum antibody in our studies, function only with those hapten-carrier systems in which optimal structural associations are presumed to exist.

In addition to the mode of inheritance, findings which are consistent with our interpretation of the responder-nonresponder phenomenon are: (*a*) the responder trait is, to a degree, quantitative, i.e., larger doses of antigen stimulate a response in "nonresponders" (39, 40). This feature suggests that with larger quantities of antigen the NRA is bound, and effectively removed, and the excess quantity of free antigen is available for stimulation; (*b*) the nonresponder state is highly specific and may depend on single amino acid substitutions in the antigen (41, 42), a degree of specificity characteristic of antibodies; (*c*) the lack of evidence for recombination between "responder genes" and major histocompatibility genes; and (*d*) the high frequency of cells capable of responding to foreign major histocompatibility antigens (43), a finding which would allow for a sufficient quantity of NRA.

The abrogation of the nonresponder state after the induction of immunological tolerance to responder histocompatibility antigens would support the proposed interpretation. The method of tolerance induction would have to preclude the production of lymphoid cell chimerism.

SUMMARY

The isoimmune response of fowl inoculated with RBC coated with antibody was investigated. Anti-B antiserum from a single animal was used to coat different donor type RBC. With each donor type RBC the immune response to the coated determinants is suppressed. Enhancement of the immune response to noncoated determinants occurs when they are products of an allelic gene or belong to a different blood group system. Coating some B antigen determinants suppresses the response to noncoated determinants of the same antigen, i.e., determinants which are products of the same *B* gene. Varying the quantity of passive antibody revealed that the degree of suppression and the degree of enhancement are negatively correlated.

These findings support the concept that antibody-coated determinants function as carrier for noncoated determinants, provided a certain physical asscciation exists between them. A further interpretation of these studies is that in certain situations an antibody to one antigen may interfere with events which lead to an immune response to a different antigen. The possibility, that the protection afforded by ABO incompatibility against Rh isoimmunization is because of a similar phenomenon, is discussed. A hypothesis is presented which states that where the immune response to certain antigens behaves as a dominantly inherited trait, and is associated with histocompatibility type, the non-

responder animals possess an antibody (perhaps cell bound) which interferes with the response to determinants for which it does not have specificity. Responders are assumed to lack this antibody because it has specificity for their major histocompatibility antigens.

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