INFLUENCE OF GENETIC FACTORS ON THE MAGNITUDE AND THE HETEROGENEITY OF THE IMMUNE RESPONSE IN THE RABBIT*

By KLAUS EICHMANN, M.D., DIETMAR G. BRAUN, M.D., and RICHARD M. KRAUSE, M.D.

(From The Rockefeller University, New York 10021)

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Certain rabbits immunized with streptococcal and pneumococcal vaccines produce high concentrations of antibodies to the carbohydrate antigens (1, 2). These antibodies may have a remarkable molecular uniformity, and studies on their primary structure are currently underway (3, 4). Since only a small percentage of random-bred rabbits produced uniform antibodies in quantities which were sufficient for extensive structural studies (1, 2), selective breeding of these special rabbits was begun in order to increase the number of rabbits which respond in this way. It was shown previously that an antibody response similar to that in the parents is commonly seen in the offspring after imunization (1, 5, 6). These studies have been extended to examine in greater detail the genetic control of the immune response to the streptococcal carbohydrates. It is now clear from the data reported here that the amount of antibody produced after immunization, as well as the degree of heterogeneity of these antibodies, are under genetic control. The degree of heterogeneity and the magnitude of the immune response appear to be independent variables.

Materials and Methods

Rabbits.—A random-bred population of New Zealand Red rabbits was obtained from the Carver Rabbitry, Somerville, N. J.

Vaccine.—Vaccines from Group A, strain J17A4, and Group C, strain C74, streptococci were prepared as previously described (6, 7). The concentration of the Group A vaccine was adjusted so that a 1:10 dilution of the final suspension employed for immunization had an OD at 660 mμ of 0.8 when measured in a 1 cm cuvette. A 1:10 dilution of the Group C vaccine had an OD at 660 mμ of 1.2. The vaccines were streaked on blood agar plates to test for sterility.

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Immunization.—Each rabbit received two courses of intravenous injections. At 4 months of age a first course of injections was given for 4 wk (three injections of 1 ml/wk). After a rest period of 4 months a second course was given for 3 wk (three injections of 1 ml/wk). The rabbits were bled after the 3rd and 4th wk of the first course and after the 2nd and 3rd wk of the second course (1).

Determination of Antibody Concentration.—The antibody concentration of each serum was determined by the quantitative precipitin analysis, as previously described (1, 8). This employed the soluble group-specific carbohydrates of Groups A and C streptococci, extracted from streptococcal cells walls as described by Krause and McCarty (9).

Determination of Gamma Globulin Concentrations.—The IgG concentration of each antiserum was estimated by integration of the densitometric tracing of the microzone electrophoretic pattern (1, 7) and from the total protein of the antiserum, determined by the biuret test (10.)

Classification of the Heterogeneity of the Immune Response.—The immune responses were classified into three categories which were designated as "heterogeneous," "restricted," and "monoclonal." Quantitative and qualitative estimation of the electrophoretic heterogeneity of the antibodies in an antiserum was achieved by comparison of the densitometric tracings developed from the microzone electrophoretic patterns of the preimmune serum and the secondary response antiserum of each rabbit. The following criteria were applied: The response was designated monoclonal when at least 60% of the total serum antibody was contained in a single narrow electrophoretic component. Experience has shown that the antibody recovered from such a single component has many other properties which indicate molecular uniformity (3, 7, 8, 11). The response was designated restricted when the antibodies were distributed within several components, one of which contained at least 30% of the total serum antibody. Commonly the antibodies recovered from such components also have molecular uniformity (3, 11–14). All other responses were designated heterogeneous even though distinct but minor antibody components were observed by electrophoresis.

Selective Breeding.—42 random New Zealand Red rabbits were immunized with Group C vaccine and 49 random New Zealand Red rabbits were immunized with Group A vaccine (1). Rabbits were selected from these initial groups for two major series of breeding experiments.

The first series was performed to reveal information on genetic influences on the magnitude of the immune response. For this series, "low"- and "high"-response breeding pairs were selected. Low-response breeding pairs had less than 10 mg precipitating antibody per milliliter in second immunization antisera. High-response breeding pairs had more than 15 mg precipitating antibody per milliliter in second immunization antisera. The F_2 generations were achieved by breeding low-responder siblings from the low-response F_1 generation and high-responder siblings from the high-response F_1 generation.

A second series of breeding experiments was performed to reveal genetic influences on the restriction of the heterogeneity of the immune response. For this series rabbits were selected from the random group and from the F_1 generation which were representative of heterogeneous, restricted, and monoclonal responders. These were bred in the following combinations: heterogeneous–heterogeneous, heterogeneous–restricted, restricted–restricted, restricted–monoclonal, and monoclonal–monoclonal.

RESULTS

Influence of Genetic Factors on the Magnitude of the Immune Response.—Depicted in the left frame of Fig. 1 are the precipitin concentrations of 42 random-bred New Zealand Red rabbits which had been immunized with Group C streptococcal vaccine (1). The primary response precipitin levels are indicated by the open circles. 29 of these rabbits received a second immunization, and the

results are indicated by the closed circles. The concentration of precipitins after the primary immunization varied from 1 to 24 mg/ml with an average of 8.5 mg/ml. After a second immunization the precipitin concentrations were between 2 and 34 mg/ml with an average of 14.5 mg/ml.

Illustrated in the second and third frames of Fig. 1 are the precipitin con-

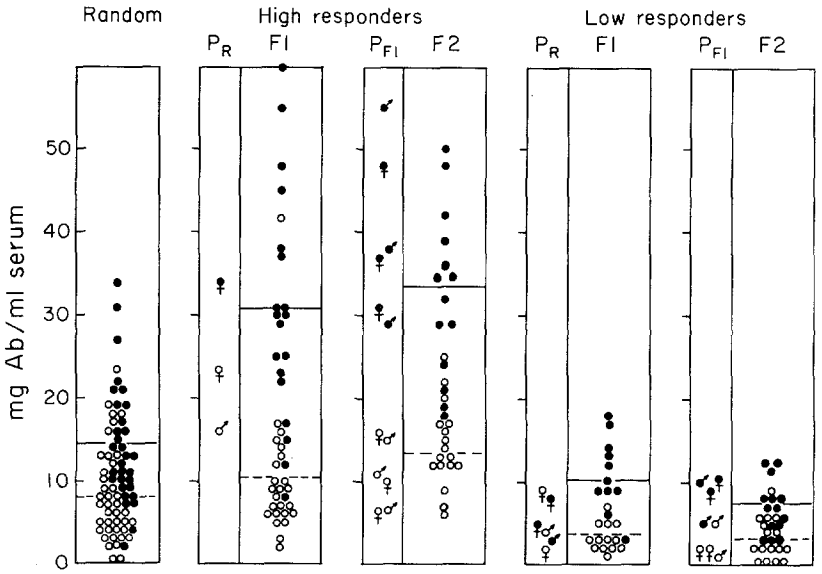

FIG. 1. Serum concentration of Group C precipitins in random rabbits and in the F_1 and F_2 generations derived from selected high- and low-response breeding pairs. P_R, parental rabbits selected from the random group. P_{F1}, parental rabbits selected from the F_1 generation. Open circles (○), precipitin concentrations after the first immunization. Closed circles (●), precipitin concentrations after the second immunization. Dotted horizontal line (- -), average precipitin concentration after the first immunization. Solid horizontal line (–), average precipitin concentration after the second immunization.

Ab = antibody.

centrations of one high-response breeding pair, which was selected from the random group, and that of the F_1 and F_2 generations derived from it. P_R designates the parental rabbits selected from the random group and P_{F1} designates the parental rabbits selected from the F_1 generation. 25 offspring in the F_1 generation received a first immunization and 19 of these survived a second immunization. The precipitin concentrations after the primary immunization were between 2 to 42 mg/ml with an average of 10.5 mg/ml. After the second immunization the precipitin concentrations were between 8 and 60 mg/ml with an average of 31 mg/ml. Three brother–sister pairs were selected from the F_1

generation, which had secondary response precipitin concentrations greater than the average. 17 offspring of these three pairs received a primary immunization and 14 survived an additional secondary immunization. The precipitin concentrations in the F_2 generation were between 6 and 24 mg/ml after the primary immunization and between 18 and 51 mg/ml after the secondary immunization. The average values are 13.5 mg/ml and 33 mg/ml, respectively.

In the fourth and fifth frames of Fig. 1 are depicted the precipitin concentrations of two low-response breeding pairs and those of the F_1 and F_2 generations. 13 F_1 generation rabbits received a first immunization and 11 received a second immunization. After the primary immunization the precipitin levels were between 1 and 7 mg/ml with an average of 3.5 mg/ml. After the secondary immunization the precipitin concentrations were between 3 and 18 mg/ml with an average of 10.5 mg/ml. Two brother–sister pairs which had secondary response precipitin concentrations lower than the average were selected from the F_1 generation. 16 F_2 generation rabbits were derived from these two pairs. The primary response precipitin concentrations were between 1 and 8 mg/ml, and the secondary response precipitin concentrations were between 4 and 13 mg/ml. The average values were 4 mg/ml and 7.5 mg/ml, respectively.

It is evident from these data that the progeny of rabbits which had a high response to Group C streptococcal carbohydrate also respond with predominantly high levels of precipitating antibodies to this antigen. The same correlation is observed for low-responding rabbits and their progeny. The difference between the progeny of the low-and high-response breeding pairs is statistically significant with a *P*-value of <0.001. It is also evident that the average concentration of precipitins increases from the F_1 to the F_2 generation for the high responders and decreases from the F_1 to the F_2 generation for the low responders. Comparison of the secondary response precipitin levels between low and high responders of the F_2 generation reveals that both populations have been completely segregated. Among the F_2 generation the lowest high responder had 18 mg/ml precipitating antibody, whereas the highest low responder had 13 mg/ml. Thus two generations of selective breeding were sufficient to segregate a low- and a high-responding population out of a random group of rabbits.

Depicted in the left frame of Fig. 2 are the precipitin concentrations measured in the antisera of 49 random-bred rabbits which had been immunized with Group A streptococcal vaccine (1). 19 of these rabbits survived a second immunization. The precipitin concentrations after the primary immunization varied from <1 to 30 mg/ml with an average of 6.5 mg/ml. After the second immunization the precipitin concentrations were between 3 and 15 mg/ml with an average of 8 mg/ml.

Illustrated in the second frame of Fig. 2 are the precipitin concentrations of three high-response breeding pairs (designated P), which were selected from the random group, and that of their F_1 generation. 20 offspring from the three

pairs received a first immunization and six survived a second immunization. The precipitin concentrations after primary immunization were between 7 and 38 mg/ml with an average of 14.5 mg/ml. After a second immunization the precipitin concentrations were between 8 and 21 mg/ml with an average of 16.5 mg/ml. Low responders to Group A carbohydrate have not been bred. However, the average precipitin concentration in the high-response F_1 generation

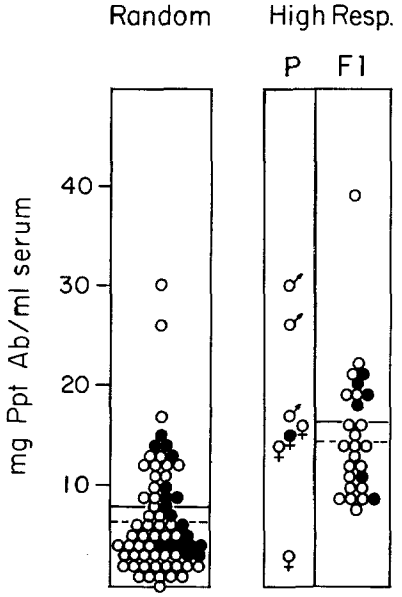

FIG. 2. Serum concentrations of Group A precipitins in random rabbits and in the offspring (F_1) derived from selected high-response breeding pairs. P, parental rabbits selected from the random group. Open circles (○), precipitin concentrations after the first immunization. Closed circles (●), precipitin concentrations after the second immunization. Dotted horizontal line (- -), average precipitin concentration after the first immunization. Solid horizontal line (–), average precipitin concentration after the second immunization.

Ppt Ab = precipitating antibody.

to Group A carbohydrate is twice as high as that observed in random rabbits. The statistical difference between the two groups is significant with a P-value of <0.001.

Influence of Genetic Factors on the Heterogenetiy of the Immune Response.—Depicted in Fig. 3 are the microzone electrophoretic patterns of the antisera developed in a selected pair of breeders and their 19 offspring. All antisera shown here were collected after a second immunization with Group C streptococcal vaccine. The breeders had been selected from the random group because their antibodies had restricted heterogeneity. As can be seen in Fig. 3, three major

types of responses are observed in the offspring. The antisera of five rabbits which produced a single predominant antibody component are depicted in the left group. Each of these antisera contained a single narrow band in the gamma globulin region, which appeared as a narrow peak in the densitometric tracings of the electrophoretic patterns. Integration of the densitometric tracings re-

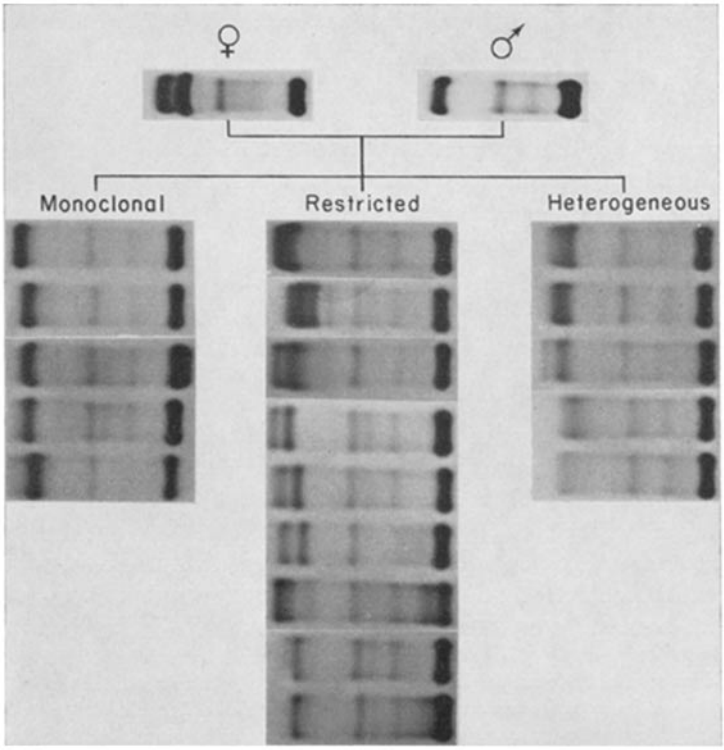

FIG. 3. Microzone electrophoretic patterns of the second immunization antisera of one pair of breeders, which was selected from a random group, and of their 19 offspring. All rabbits were immunized with Group C vaccine.

vealed that the protein under the peak of each antiserum accounted for more than 60% of the total antibody and absorption studies revealed this protein to be precipitating antibody to the carbohydrate antigen. By other criteria these antibodies have molecular uniformity (3, 7, 8, 11), and this type of response has been designated as monoclonal. In the middle group are the electrophoretic patterns of the antisera of 9 offspring which responded with the production of multiple antibody components. One of the components in each case apeared as a narrow peak in the densitometric tracing and integration revealed that this peak component contained at least 30% of the total antibody. This type of

immune response has been designated as restricted. Although the antibodies in the first two sera of this category do not appear to fulfill the criteria for restriction, this is due to the loss of detail in the photograph. The original electrophoretic patterns and densitometric tracings clearly revealed these sera to contain restricted antibodies. The patterns of the two antisera at the bottom of this group appear to show primarily a single antibody component, but they were designated restricted responders because the major component was less than 60% of the total antibody. The antibodies in these recognizable components in these restricted antisera also have other properties which indicate uniformity (3, 11–14). In the right group are the electrophoretic patterns of five antisera which contained antibodies distributed in a polydisperse fashion. Such antisera may contain minor restricted antibody components (i.e. the third pattern) but such components represent only a small proportion of the total antibody and therefore these immune responses are designated as heterogeneous.[1]

The proportion of rabbits with restricted and monoclonal responses in the offspring of this selected pair of breeders is much higher than was observed in random rabbits. As shown in Table I, one out of 42 random rabbits developed a monoclonal response and seven a restricted response to Group C carbohydrate. The more common occurance of restricted and monoclonal responders among offspring of selected breeders than among random rabbits suggests that the degree of heterogeneity of the immune response to Group C carbohydrate is genetically determined.

In order to collect further information on genetic influences on antibody heterogeneity, rabbits were selected from the three types of antibody responses described above and were bred in five different combinations. The results for

[1] Previous studies have shown that microzone electrophoresis on cellulose acetate membranes of whole antiserum affords a reasonable basis for evaluating the degree of antibody heterogeneity. Antibodies in a single electrophoretic antibody component have been isolated by affinity chromatography and preparative electrophoresis (13) from the majority of the antisera shown in Fig. 3 and have been extensively studied by a variety of methods for the evaluation of antibody uniformity such as polyacrylamide disc electrophoresis (8, 11–14), quantitative idiotypic precipitation (14), quantitative allotypic precipitation (15), and amino acid sequence analysis (3, 8). For example, a single amino acid sequence was seen in the first 20–30 N-terminal residues of the L chains derived from the antibody in the first serum of the monoclonal group (R26-90), and from the isolated fast components in the fourth and fifth serum (R24-36, R24-61) of the restricted group (3). Taken together, such data indicate that a discrete antibody component, recognizable by microzone electrophoresis, commonly has those other properties which are indicative of molecular uniformity.

At least 60% of the L chain protein of all of the antibodies in an antiserum of the monoclonal type migrated in one or two bands by disc electrophoresis, whereas L chains from all of the antibodies recovered from restricted antisera were distributed in three or four bands. L chains of all of the antibodies in heterogeneous antisera migrated in six or more bands (8, 11–14).

all the breeding experiments are summarized in Table I, and microzone electrophoretic patterns of the antisera from representative breeding pairs and from

TABLE I

The Frequency of Heterogeneous, Restricted, and Monoclonal Responses to Group C Carbohydrate in Random- and Selectively Bred Rabbits

Parents			Offspring				
Pair	Response*	Generation	Generation	Total No.	Response*		
					Het	Restr	Mon
—	—	—	Random	42	34	7	1
I	Het–het	R	F_1	6	6	—	—
II		R	F_1	7	6	1	—
III		R	F_1	6	6	—	—
IV		F_1	F_2	7	7	—	—
V		F_1	F_2	5	5	—	—
VI		F_1	F_2	6	6	—	—
Total				37	36	1	—
VII	Het–restr	R	F_1	4	2	2	—
VIII		R	F_1	7	3	3	1
Total				11	5	5	1
IX	Restr–restr	F_1–R	F_{1a}	4	1	3	—
X		F_1–R	R_{1a}	13	4	6	3
XI		F_1	F_2	4	1	1	2
XII		F_1	F_2	3	1	2	—
Total				24	7	12	5
XIII	Restr–mon	R	F_1	19	5	9	5
XIV		F_1–R	F_{1a}	2	—	1	1
XV		F_1	F_2	2	—	2	—
XVI		F_1	F_2	4	—	2	2
XVII		F_1	F_2	6	1	2	3
Total				33	6	16	11
XVIII	Mon–mon	F_1	F_2	3	—	1	2

* Het = heterogeneous immune response; restr = restricted immune response; mon = monoclonal immune response, after second immunization. See text for definitions of other abbreviations.

one of their litters are depicted in Fig. 4. All rabbits included in this table have been immunized with Group C streptococcal vaccine and the limitation in heterogeneity of the immune responses was judged exclusively from second immunization antisera. When the parental rabbits were selected from the ran-

dom group (designated R)[2] the offspring were designated as F_1. When both parents were selected from the F_1 generation and were in addition siblings, the offspring were designated F_2. When one parent was selected from the F_1 generation and the other parent was a random rabbit, the offspring were designated F_{1a}.

Both mates in breeding pairs I to VI had heterogeneous responses. A total of 37 offspring was obtained. 36 of these had heterogeneous responses, whereas one had a restricted response. Microzone electrophoretic patterns of the antisera from breeding pair IV and its first litter are shown in the top frame of Fig. 4. One of the parents in breeding pairs VII and VIII had a heterogeneous and the other parent a restricted response. From a total of 11 offspring of these two breeding pairs, five had a heterogeneous, five had a restricted, and one had a monoclonal response. Microzone electrophoretic patterns of the antisera of breeding pair VII and its litter are shown in the second frame of Fig. 4. Both parental rabbits in breeding pairs IX to XII had a restricted response. Out of 24 offspring obtained from these breeding pairs, seven had heterogeneous, 12 had restricted, and five had monoclonal responses. Microzone electrophoretic patterns of the antisera of breeding pair X and its first litter are shown in the third frame of Fig. 4. 23 offspring were obtained from breeding pairs XIII to XVII in which one parent had a restricted and the other a monoclonal response. Six offspring had heterogeneous, 15 had restricted, and 11 had monoclonal responses. Microzone electrophoretic patterns of the antisera from breeding pairs XVI and XVII and their offspring are shown in the fourth and fifth frames of Fig. 4. A very low number of surviving offspring was obtained from the breeding pairs in which both parents had monoclonal responses, and only three offspring from one pair survived the second immunization. One had a restricted and two had a monoclonal response.

It is evident from these data that there is a clear correlation between the degree of heterogeneity of the immune responses to streptococcal Group C carbohydrate in parental rabbits and in their offspring. A monoclonal response was not observed in the progeny of rabbits which responded with heterogeneous antibodies, but this type of response is observed more commonly in the offspring of rabbits with restricted responses. 33% monoclonal responses were observed in a population derived from breeding pairs in which one parent had a restricted and the other a monoclonal response. Two out of three offspring from two monoclonal parents had a monoclonal response. This suggests an even higher proportion of monoclonal responders in this breeding combination but a sufficient number of surviving offspring has not yet been obtained to test this point

[2] *Abbreviations used in this paper:* F_1, offspring from random rabbits; F_{1a}, offspring from breeding pairs composed of one random and one F_1 generation rabbit (F_1–R); F_2, offspring from two F_1 generation rabbits which were brother and sister; R, random rabbits; RBC, red blood cells.

further. The proportion of rabbits with a restricted response is about 3% in the offspring of rabbits which produced heterogeneous antibodies. This proportion

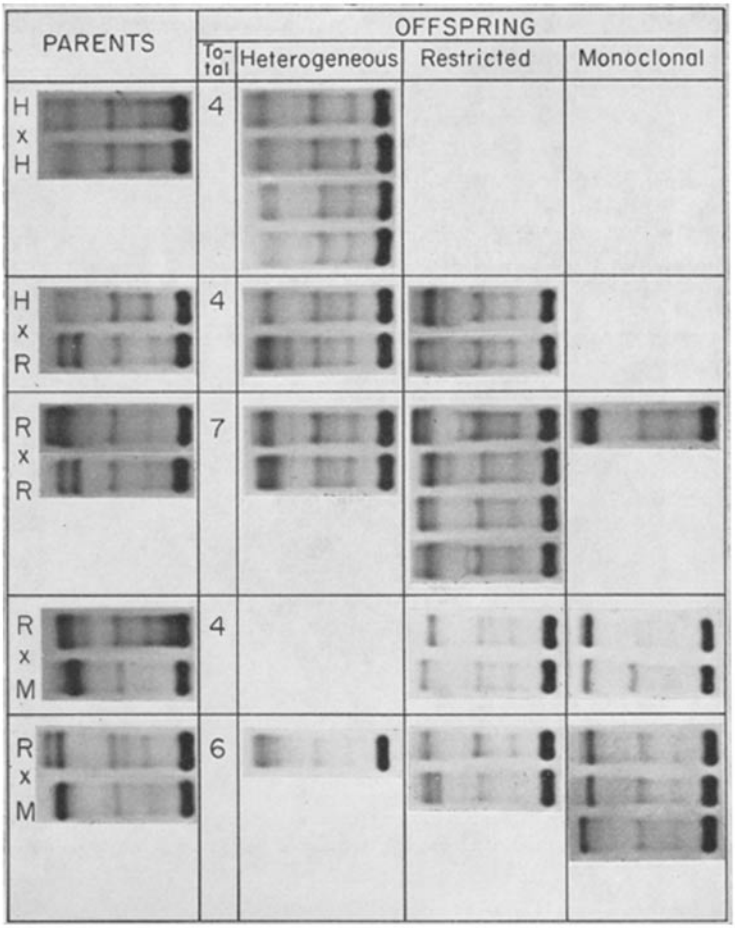

FIG. 4. Microzone electrophoretic patterns of the second immunization antisera of five breeding pairs and of one litter of each. The breeding pairs shown in the above frames are listed in Table I with the following roman numeral designations. First frame, pair IV. Second frame, pair VII. Third frame, pair X. Fourth frame, pair XVI. Fifth frame, pair XVII. All rabbits were immunized with Group C vaccine. The parental rabbits were selected to represent heterogeneous (H), restricted (R) and monoclonal (M) responders.

is about 50% in all other breeding combinations. The proportion of rabbits with a heterogeneous response is higher than 95% in the offspring of heterogeneous responders, and the decrease in frequency of this type of response matches the increase in the proportion of monoclonal responders.

The proportion of rabbits which develop restricted and monoclonal responses is much lower when Group A streptococcal vaccine is used. As shown in Table II, five out of 49 random rabbits developed a restricted response to Group A streptococcal carbohydrate, but no monoclonal response was observed. For this reason only three combinations of breeding pairs were possible. One breeding pair of heterogeneous responders yielded three offspring with heterogeneous

TABLE II

The Frequency of Heterogeneous, Restricted, and Monoclonal Responses to Group A Carbohydrate in Random- and Selectively Bred Rabbits

Parents			Offspring				
Pair	Response*	Generation	Generation	Total No.	Response		
					Het	Restr	Mon
—	—	—	Random	49	44	5	—
I	Het–het	R	F_1	3	3	—	—
II	Het–restr	R	F_1	2	—	2	—
III		R	F_1	7	5	1	1
IV		R	F_1	1	—	1	—
V		F_1–R	F_{1a}	4	3	1	—
VI		F_1–R	F_{1a}	3	2	1	—
Total				17	10	6	1
VII	Restr–restr	R	F_1	1	—	1	—
VIII		R	F_1	6	3	3	—
IX		R	F_1	7	2	4	1
X		F_1–R	F_{1a}	7	4	2	1
Total				21	9	10	2

* Het = heterogeneous immune response; restr = restricted immune response; mon = monoclonal immune response, after second immunization. See text for definitions of other abbreviations.

responses. 17 offspring were obtained from five breeding pairs in which one parent had a heterogeneous and the other a restricted response. 10 of these offspring had a heterogeneous, six had a restricted, and one had a monoclonal response. 21 offspring were obtained from four breeding pairs in which both parents had restricted responses. 9 of these had a heterogeneous, 10 had a restricted, and two had a monoclonal response. Thus a restricted or a monoclonal response to Group A carbohydrate is more likely to occur in the offspring if both parents had a restricted response than if only one had responded in this fashion. When only one of the parents had a restricted immune response, about 30% of the offspring responds with restricted antibodies. When both parents

had restricted responses, this percentage increased to 50. Monoclonal responders were not observed in a group of 49 random rabbits but occurred in a frequency of about 10% when both parental rabbits had a restricted response.

Taken together, these data suggest that both the degree of heterogeneity and the magnitude of the immune response to the group-specific streptococcal carbohydrates are under genetic control. This then raised the question whether a monoclonal antibody response is exclusively associated with a high response, or whether a similar degree of restriction can be observed in the antibodies of low responders as well. Compared in Fig. 5 are the microzone electrophoretic patterns of the antisera of four breeding pairs and one of their litters. These breeding pairs are listed in Tables I and II as follows: upper left: pair VI, Table I; lower left: pair XII, Table I; upper right: pair I, Table II; lower right: pair XVIII, Table I. Both breeding pairs at the left were low responders with precipitin concentrations of less than 13 mg/ml. Both breeding pairs at the right were high responders with precipitin concentrations of more than 18 mg/ml. These microzone electrophoretic patterns clearly show that the immune responses of the offspring of each pair resemble the immune responses of their parents with respect to both antibody concentration and degree of heterogeneity. Furthermore, antibodies of restricted heterogeneity can occur in both low and high responders. These findings suggest that the genetic control on the magnitude of the immune response is independent from that on the degree of antibody heterogeneity.

DISCUSSION

The genetic control of the immune response to a variety of antigens had been examined for several different species, and this subject has been reviewed recently by McDevitt and Benacerraf (16). In much of the previous work, several inbred strains of a species were immunized with a given antigen to identify responder and nonresponder strains. An examination of the immune responses to the same antigen in the offspring of cross- and backcross experiments led to the identification of several allelic pairs of Ir-genes, each controlling the response to a given set of antigenic determinants (16–19). For structurally restricted antigens, such as synthetic peptides, the alleles coding for response behaved like single autosomal dominant mendelian factors (17, 18). For antigens with multiple determinants, such as insulin, high and low responses were transmitted in a polygenic fashion (20).

As an alternative approach to genetic studies which employed inbred strains of animals, selective breeding of outbred animals was employed by several groups of investigators. In an early report Scheibel succeeded in segregating high- and low-responder guinea pigs to diphtheria toxoid from random-bred populations (21). Recently Biozzi et al. (22) reported the segregation of low- and high-responder mice to sheep red blood cells (RBC) after several genera-

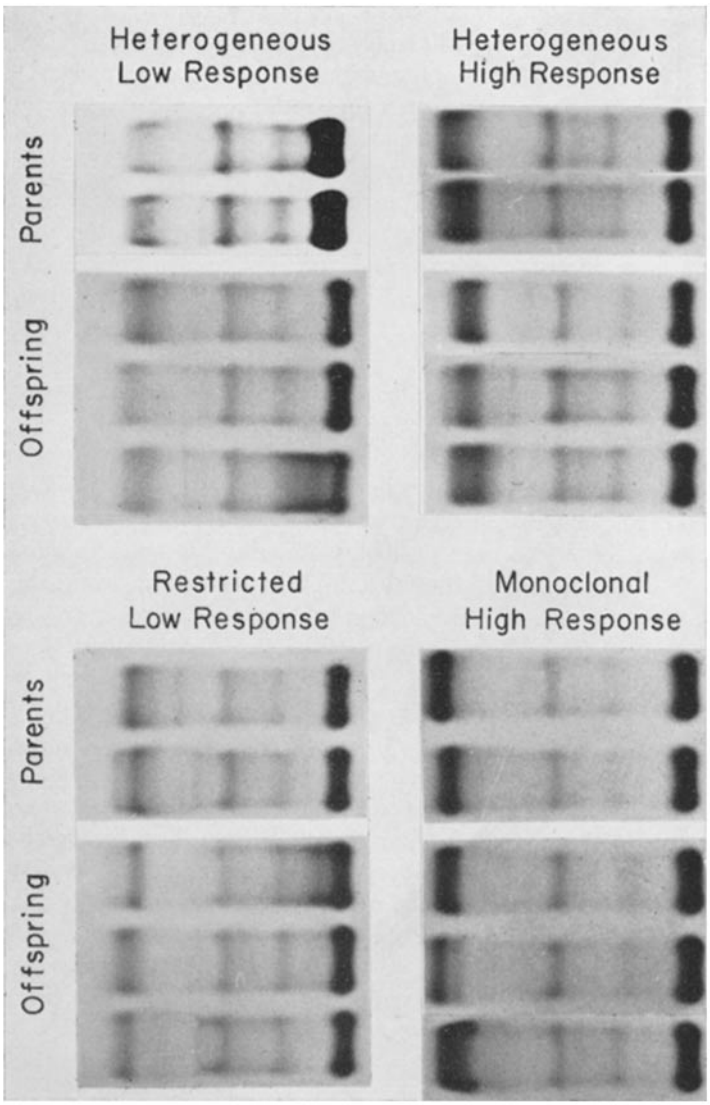

FIG. 5. Microzone electrophoretic patterns of the second immunization antisera of four breeding pairs and of one litter of each. The breeding pairs are listed in Tables I and II as follows: Upper left: pair VI, Table I. Lower left: pair XII, Table I. Upper right: pair I, Table II. Lower right: pair XVIII, Table I. The parental rabbits were selected to represent heterogeneous low responders (upper left), restricted low responders (lower left), heterogeneous high responders (upper right), and monoclonal high responders (lower right).

tions of selective breeding. The antigens used in these studies bear a large number of different antigenic determinants, and six generations of inbreeding in the first experiment (21) and 16 generations in the second experiment (22) were needed to segregate low- and high-responder populations. Since the immune response to a given antigenic determinant is controlled by one pair of alleles (16), the number of generations required to segregate low and high responders should be related to the number of different antigenic determinants on the antigen. In the present studies only two generations of selective breeding were needed to completely segregate high and low responders to Group C streptococcal carbohydrate. This is consistent with what is known about the antigenic structure of the Group C carbohydrate. The primary antigenic determinant is a terminal *N*-acetyl-galactosamine residue (9).

Similar, though less complete, breeding studies have been undertaken to obtain high responders to the carbohydrate antigens of Group A streptococci. Antigenic specificity of the Group A carbohydrate is determined by terminal *N*-acetyl-glucosaminide residues (23). Despite their chemical similarity, there is minimal cross-reactivity between the Groups A and C carbohydrates (6). It will therefore be of interest to see whether rabbits bred for either high or low responses to one antigen have a corresponding response to the other antigen. From such experiments it will be possible to identify immune response genes specific for carbohydrate determinants, as it has been possible to do for amino acid determinants (16–18).

After immunization of a random group of rabbits with streptococcal vaccines, a certain proportion of the rabbits produce antibodies of restricted heterogeneity to the carbohydrate antigen (1). This is most strikingly noted by the division of the IgG into electrophoretically distinct bands on cellulose acetate electrophoresis of the hyperimmune sera. Earlier detailed studies have shown that the degree of antibody heterogeneity as judged by cellulose acetate electrophoresis is in good agreement with that detected by other procedures, including polyacrylamide disc electrophoresis, quantitative determination of allotypes and idiotypes, and amino acid sequence analysis (3, 8, 11–15). Because of this correlation, reliance has been placed on cellulose acetate electrophoresis for genetic studies which require screening a large number of antisera for the occurrence of restricted antibodies. The classification of the immune responses into the three groups monoclonal (>60% homogeneous), restricted (>30% and <60% homogeneous), and heterogeneous (<30% homogeneous) is arbitrary and in some respects artificial. However, in the present studies this classification has been useful to assess the genetic control of antibody heterogeneity.

Immunization of selectively bred rabbits has demonstrated that the percentage of rabbits with a restricted or monoclonal response to Group C carbohydrate in the progeny of restricted and monoclonal responders is much higher

than was observed in random rabbits or in the progeny of heterogeneous responders. Furthermore, the number of restricted and monoclonal responders to Group C carbohydrate which occur among progeny of heterogeneous responders is much lower than that in the random rabbits. The concentration of streptococci in the Group A vaccine is lower than that in the Group C vaccine, and this might in part be responsible for the lower incidence of restricted immune responses to Group A carbohydrate in a random group of rabbits. However, the selective breeding of rabbits which had a restricted response to Group A carbohydrate results in an increase of the percentage of restricted and monoclonal responses in the progeny. Taken together, these data suggest that the degree of heterogeneity of antibodies to streptococcal carbohydrate is under genetic control.

In addition to genetic factors, properties of the antigen certainly influence both the magnitude and the heterogeneity of the immune response. The number of different immunodominant groups and the regularity in their attachment to the carrier seem to exert an influence on antibody heterogeneity (24, 25). However, the antigens used to demonstrate this effect elicited relatively poor immune responses (24, 25), and the question has been raised whether this is the reason for the recruitment of a limited number of cell clones (6). Antigens with low immunogenicity have also been used in most studies on the genetic control of the magnitude of the immune response, but these antigens did not elicit antibodies with any detectable limitations in heterogeneity (17, 18). The rabbit anti-streptococcal system offers the unique opportunity to study genetic influences on both the magnitude of the immune response and the heterogeneity of the immune response. The present experiments suggest that the limitation in heterogeneity of specific antibodies varies independently from the magnitude of the antibody response, and cross-experiments are underway to show a genetic segregation of genes controlling each of these aspects of the immune response.

Genes controlling the magnitude of the immune response have been shown to be primarily linked to histocompatibility genotypes rather than to genes controlling antibody structure (16), and it has been suggested that these genes control the immune response in the early stage of antigen recognition (16, 19, 22). Genetic factors which appear to place limits on antibody heterogeneity seem to directly influence antibody structure because rabbit strains bred for a high incidence of monoclonal responses show a limited idiotypic variability (26). This suggests that antibody heterogeneity is determined by the number of different genes which can be expressed with antigenic stimulation. Phenotypic limitation in the number of different antibody molecules could be achieved by two possible mechanisms. It is conceivable that a monoclonal responder possesses only a limited portion of the gene pool of the heterogeneous responders. Alternatively, the number of genes may be equally large but a hereditary suppression mechanism inhibits the phenotypic expression of a certain fraction of

the genetic information. The former hypothesis can be tested because in this case one would expect to find structually identical antibodies in specially bred rabbits. Such an event might be observed with high frequency especially in rabbit strains with a high proportion of monoclonal responders because in these strains the antigenic selection would occur among a limited number of available gene products. Studies to assess this question are currently underway.

SUMMARY

Selective breeding of rabbits immunized with Group C and Group A streptococcal vaccines was employed to reveal genetic influences on the magnitude and on the restriction in heterogeneity of the immune response to the group-specific carbohydrates. After two generations of selective breeding, complete segregation was achieved between a high-response population (>18 mg precipitins/ml serum, average 33 mg/ml) and a low-response population (<13 mg precipitins/ml serum, average 7.5 mg/ml) to Group C carbohydrate. This suggests that a limited number of genes controls the magnitude of the immune response to this antigen.

Selective breeding of rabbits which were representative of heterogeneous, restricted, and monoclonal responses revealed that the degree of antibody heterogeneity in the parental rabbits is reflected in the offspring. More than 95% of the offspring derived from rabbits which had a heterogeneous immune response developed heterogeneous antibodies. 33% of the offspring derived from rabbits which had restricted and monoclonal immune responses developed monoclonal antibodies. This suggests that the degree of heterogeneity of the antibody response to the streptococcal carbohydrates is under genetic control. The degree of heterogeneity and the magnitude of the immune response appear to be independent variables.

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