

SERUM CONCENTRATIONS AND ALLOTYPES OF IMMUNO-
GLOBULINS IN TWO LINES OF MICE GENETICALLY
SELECTED FOR "HIGH" OR "LOW" ANTIBODY
SYNTHESIS

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The immune response is a complex process which must be controlled by many genes acting at different levels. Genetic studies of the immune response have followed three different approaches: (a) The structural (1) and antigenic analysis of individual immunoglobulins (2) to identify and investigate the structural genes which control their synthesis. (b) The genetic analysis of the ability of random-bred or inbred strains to recognize as immunogens different antigens with relatively simple structures, usually synthetic polypeptides (3). Responsiveness to such antigens has been shown to be under the control of single dominant autosomal genes. These "specific immune response genes" appear to be concerned with recognition of immunogenicity at an early stage of the response and are linked to histocompatibility genotype rather than to genes controlling the structure or the synthesis of individual immunoglobulins (3, 4). (c) The study of the genetic regulation of antibody synthesis which has been investigated by breeding random-bred animals for the production of either "high" or "low" amounts of antibodies against selected strong multideterminant antigens. This third approach was initially introduced by Scheibel (5) who selected random-bred guinea pigs for their ability to respond to diphtheria toxoid and succeeded by selective breeding for six generations in developing two populations of animals which differed in their ability to produce anti-diphtheria toxoid antibodies. More recently Biozzi et al. (6-8) undertook to breed selectively random-bred Swiss mice for their ability to produce anti-sheep erythrocyte antibodies for six generations, and anti-sheep erythrocyte and anti-pigeon red cell antibodies alternatively for 10 additional generations. The animals separated progressively into high and low responder lines. By the ninth generation, the two populations differed 30-fold, and by the 16th generation, 280-fold in the mean agglutinin titers of the antibodies produced against sheep erythrocytes. The number of generations required for effective separation of the lines in these experiments and those of Scheibel, indicate that a relatively small number of genes are involved in the control of the process investigated.

A most remarkable finding in the experiments of Biozzi *et al.* was that the

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high and low lines were shown to also be separated with respect to their antibody responses to antigens unrelated to those used in the selection process, such as the O and H antigens of *Salmonella typhi* (8) and hen ovalbumin.¹ However, the responses of the high and low lines to these antigens were not as markedly different as their responses to sheep erythrocytes.

This observation suggests that the selection had operated primarily at the level of genes concerned with antibody synthesis irrespective of immunological specificity. If this is correct, the serum levels of individual immunoglobulins, both before and after immunization, would be expected to differ markedly in the high and low lines of mice. Differences in the allotypes of the immunoglobulins of the two lines might also be expected to occur. The present study demonstrates that this is indeed the case.

Materials and Methods

Selection and Breeding.—62 random-bred Swiss mice of both sexes were obtained from several different breeders to initiate the selection. Two lines of mice were selected for their ability to respond to sheep erythrocytes with the formation of either high or low serum titers of hemagglutinating antibodies. After six generations it was noted that the ability of offspring of the high line to form antibodies against sheep erythrocytes was inhibited by maternal antibodies passively transmitted. The selection was therefore continued by immunizing alternate generations with pigeon red cells which do not cross-react with sheep erythrocytes. The alternate selection with two antigens was justified by the observation that the two lines, which had been selected for their response to sheep erythrocytes, were also similarly separated with respect to their antibody response to pigeon red cells (6, 8).

Breeding was carried out from the first generation on the basis of the production of hemagglutinating antibodies. Interline crossing and brother-sister mating were excluded. Nevertheless, a certain degree of inbreeding resulted in each line because of the small size of the initial colony and because occasionally several members of the same litter were used to produce the next generation. Five couples of each respective line were selected initially at each generation and mated. However, in the last generations, when more homogeneous populations were obtained, a greater number of couples were used (6, 8). The 15th generation, which is studied in detail in this report, consisted of 12 families of the high line with 42 male and 35 female offspring, and of 7 families of the low line with 18 male and 25 female offspring.

The selection has proceeded until the 16th generation. The 14th and 16th generations were challenged with sheep erythrocytes, whereas the 15th generation was immunized with pigeon red cells. This selection resulted in the clear separation of a high and low responder line with respect to antibody synthesis (Fig. 1).

Immunization.—The mice were immunized until the 13th generation with 10^8 sheep erythrocytes or 10^8 pigeon red cells administered in a single dose intravenously. The immunizing dose of sheep erythrocytes was increased to 5×10^8 cells for the 14th and 16th generation. Mice were generally immunized between 30 and 45 days of age.

Antibody Assay.—The mice were bled 7 and 14 days after immunization, and the titer of antibodies was determined by a microhemagglutination technique on standard plates. The erythrocyte suspensions used contained 2×10^8 sheep cells or 2×10^7 pigeon cells. To 0.05 ml of serial dilutions of antisera was added 0.05 ml of erythrocyte suspension.

¹ Prouvost-Danon, A., C. Stiffel, D. Mouton, and G. Biozzi. Anaphylactic antibodies in mice genetically selected for antibody production. In preparation.

In each individual case the highest titer observed either at 7 or 14 days was recorded as the response of the animal. The highest titers were usually observed at 14 days, except in the last generations of the low line which showed peak titers at 7 days.

Measurement of Immunoglobulin Concentration.—Myeloma tumors originating in BALB/c

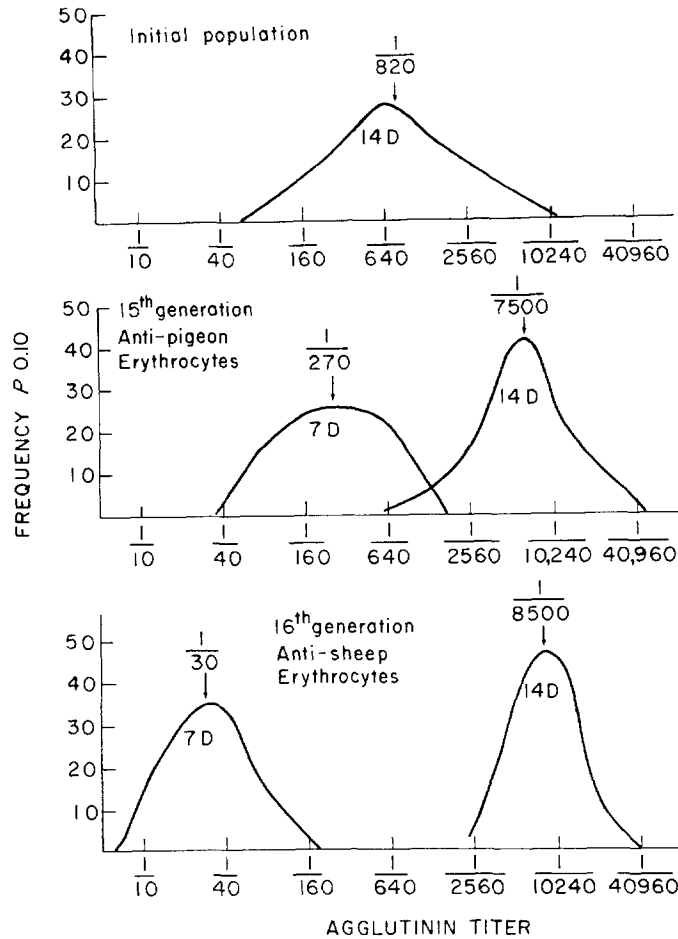


FIG. 1. Agglutinin production by the initial population and by the 15th and 16th generations of high and low responder mouse lines. The initial population and the 16th generation were immunized with sheep erythrocytes; the 15th generation was immunized with pigeon red cells. Peak titers in the low responder line were observed 7 days (7d) after immunization; and in the high responder line 14 days (14d) after immunization.

mice were obtained from Doctors M. Potter and J. L. Fahey, National Cancer Institute. They were transplanted in ascites form. Myeloma proteins were purified from ascites by means of preparative electrophoresis on Pevikon/geon blocks, followed when needed by Sephadex G-200 filtration. Fc fragments of γ_1 (F), γ_{2a} (G), and γ_{2b} (H) proteins were prepared as described by Porter (9), except that the incubation time was reduced to 1 hr. In

most cases the Fc fragments were isolated from the digest by preparative electrophoresis in agar (10).

Antisera were raised in goats by repeated immunization with myeloma proteins or their Fc fragments in complete Freund's adjuvant. The antisera were rendered specific by appropriate absorption with purified myeloma proteins and germfree mouse serum. One antiserum prepared against a γ_{2a} Fc fragment reacted equally well with γ_{2a} and γ_{2b} proteins, and was used to determine total γ_2 levels.

Standards.—Standard mixtures with known concentrations of all of the mouse immunoglobulins were prepared by mixing ascites fluid from mice bearing transplantable myeloma tumors of each class. Using the radial diffusion technique, each standard mixture was in turn calibrated for immunoglobulin content by comparison with at least two purified myeloma protein preparations of each immunoglobulin class whose concentration had been determined independently. A modification (11) of the method of single radial diffusion (12) was used to determine immunoglobulin levels. Measurements with this method were reproducible to $\pm 7\%$. Determinations were made of levels of IgM, IgA, γ_1 , γ_{2a} , γ_{2b} , and total γ_2 . The antiserum specific for γ_{2a} reacted poorly with sera containing immunoglobulin of the unassigned 2 determinant; in such sera, γ_{2a} levels were obtained by subtracting the γ_{2b} level from the total γ_2 level. In mice lacking the unassigned 2 determinant, γ_{2a} levels obtained in this way did not differ by more than 10% from γ_{2a} levels measured directly.

The sera from 40 males and 35 females from the 15th generation high responder line, and of 17 males and 25 females from the 15th generation low responder line were analyzed. The sera were obtained 14 days after immunization with pigeon red cells.

Pooled sera from mice of the 14th generation high and low responder lines respectively were obtained 14 days after immunization with sheep red cells. In addition, pooled sera from mice of the 13th generation high and low responder lines respectively were obtained before immunization with pigeon erythrocytes. These various pools were analyzed for the levels of individual immunoglobulins.

Serologic Typing of Immunoglobulin Allotypic Determinants.—Immunoglobulin phenotypes of inbred strains of mice are based on the immunoglobulin determinants identified on the heavy-chain Fc regions by homologous antisera (13). These homologous antisera (alloantisera) are prepared with normal and myeloma immunoglobulins. Most of the alloantisera used to type the mice for immunoglobulin determinants in this study have been described (14), and include antisera identifying γ_G (γ_{2a}), γ_H (γ_{2b}), γ_A , γ_F (γ_1) determinants and immunoglobulin determinants not yet assigned to specific heavy chains.

Individual sera of 76 mice from the 15th generation high responder line were analyzed for immunoglobulin allotypic markers. All sera from mice of the high responder line reacted in double gel diffusion plates with the following antisera:

Antisera	Determinants identified	Reference strain
C57BL anti-BALB/c	G ¹	BALB/c
C57BL anti-AL	G ⁶	BALB/c
DBA/2 anti-NH	G ⁷	BALB/c
C57BL anti-DBA/2	G ⁸	BALB/c
PL anti-SJL	2	C57BL
AL anti-MOPC 195 (BALB/c)	H ⁹	NH
AL anti-C58	H ¹¹	NH
YBR anti-MOPC352 (BALB/c-2)	H ¹⁶	C57BL
A/He anti-PC6A (BALB/c)	A ^{12, 14}	BALB/c, NH
DE anti-MOPC209 (BALB/c)	A ^{12, 13}	BALB/c, AL
AL anti-MOPC320 (BALB/c-2)	A ¹⁵	C57BL

Individual sera of 43 mice from the 15th generation low responder line were analyzed for immunoglobulin allotypic markers. The antisera used to identify their allotypes included:

Antisera	Determinants identified	Reference strain
C58 anti-RF	G ³	DBA/2
SJLxBALB/c anti-CE	G ⁵	NH
C57BL anti-BALB/c	G ⁷	NH
C57BL anti-BALB/c	G ⁸	DBA/2
AL anti-C58	H ⁹	C57BL
AL anti-C58	H ¹¹	NH

The γ F (γ_1) immunoglobulin allotypes were determined on the basis of electrophoretic mobility. Immunoelectrophoresis was developed with a specific goat anti-mouse myeloma γ F-Fc (MOPC 31) antiserum (14). MOPC 195- γ H myeloma protein and MOPC-

TABLE I
Serum Immunoglobulin Levels in Pooled Sera from the 13th and 14th Generation of High and Low Responder Mouse Lines

Generation	Line	Immunoglobulin level			
		IgM	IgA	γ_1	γ_2
		mg/ml	mg/ml	mg/ml	mg/ml
13	High	0.165	0.54	0.78	1.45
	Low	0.14	0.34	0.31	0.61
14	High	0.40	0.90	3.6	6.0
	Low	0.14	0.46	0.24	0.75

13th generation sera were obtained before immunization, and 14th generation sera 2 wk after immunization with sheep erythrocytes.

209 and PC-6A γ A myeloma proteins of BALB/c mice, and MOPC 352 γ H myeloma protein and MOPC 320 γ A myeloma protein from BALB/c-2 variant were obtained from Dr. Michael Potter.

RESULTS

Serum Immunoglobulin Levels.—Immunoglobulin levels in pooled sera from the 13th generation before immunization, and in pooled sera from the 14th generation after immunization with sheep erythrocytes are shown in Table I. The table illustrates two points. First, immunoglobulin levels in the high responders before immunization were about double those in the low responders, except for IgM, where the difference was much smaller. Second, the magnitude of the differences were increased following immunization with sheep erythrocytes. Low responders showed little increase in immunoglobulin levels, whereas in high responders the increase was large. A more detailed study was made of the 15th generation. Fig. 2 presents the serum immunoglobulin levels of individual

mice of the high and low responder lines 2 wk after immunization with pigeon erythrocytes. In this analysis both constituents of γ_2 , (γ_{2a} and γ_{2b}) were determined. The mean immunoglobulin levels of the high responders were at least double those found in the low responders except for IgA where the difference was not as marked (Table II). These differences were highly significant ($P < 0.001$) in each instance.

Fig. 3 shows an analysis of total IgG ($\gamma_1 + \gamma_{2a} + \gamma_{2b}$) in the sera of mice from

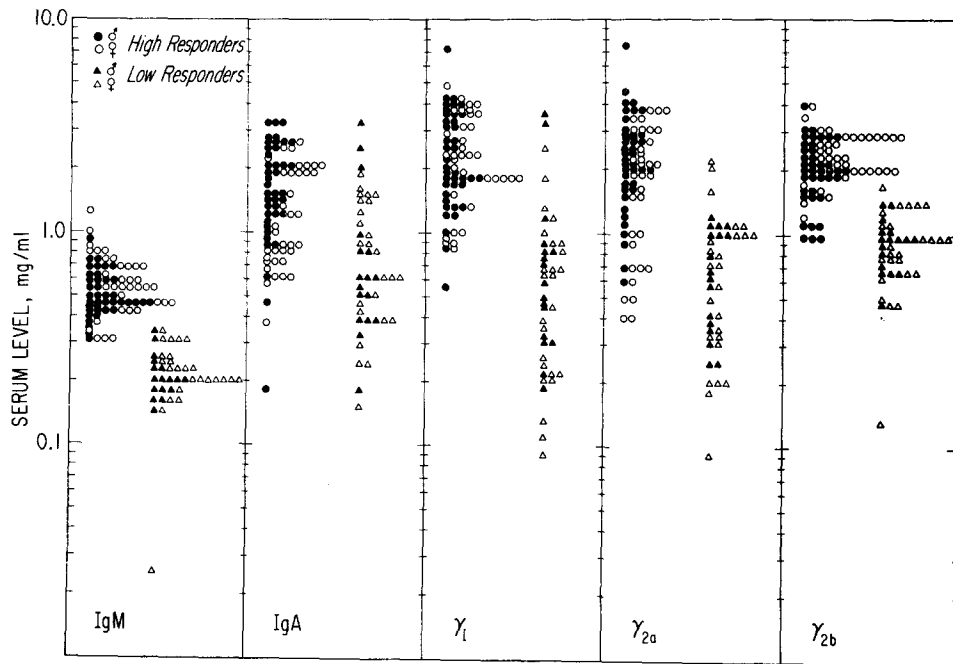


FIG. 2. Serum levels of five immunoglobulins (IgM, IgA, γ_1 , γ_{2a} , and γ_{2b}) in mice of the 15th generation high responder and low responder lines 14 days after immunization with pigeon erythrocytes. Each point indicates the level in an individual mouse. Ordinate: serum immunoglobulin level in mg/ml, logarithmic scale.

individual families (litter mates). The differences in levels between the two lines are clearly seen. In addition, there was considerable variation from family to family in both lines, and in both instances this variation was statistically significant ($P < 0.02$). Since γ_1 , γ_{2a} , and γ_{2b} tended to vary together and in the same direction, a similar result was obtained when these components were analyzed separately. Significant variation among families was found with respect to IgA levels, but in this case the levels were not covariant with IgG levels. Family variation in IgM levels was of borderline ($P = 0.05$) or no ($P > 0.05$) significance in the high and low lines, respectively.

In order to investigate more closely the correlation between antibody response and serum immunoglobulin levels in the two mouse lines as well as the relationship between the concentrations of individual immunoglobulins in high and low responder mice, the serum immunoglobulin levels in two representative families of the high and of the low line are presented in Table III together with the corresponding hemagglutinin titers against pigeon red cells. There was no apparent correlation within either line between the levels of immunoglobulins and the antibody titers against pigeon erythrocytes.

TABLE II
Statistical Analysis of Immunoglobulin Levels in Mouse Sera from the 15th Generation High and Low Responder Lines

Immunoglobulin	Line	Mean level	SEM	T	P
		<i>mg/ml</i>			
IgM	High	0.55	0.02	12.4633	<0.001
	Low	0.21	0.01		
IgA	High	1.55	0.09	5.0852	<0.001
	Low	0.85	0.10		
γ 1	High	2.44	0.14	8.1686	<0.001
	Low	0.76	0.12		
γ 2 _a	High	2.23	0.14	7.6232	<0.001
	Low	0.73	0.07		
γ 2 _b	High	2.23	0.08	11.8569	<0.001
	Low	0.93	0.05		

Sera were obtained 2 wk after immunization with pigeon erythrocytes. 75 sera of the high responder line and 42 sera of the low responder line were analyzed. Arithmetic means and standard errors are shown. Geometric means did not differ from arithmetic means by more than 10%.

Immunoglobulin Phenotypes.—Progeny from the 12 different families of the 15th generation high response line were serologically typed to determine their immunoglobulin phenotypes (Table IV). Of the 76 progeny tested, six had phenotypes resembling those found in the group of which the inbred BALB/c is a prototype, 33 progeny had phenotypes similar to the group of strains of which C57BL inbred strain is a prototype, and 37 appeared to be hybrids comparable to an F₁ of these two genotypes (13).

In contrast, all mice of the 15th generation low responder line exhibited the identical phenotype G^{3, 5, 7, 8}, H⁹, 11A-F^f, distinct from those of the high responder line (Table IV). While the low line phenotype at first appears to be similar to a hybrid produced by crossing inbred strains,

e.g. DBA/2 \times NH, it became immediately apparent that the A¹⁴ determinant found in inbred mice having the G⁵ determinant, e.g. NH, was absent. Furthermore, the lack of segregation of the G³ and the G⁵ genes appears to indicate that these genes are linked in the low responder line. This new

TABLE III
Immunoglobulin Concentrations and Antibody Titers in Sera from 15th Generation Families of High and Low Responder Mouse Lines Immunized with Pigeon Erythrocytes

Line	Family	Sex	Immunoglobulin Concentration				Hem Titer*
			IgM	IgA	γ 1	γ 2	
			<i>mg/ml</i>	<i>mg/ml</i>	<i>mg/ml</i>	<i>mg/ml</i>	
High	V	M	0.54	1.1	3.6	7.2	5,120
		M	0.42	1.2	7.2	3.6	5,120
		M	0.50	1.3	4.2	7.2	5,120
		M	0.42	1.05	4.0	5.0	7,680
		F	0.68	0.82	4.0	5.8	5,120
		F	0.54	0.73	6.7	5.4	15,360
High	VIII	M	0.46	0.46	2.5	10.0	15,360
		M	0.62	1.5	3.1	6.7	15,360
		M	0.59	1.4	4.0	3.6	15,360
		M	0.68	2.0	3.7	5.0	15,360
		F	0.59	0.73	3.6	4.8	15,360
		F	0.46	0.61	3.7	5.4	15,360
		F	0.59	0.82	2.3	6.3	3,840
		F	0.59	0.82	2.3	6.3	3,840
Low	B	M	0.20	0.55	0.50	1.11	160
		M	0.34	0.51	0.84	1.66	80
		F	0.31	1.5	1.3	2.1	80
		F	0.23	0.61	0.21	1.3	40
		F	0.34	0.87	0.22	2.7	80
		F	0.23	1.6	1.2	2.2	120
Low	I	F	0.2	0.88	0.24	1.18	320
		F	0.2	0.61	0.09	1.26	160
		F	0.16	0.98	0.36	2.7	160
		F	0.26	1.5	1.8	3.2	160
		M	0.16	0.51	0.48	2.08	160

* Reciprocal of hemagglutination titer against pigeon red cells at 14 days for the high line and 7 days for the low line.

type of heavy-chain linkage group was recently reported in wild mice in the United States and probably represents a recombinant type involving a cross-over between the G³ (DBA/2) and the G⁵ (NH) genes found in inbred strains (14).

DISCUSSION

Selective breeding of random-bred Swiss mice for high or low antibody response to sheep and pigeon erythrocytes resulted, after 16 generations, in the

development of two lines which differed markedly in the amounts of antibody produced against these antigens. It was later shown that these two mouse lines, referred to respectively as high and low responder lines, showed similar differences in their antibody response to other noncross-reacting complex antigens

TABLE IV
Immunoglobulin Phenotypes of Mice from the 15th Generation Responder Lines*

Family	G ^{1,6,7,8} H ^{9,11} A ^{12,13,14} F ^f		2G ^{1,6,7,8} H ^{9,11,16} A ^{12,13,14,15} F ^{fs}		2G-H ^{9,16} A ¹⁵ F ^{fs}	
	♂	♀	♂	♀	♂	♀
High line						
I	0	0	1	2	2	2
II	1	0	2	2	1	1
III	0	0	0	1	1	0
IV	0	0	1	1	0	1
V	0	1	3	1	1	0
VI	1	0	1	0	2	0
VII	0	0	1	2	1	2
VIII	0	0	3	3	1	0
IX	0	0	0	0	6	6
X	0	0	2	1	2	0
XI	2	1	2	3	1	0
XII	0	0	2	3	1	2
Total	4	2	18	19	19	14
<hr/>						
			G ^{3,5,7,8} H ^{9,11} A-F ^f			
			♂	♀		
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Low line						
A			3	2		
B			2	4		
C			3	3		
F			3	5		
G			3	5		
H			2	2		
I			2	4		
Total			18	25		

* G, H, A, and F designate respectively γ_{2a} , γ_{2b} , γ_A , and γ_1 .

which had not been used for the selection, such as the O and H antigens of *S. typhi* (8) and hen ovalbumin.¹

These observations suggested that these lines differ in their ability to synthesize immunoglobulin rather than in their capacity to form specific antibodies against determinants of the antigens used for selective breeding. The data presented in this study establish that the levels of all immunoglobulins are signifi-

spring of the same family show serum IgG concentrations which are very similar and significantly different as a group from those found in mice of other families of the same line, suggests that a further level of fine genetic regulation operates in this process.

The line of low responder mice, which can be considered established after 16 generations, may provide a very useful experimental tool to study the factors which regulate immunoglobulin synthesis, as well as a model for certain types of human hypogammaglobulinemia (16).

Another most interesting finding concerns the phenotypic markers detected on the immunoglobulins of the two lines. Thus, different phenotypes were detected in mice of the high responder line: one resembled that of BALB/c, the other that of C57BL mice, and the third appeared comparable to that of a (BALB/c \times C57BL) cross. This third phenotype, which had the allotypic markers of the other two, could therefore be presumed to belong to mice heterozygous for the heavy-chain linkage group. Breeding studies are in progress to establish the validity of this interpretation. In contrast, all the mice of the low responder line exhibited an identical immunoglobulin phenotype distinct from those found in mice of the high responder line, and therefore can be presumed to be homozygous for this linkage group. This particular heavy-chain linkage group of the low responder line, which may be considered to have both G³ and G⁵ genes on the same chromosome, is of considerable interest. This combination was never found in inbred strains or outbred Swiss mice in the United States, but was recently detected in wild mice and probably represents a recombinant type involving a crossover between the G³ and G⁵ genes of inbred strains (14).

Considering (a) the apparent homozygous character of the heavy-chain linkage group of the low responder line, (b) the peculiar immunoglobulin phenotype observed in all mice of this line, and (c) the finding that only the low line is different from normal mice with respect to serum immunoglobulin levels, the question must be raised whether there is a relationship between this heavy-chain linkage group and the depressed immunoglobulin levels which characterize this line of mice. The alternative possibility must also be considered, that the presence of distinct immunoglobulin phenotypes in the two lines may be fortuitous. A certain degree of inbreeding has indeed resulted in each line from the selection process, as witnessed by the finding that skin grafts are tolerated significantly longer within each respective line than between both lines.² Unfortunately, sera from mice of the early generation are not available for analysis. Breeding experiments have therefore been initiated to resolve this question. Crosses between mice of the low and high responder lines will be made, and the F₁ generation will be backcrossed with both parental lines to investigate whether there exists a linkage between the genes controlling the structure of immunoglobulin heavy chains and the regulation of immunoglobulin synthesis.

² Biozzi, G., C. Stiffel, and D. Mouton. Unpublished results.

SUMMARY

Random-bred Swiss mice were selectively bred for 16 generations; selection was based on their agglutinin response to sheep and pigeon erythrocytes to produce a high and a low responder line.

The serum levels of individual immunoglobulins differed significantly in these two lines before immunization. The differences in the levels of immunoglobulins were much more marked after immunization with pigeon or sheep erythrocytes. Greater differences between the two lines were noted in IgM and IgG levels than in IgA.

Another remarkable finding was the presence of different immunoglobulin phenotypes in the two lines. The high responders were homozygous or heterozygous for heavy-chain linkage groups found separately in the prototype BALB/c and C57BL inbred strains. The low responders were homozygous for a heavy-chain linkage group not present in bred mice in the United States, but observed as a recombinant type among wild mice probably representing a crossover between the heavy-chain linkage groups of the prototype DBA/2 and NH inbred mice.

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