

GENETIC FACTORS CONTROLLING ANTI-SHEEP ERYTHRO-  
CYTE ANTIBODY RESPONSE AND IMMUNOGLOBULIN  
SYNTHESIS IN BACKCROSS AND F<sub>2</sub> PROGENY OF  
MICE GENETICALLY SELECTED FOR "HIGH" OR  
"LOW" ANTIBODY SYNTHESIS

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Random-bred Swiss mice were bred selectively for their agglutinin responses to sheep and pigeon erythrocytes to obtain high and low responder lines for those antigens (1). After 20 generations, the two lines differed markedly in their mean agglutinin titers against sheep erythrocytes and were considered established, since the difference in agglutinin response could not be increased by further breeding (2). The two lines differed remarkably in their humoral responses not only against the antigens used for their selection but also against every antigen with which they have been tested: hen ovalbumin (3), *Salmonella typhi* (1), pneumococcal polysaccharide SIII (4), transplantation antigens (5), DNA hapten (6), and T<sub>4</sub> bacteriophages (J. G. Howard, unpublished observations). Nevertheless, the high and low lines behaved alike in their cellular immune responses that are known to be expressions of thymus-derived cell function, such as contact reactivity (C. Stiffel, unpublished observations), graft-vs.-host reaction,<sup>1</sup> allograft rejection (2), and phytohemagglutinin responsiveness (7). These findings suggested that the selection had operated primarily at the level of the bone marrow-derived cell, on genes concerned with differentiation for antibody synthesis regardless of immunological specificity (1).

We confirmed this interpretation in an earlier study (8). The serum levels of individual immunoglobulins were measured in the two lines before and after antigenic challenge, and the immunoglobulin allotypes were determined. The levels of all immunoglobulins were shown to be depressed in the low line compared with the high line both before and after immunization with pigeon or sheep erythrocytes. The differences in immunoglobulin levels were more marked for IgM and IgG classes and subclasses than for IgA, and were considerably greater after immunization than before. The differences in immunoglobulin concentrations were considered to result from a deficiency in the low line, since in the high line the serum concentrations of

<sup>1</sup> Byfield, P. E., and J. G. Howard. 1972. Manuscript submitted to *Transplantation*.

the various immunoglobulins were within the range observed for normal Swiss mice of the same age and development. Different phenotypic markers were detected on the immunoglobulin heavy chains of the two lines. The low responder mice were homozygous for a heavy chain linkage group ( $G^{3, 5, 7, 8}, H^9, 11 A^{-} F^1$ ) not present in inbred mice in the United States but observed as a recombinant type among wild mice (9) and recently in rare Swiss mice (8). This linkage group probably represents a crossover between the heavy chain linkage group of the prototypes DBA/2 and NH inbred strains. The high responder mice were heterozygous or homozygous for heavy chain linkage groups ( $G^{1, 6, 7, 8} H^9, 11 A^{12, 13, 14} F^1$ ) and ( ${}^2G^{-} H^9, {}^{16}A^{15} F^8$ ) found separately in the prototypes BALB/c and C57BL inbred mice, respectively.

Questions raised by these findings were whether the distinct immunoglobulin phenotypes characteristic of the low and high lines resulted from the selective breeding for anti-sheep and anti-pigeon erythrocyte agglutinins, and whether some insight could be gained about the number and types of genes concerned with the control of immunoglobulin synthesis by a test of the  $F_1$ ,  $F_2$ , and backcross generations from the two lines. The present study reports the levels of immunoglobulins of the various classes and subclasses in mice of these generations. In addition, a highly significant correlation is demonstrated between the anti-sheep erythrocyte agglutinin responses and the presence of the low line immunoglobulin heavy chain linkage group in the  $F_2$  generation and in the generation resulting from a backcross of  $F_1$  with the low line. This indicates that one of the genes concerned in the selection process is linked to the immunoglobulin heavy chain structural genes.

#### *Materials and Methods*

*Selection and Breeding.*—The selection process used to develop the high and low responder lines has been described (1).

*Immunization.*—Mice were immunized with  $5 \times 10^8$  sheep erythrocytes in a single dose intravenously between 30 and 45 days of age.

*Antibody Assay.*—The mice were bled 5 and 17 days after immunization and the antibody titer of the sera was determined by a microhemagglutination technique as described previously (8).

*Measurement of Immunoglobulin Concentrations.*—The concentrations of immunoglobulin were measured on sera obtained 10 or 11 days after immunization. Serum immunoglobulin levels were determined by the method of radial immunodiffusion (10) as described previously (8). Levels were determined for IgM, IgA,  $\gamma_1$ , and  $\gamma_2$ . Levels of  $\gamma_2a$  and  $\gamma_2b$  were not determined separately. Results are shown as the geometric mean  $\pm$  1 standard deviation.

*Serologic Typing of Immunoglobulin Allotypic Determinants.*—Typing for allotypic determinants was carried out as described in our earlier study (8) using precisely the same antisera as reagents, to determine whether individual  $F_2$  or backcross offspring had inherited the low line ( $G^{3, 5, 7, 8} H^9, 11 A^{-} F^1$ ) or the high line ( ${}^2G^{-} H^9, {}^{16}A^{15} F^8$ ) heavy chain linkage groups. Since ( ${}^2G^{-} H^9, {}^{16}A^{15} F^8$ ) is by far the most frequent of the two heavy chain linkage groups of the high line, the few mice with the other high line heavy chain allotype ( $G^{1, 6, 7, 8} H^9, 11 A^{12, 13, 14} F^1$ ) were not considered in order to permit a more adequate statistical analysis of the data in Tables I–III.

## RESULTS

*Serum Immunoglobulin Levels.*—Sera from 35 high responders, 18 low responders, 32  $F_1$  and 209  $F_2$  progeny between these two lines, 68 progeny of backcross of  $F_1$  to low responders, and 48 progeny of backcross of  $F_1$  to high responders were analyzed for content of  $\gamma_1$ ,  $\gamma_2$ , IgA, and IgM. No significant

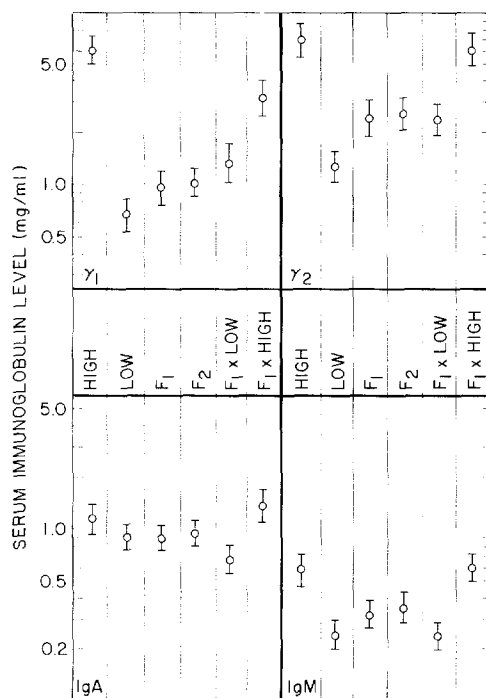


FIG. 1. Geometric mean immunoglobulin levels in sera of various groups of mice. Sera in each group were analyzed regardless of sex or allotype (see text). Each point (○) shows the mean in the indicated group; brackets denote the limits of  $\pm 1$  standard deviation. Upper left: serum  $\gamma_1$  levels; upper right:  $\gamma_2$  levels; lower left: IgA levels; lower right: IgM levels.

differences were observed in these various groups in levels of protein between males and females, except for one of two groups of  $F_2$  mice, in which  $\gamma_1$  and IgA levels were significantly higher ( $P < 0.01$ ) in females. In the second group of  $F_2$  mice levels of these proteins were identical in males and females. Furthermore, no significant difference among different allotypes was observed, either in  $F_2$  or backcross progeny.

The pooled data from each group are summarized in Fig. 1. Serum IgM levels showed a simple pattern of inheritance. Levels in  $F_1$  and  $F_2$  progeny were intermediate between those found in the parental strains. Levels equiv-

alent to those found in parental strains were seen in the first backcross to each. Levels of  $\gamma 1$ ,  $\gamma 2$ , and IgA were also intermediate in  $F_1$  and  $F_2$  mice. Backcross of  $F_1$  animals to high responders produced progeny with  $\gamma 1$  and  $\gamma 2$  levels intermediate between those of the  $F_1$  and parental line animals, but closer to

TABLE I  
*Relationship between Immunoglobulin Allotypes and Anti-Sheep Erythrocyte Response in  $F_2$  Generation Mice from Crosses between High and Low Responder Lines*

No. of Exp. group	Allotype	No. of animals	Rec. geom. mean titer and se	
			Day 5	Day 17
Female mice				
I	3, 5/3, 5*	33	491 + 73 - 65	438 + 113 - 100
II	2/3, 5	66	525 + 58 - 52	551 + 83 - 72
III	2/2*	17	921 + 144 - 122	875 + 217 - 174
Male mice				
IV	3, 5/3, 5	37	536 + 61 - 55	320 + 57 - 49
V	2/3, 5	52	826 + 81 - 74	682 + 103 - 92
VI	2/2	20	1077 + 116 - 98	492 + 103 - 86

Student's *t* test:

Differences between group I and III at 5 days  $0.005 > P < 0.01$ ;

“ “ “ IV and VI at 5 days  $P < 0.001$ ;

“ “ “ I and II at 5 days are not significant;

“ “ “ II and III at 5 days  $0.01 > P < 0.02$ ;

“ “ “ IV and V at 5 days  $0.001 > P < 0.005$ ;

“ “ “ V and VI at 5 days  $0.1 > P < 0.2$ ;

“ “ “ II and V at 5 days  $0.001 > P < 0.005$ .

\* 3, 5, and 2 indicate the allotypes of the heavy chain linkage groups of the low line  $G^3, 5, 7, 8H^9, 11A^-F^f$  and of the high line  $2G^-H^9, 16A^{15}F^s$ , respectively.

the latter. On the other hand, backcross to low responders resulted in progeny with  $\gamma 1$  and  $\gamma 2$  levels similar to those found in  $F_1$  and  $F_2$  mice. There was a statistically significant difference in IgA levels between high and low lines but the difference was small. Progeny of  $F_1$ ,  $F_2$ , and backcrosses showed no obvious pattern of inheritance indicating that there had been little effective selection for the synthesis of this immunoglobulin class.

*Relationship between Immunoglobulin Allotype and Anti-Sheep Erythrocyte Agglutinin Response.*—The F<sub>2</sub> and backcross generations were immunized with sheep erythrocytes and the agglutinin titer of the sera measured on days 5 and 17. The sera were then typed for the heavy chain allotype linkage groups of the high and low lines. The results obtained with the F<sub>2</sub> generation are shown in Table I. The titers of the female mice (groups I–III) and of the male mice (groups IV–VI) were analyzed separately. The data were grouped according to the immunoglobulin allotype of the individual F<sub>2</sub> animal. Groups I and IV

TABLE II  
*Relationship between Immunoglobulin Allotypes and Anti-Sheep Erythrocyte Response in Progeny of F<sub>1</sub> Backcrossed with High Line*

Exp. group	Allotype	No. of animals	Rec. geom. mean titer and SE	
			Day 5	Day 17
Female mice				
A	2/2*	10	1258 + 159 – 142	1632 + 243 – 212
B	2/3, 5*	12	698 + 198 – 155	1479 + 162 – 147
Male mice				
C	2/2	11	990 + 78 – 73	2109 + 287 – 262
D	2/3, 5	10	1095 + 123 – 111	2960 + 364 – 332

Statistical analysis by Student's *t* test:

Differences between group A and B at 5 days are significant  $0.05 > P < 0.1$ ;

“ “ “ “ “ “ at 17 days are not significant;

“ “ “ C and D at 5 days are not significant;

“ “ “ “ “ “ at 17 days are not significant.

\*3, 5, and 2 indicate the allotype of the heavy chain linkage groups of the low line G<sup>3</sup>, 5, 7, <sup>8</sup>H<sup>9</sup>, <sup>11</sup>A–F<sup>1</sup> and of the high line <sup>2</sup>G–H<sup>9</sup>, <sup>16</sup>A<sup>15</sup>F<sup>8</sup>, respectively.

were made of animals homozygous for the heavy chain linkage group of the low line; groups III and VI were constituted with animals homozygous for heavy chain linkage group of the high line. Groups II and V comprised animals heterozygous for this character. The data are presented as the reciprocal of the geometric mean titers for each group with the corresponding standard errors. The peak antibody titers were generally obtained on day 5. The mean agglutinin titers of mice with only allotype markers characteristic of the low line were always lower than those of mice homozygous for high line allotypes. These differences were highly significant statistically in both male and female groups. Statistically significant differences were also noted between the titers

of the heterozygous groups and the titers of mice with high line allotypes in females and with low line allotypes in males. The results obtained with  $F_1$  backcrossed with the high line and with the low line are found, respectively, in Tables II and III. The mice were again grouped according to the allotype markers which they inherited from the high or the low line. These data afford a better comparison between animals heterozygous for the heavy chain linkage groups and animals homozygous for either the high or the low line linkage group. The only highly significant differences were obtained with the male

TABLE III  
*Relationship between Immunoglobulin Allotypes and Anti-Sheep Erythrocyte Response in Progeny of  $F_1$  Backcrossed with Low Line*

Exp. group	Allotype	No. of animals	Rec. geom. mean titer and SE	
			Day 5	Day 17
Female mice				
A	3, 5/3, 5*	12	127 + 37 - 29	159 + 62 - 44
B	2/3, 5*	9	220 + 50 - 41	354 + 159 - 110
Male mice				
C	3, 5/3, 5	21	133 + 23 - 19	129 + 38 - 29
D	2/3, 5	18	214 + 18 - 17	286 + 69 - 56

Statistical analysis by Student's *t* test:

Differences between groups A and B at 5 days are significant  $0.1 > P < 0.2$ ;

“ “ “ “ “ “ at 17 days are significant  $0.1 > P < 0.2$ ;

“ “ “ C and D at 5 days are significant  $0.01 > P < 0.02$ ;

“ “ “ “ “ “ at 17 days are significant  $0.025 > P < 0.05$ .

\* 3, 5, and 2 indicate the allotypes of the heavy chain linkage groups of the low line  $G^3, 5, 7, 8H^9, 11A-F^f$  and of the high line  $2G-H^9, 16A^{15}F^s$ , respectively.

offspring from  $F_1$  backcrossed to the low line. In these groups, mice with only low line allotypes had clearly lower agglutinin titers as a group than the mice heterozygous at this locus. If, however, a comparison is made of the titers of mice with identical heavy chain linkage group but differing at other genes, considerable differences were noted. Thus, heterozygous mice resulting from both types of backcrosses differed markedly from each other in this respect. The heterozygous low line backcross mice showed agglutinin titers very much lower than the heterozygous high line backcross or the  $F_2$  mice. In contrast to the apparent relationship between anti-sheep erythrocyte agglutinin titers and immunoglobulin allotype shown above, a similar relationship between immunoglobulin allotype and immunoglobulin concentrations was not observed for any of the immunoglobulin classes or subclasses.

## DISCUSSION

The data presented indicate that one of the several genes concerned with the selection for anti-sheep erythrocyte agglutinin production is linked to the mouse immunoglobulin heavy chain linkage group and that the segregation of different sets of immunoglobulin allotypes in the two mouse lines bred for anti-sheep erythrocyte response was not the result of chance but was determined by the selection process. Genes controlling immune responses to  $\alpha$ -1,3 glucosyl linkage in dextrans have also been found linked to the immunoglobulin heavy chain locus in the mouse (11). The most significant findings were obtained in the F<sub>2</sub> generation when the agglutinin titers of animals homozygous for the heavy chain linkage group of the high and of the low line were compared with each other. In selected cases significant differences were also observed in the backcross studies. We must, nevertheless, stress that the gene linked to the immunoglobulin heavy chain structural genes identified in these experiments is only one of the several genes involved in the selection. This was clearly shown by the observation that animals similarly heterozygous at this locus but with different genetic background had very different agglutinin responses. The fact that this is a multigenic system probably accounts for the failure of agglutinin responses to associate with allotypes in most of the backcross progeny.

A similar relationship between allotype and serum immunoglobulin levels was not detected for any of the immunoglobulin classes in our experiments. This could be due to the fact that the differences in immunoglobulin concentrations between the two lines were not as marked as the difference in antibody titers. In addition, we must note that the immunoglobulin levels were measured on the 10-11 day whereas the agglutinins peaked on day 5 after immunization. However, in an earlier study in man, Yount, Kunkel, and Litwin (12) observed a definite relationship between the serum concentration of the IgG3 subclass and the allotype on this immunoglobulin. Gm(b<sup>+</sup>) individuals showed a higher concentration of this Ig subclass than those who were Gm(b<sup>-</sup>).

In many cases the serum immunoglobulin levels appeared to follow a surprisingly simple pattern of inheritance, as shown by the intermediate levels found in F<sub>1</sub> and F<sub>2</sub> progeny, and the high immunoglobulin levels observed in progeny of F<sub>1</sub> and high responder mice. Progeny of F<sub>1</sub> and low responder mice had IgM levels in the range of the low responders. However, in this backcross,  $\gamma$ 1 and  $\gamma$ 2 levels were not different from those in F<sub>1</sub> and F<sub>2</sub> mice, while IgA levels were significantly lower than those found in low responders. The latter findings may be related to the variation in immunoglobulin levels among "families" seen in both the high responder and low responder lines (8). Highly significant variations both in IgG ( $\gamma$ 1 and  $\gamma$ 2) and IgA levels were observed comparing the progeny of individual matings. Further, the variations in IgG and IgA were not covariant. It is therefore possible that the low responder mice

selected at random for mating with F<sub>1</sub> mice belonged to groups with relatively high IgG and relatively low IgA levels. Such families, in retrospect, can be identified among the low responders described previously: they show IgG levels from 25 to 50% higher, and IgA levels lower by a similar amount than the low responder group as a whole. Immunoglobulin levels in specific mice chosen for breeding and their littermates will require closer attention in future experiments.

A lack of correlation between antibody response and immunoglobulin levels was found previously (8) both in the high and low responder lines. The present study demonstrates an immune response gene for anti-sheep erythrocytes linked to the immunoglobulin heavy chain genes. Immunoglobulin levels were identical in mice with different allotypes in F<sub>2</sub> and in both backcrosses. Thus, although differences in capacity to synthesize immunoglobulins undoubtedly accounts for the major part of the difference in response between the two lines (13), genetic selection for the capacity to respond specifically to sheep erythrocytes cannot be excluded.

#### SUMMARY

Agglutinin responses to sheep erythrocytes and immunoglobulin heavy chain phenotypes determined in F<sub>1</sub>, F<sub>2</sub>, and backcross progeny of mice genetically selected for high and low antibody synthesis indicated that an immune response gene for sheep erythrocytes is linked to the immunoglobulin heavy chain allotype. Mice homozygous for the phenotype of the high line had significantly higher titers than mice homozygous for the phenotype of the low line. An association was also observed in some progeny of the backcross of the F<sub>1</sub> generation with the low line. However, the control of the immune response was clearly multigenic since heterozygous mice of the same phenotype (2/3, 5) resulting from the two backcrosses (high and low) had very different immune responses.

Immunoglobulin levels in the same progeny showed no linkage to the immunoglobulin allotype but a rather simple pattern of inheritance.

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