

THE GENETIC CONTROL OF ANTIBODY SPECIFICITY*

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Genetic and structural analyses of immunoglobulin molecules, by means of allotypes and amino acid sequencing, indicate that both light and heavy chains have an N-terminal variable region and a C-terminal constant region, each coded for by separate structural genes (1-5). However, the genetic origin of antibody specificity is still not understood, and it is not yet possible to choose between two alternative hypotheses—a large number of germ line variable region genes (5), or a very few germ line variable region genes with a high degree of somatic variation (6, 7).

In theory, this problem could be approached by studying the genetic control of the ability to recognize specific antigenic determinants and to synthesize specific antibodies (8). In practice, these studies are complicated, because genetic control of the ability to respond to a particular antigen is a phenotypic marker for a very complex process, and the observed genetic control may have no relationship to the genetic origin of antibody specificity or to antibody variable region genes. This is true even for those situations in which the genetic control can be shown to affect not only the ability to respond, but also the specificity of the antibodies produced, since genes controlling antigen "processing" and the recognition of antigenic determinants would indirectly affect antibody specificity.

There is, however, considerable evidence that antibody specificity is under heritable control. Arquilla and Finn presented some of the earliest evidence supporting the concept of genetic control of the ability to produce antibodies of different specificities against the same antigen by showing that strain 2 guinea pigs produce antibodies to portions of the insulin molecule to which strain 13 animals do not (9, 10). Further evidence for this concept was obtained through the use of modified insulin derivatives. Strain 2 guinea pigs produce antibodies which appear to react preferentially with the

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N-terminus of the insulin A and B chains, while strain 13 guinea pigs produce antibodies which seem to react preferentially with the C-terminal portion of the insulin molecule (11). Pinchuck and Maurer made similar observations in inbred mice immunized with random linear synthetic polypeptides. Several inbred strains of mice were immunized with Glu⁶⁰Ala³⁰Tyr¹⁰, or Glu³⁶Lys²⁴Ala⁴⁰, and the cross-reactions of these antisera with copolymers of glutamic acid respectively with alanine, tyrosine, or lysine were investigated. The pattern of cross-reactivity was found to be characteristic of the particular inbred strain, suggesting that the capacity to produce antibodies or to recognize specific determinants on these molecules is under genetic control (12).

The present study was suggested by similar results obtained upon immunizing DBA/1 and SJL mice with a synthetic polypeptide built on multichain polyproline: poly-L(Phe, Glu)-poly-L-Pro--poly-L-Lys [(Phe, G)-Pro--L] (13). When anti-(Phe, G)-Pro--L sera were titrated with the two cross-reacting antigens, poly-L(Tyr, Glu)-poly-L-Pro--poly-L-Lys [(T, G)-Pro--L] and poly-L(Phe, Glu)-poly-DL-Ala--poly-L-Lys [(Phe, G)-A--L], it appeared that DBA/1 mice produced antibodies to the (Phe, G) part of the (Phe, G)-Pro--L while SJL antisera seemed to react primarily to the Pro--L part of the immunogen. The present study is a genetic analysis of the suggestion inherent in these results—that antibody specificity is under stable genetic control.

Previous studies have shown that the ability of inbred mice to respond to short, random sequences of tyrosine and glutamic acid (T, G), or histidine and glutamic acid (H, G), or phenylalanine and glutamic acid (Phe, G), attached to multi-poly-DL-alanyl-poly-L-lysine (A--L) (14–17) is a dominant genetic trait, linked to the major histocompatibility (H-2) locus (16, 17), and specific for the antigenic determinant [e.g. (T, G), (H, G), or (Phe, G)] carried on the A--L side chains.

However, the immune response of inbred mouse strains to a similar synthetic polypeptide built on multichain polyproline, (T, G)-Pro--L, differs markedly from the response of the same strains of mice to poly-L(Tyr, Glu)-poly-DL-Ala--poly-L-Lys [(T, G)-A--L] (13). SJL mice, which respond poorly to all the A--L series of antigens, are the highest responders to (T, G)-Pro--L, indicating that the prolyl residues also function as important antigenic determinants on this molecule.

In the present study, genetic analysis of the immune response to (T, G)-Pro--L in SJL and DBA/1 (low-responder) mice and their offspring indicates that response to (T, G)-Pro--L is under a genetic control which is superficially similar to that operating for (T, G)-A--L but qualitatively different in that it is not linked to the H-2 locus, while response to (T, G)-A--L is linked to the H-2 locus (16). Evidence for genetic control of antibody specificity was obtained by studying the immune response to (Phe, G)-Pro--L in the DBA/1 × SJL cross with the two cross-reacting polypeptides, (T, G)-Pro--L and (Phe, G)-A--L. This

genetic control appears to be the result of interaction between two separate gene loci, each affecting the response to (Phe,G)-Pro-L. Thus, the ability of the $F_1 \times$ DBA/1 backcross mice to produce antibodies capable of binding (T,G)-Pro-L is not linked to the $H-2^b$ allele donated by the high-responding SJL parent, whereas the ability of $F_1 \times$ SJL mice to produce antibodies capable of binding (Phe,G)-A-L is linked to the $H-2^a$ allele donated by the high-responding DBA/1 parents.

Materials and Methods

DBA/1 and SJL mice were purchased from the Jackson Laboratories, Bar Harbor, Maine. (DBA/1 \times SJL) F_1 , $F_1 \times$ DBA/1, and $F_1 \times$ SJL mice were bred and maintained at Stanford University.

The following branched synthetic polypeptides were used in this study: poly-L(Tyr,Glu)-poly-L-Pro-poly-L-Lys-701, denoted (T,G)-Pro-L (13); poly-L(Phe,Glu)-poly-L-Pro-poly-L-Lys-702, denoted (Phe,G)-Pro-L (13, 18); and poly-L(Phe,Glu)-poly-DL-Ala-poly-L-Lys-223, denoted (Phe,G)-A-L (19).

Bovine serum albumin (BSA, crystallized) was obtained from Sigma Chemical Company, St. Louis, Mo.

Mice were immunized with 10 μ g of (T,G)-Pro-L or (Phe,G)-Pro-L or 100 μ g of BSA in 0.06 ml complete Freund's adjuvant in the hind footpads, followed by an aqueous injection of the same dose of antigen 3 wk later as described previously (13, 16). The mice were bled 10 days after the second injection.

Antibody response was measured by an antigen-binding capacity assay (13, 16) using iodinated [with ^{125}I (20)] or tritium-labeled [with acetic anhydride- ^3H (21)] polypeptides. 0.0025 μ g of (T,G)-Pro-L- ^{125}I , or an approximately equimolar amount of any of the other antigens, was added to each of the experimental tubes.

Allotype determinations to detect the presence or absence of the Ig-1^b allotype [found in SJL mice (22)] in (DBA/1 \times SJL) $F_1 \times$ DBA/1 sera were performed using the immunodiffusion in agar gel technique according to Ouchterlony (23). The antisera used were kindly supplied by Dr. L. A. Herzenberg.

H-2 typing was done by standard methods of hemagglutination (24), using anti- $H-2^a$ and anti- $H-2^b$ antisera kindly supplied by Dr. George D. Snell. The results were scored without knowledge of the source of red cells and with the presence in random order of known positive and negative red cell suspensions. The positive controls used were SJL ($H-2^b$), (DBA/1 \times SJL) F_1 ($H-2^{b/a}$), and DBA/1 ($H-2^a$) red cell suspensions.

Inhibition of the antigen-binding capacity assay was performed by adding a 100-fold excess of the unlabeled inhibiting polypeptide [(T,G)-Pro-L or (Phe,G)-A-L] to the same volume of anti-(Phe,G)-Pro-L sera used for the antigen-binding capacity assay, at a dilution at which the values for percentage of antigen bound were not at their maximum levels but were approximately 30-55%. After incubation for 1 hr at 37°C, the usual amount of the labeled titrating polypeptide (1% of the inhibitor) was added, followed by all the other steps described for the antigen-binding capacity test (13, 16).

Quantitative precipitin determinations were done by standard methods (25). Pools of (DBA/1 \times SJL) F_1 anti-(Phe,G)-Pro-L were used in 0.4 ml aliquots and allowed to react with differing amounts of (Phe,G)-Pro-L-(Ac)- ^3H , (Phe,G)-A-L-(Ac)- ^3H , and (T,G)-Pro-L- ^{125}I . The protein content of the washed precipitates was determined by ultraviolet absorption at 280 $m\mu$, and their antigen content was determined by radioactive counting. The amount of antibody protein precipitated was then calculated.

RESULTS

Immune Response to (T,G)-Pro-L.—The immune response of SJL, DBA/1, (DBA/1 × SJL) F_1 , F_1 × DBA/1, and F_1 × SJL mice to (T,G)-Pro-L is shown in Fig. 1, where the number of animals falling in a given percentile of “% antigen bound” is plotted for the various strains and strain combinations. DBA/1 mice give a low response to this antigen, while SJL mice are high

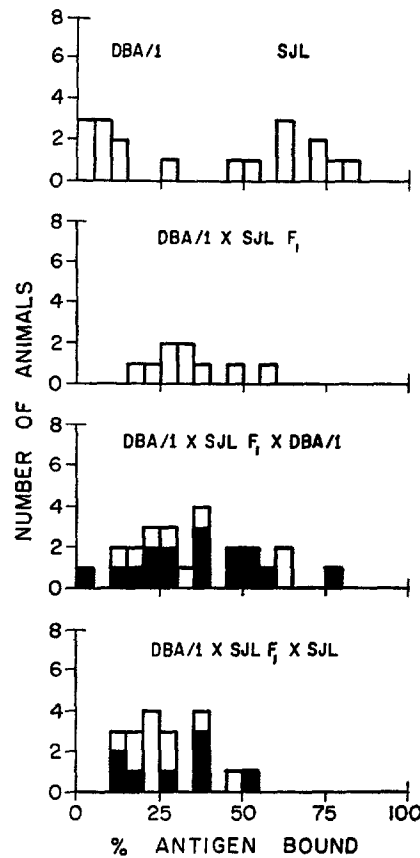


FIG. 1. The antibody response of mice immunized with (T,G)-Pro-L and titered with (T,G)-Pro-L labeled with ^{125}I . The specific activity of (T,G)-Pro-L- ^{125}I was 3.5–7.2 $\mu\text{c}/\mu\text{g}$, and 75–83% of the labeled antigen was precipitable by excess specific antibody. The horizontal axis plots the percentage of antigen bound in the antigen-binding assay, while the vertical axis plots the number of animals falling into a given percentile for bound antigen values. All antisera were titered at 1:50 dilution. In the third graph from the top, the solid bars represent those animals which carry the $H-2^s$ allele donated by the SJL grandparent. In the bottom graph, the unshaded bars indicate the animals which carry the $H-2^d$ allele donated by the DBA/1 grandparent.

responders to (T,G)-Pro-L. The (DBA/1 × SJL) F_1 mice give a response intermediate between the parental strains. (DBA/1 × SJL) F_1 × DBA/1 backcross mice segregate in their response as intermediate and low responders in a manner similar to the parents of this cross. The segregation in this case is similar to that seen for anti-(T,G)-A-L response in the CBA × C57 cross (14). The F_1 × SJL offspring are all intermediate or low, although they would be expected to be intermediate or high. No explanation has yet been found for this anomalous result, which will be referred to below.

Since immune response to all of the polypeptides belonging to the A-L series was found to be closely linked to the H-2 locus, H-2 typing by hemagglutination was performed on immunized F_1 × DBA/1 backcross mice. The

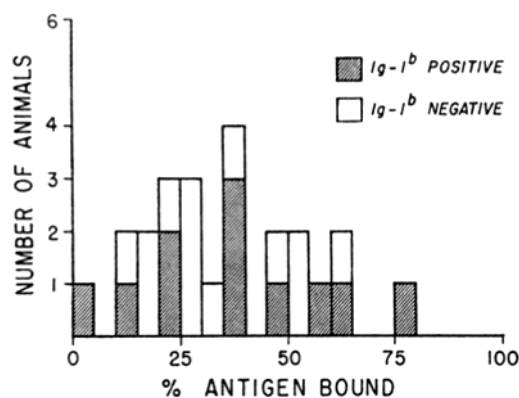


FIG. 2. Simultaneous plot of response to (T,G)-Pro-L in terms of percentage of antigen bound, and presence of the Ig-1^b allotype marker in a segregating population of (DBA/1 × SJL) F_1 × DBA/1 mice.

results show a complete absence of linkage of anti-(T,G)-Pro-L response with the H-2^s allele. In the third graph from the top, the solid bars represent those animals which carry the H-2^s allele donated by the SJL grandparent. In the F_1 × DBA/1 backcross mice, H-2^s is distributed equally in high and low responders to (T,G)-Pro-L. In the bottom graph, the unshaded bars indicate the animals which carry the H-2^a allele donated by the DBA/1 grandparent. There is no linkage between antibody response to (T,G)-Pro-L and the H-2 locus.

Lack of Correlation between Ability to Respond and the γ G Allotype of the Responding Animal.—SJL mice possess the Ig-1^b allele of the Ig-1 allotype locus described by Herzenberg et al. (22). This allotype marker is located on the Fc fragment of γ G_{2a} mouse immunoglobulins. It has been shown (26) that allotype markers on mouse γ G₁, γ G_{2a}, γ G_{2b}, and γ A Fc fragments are all

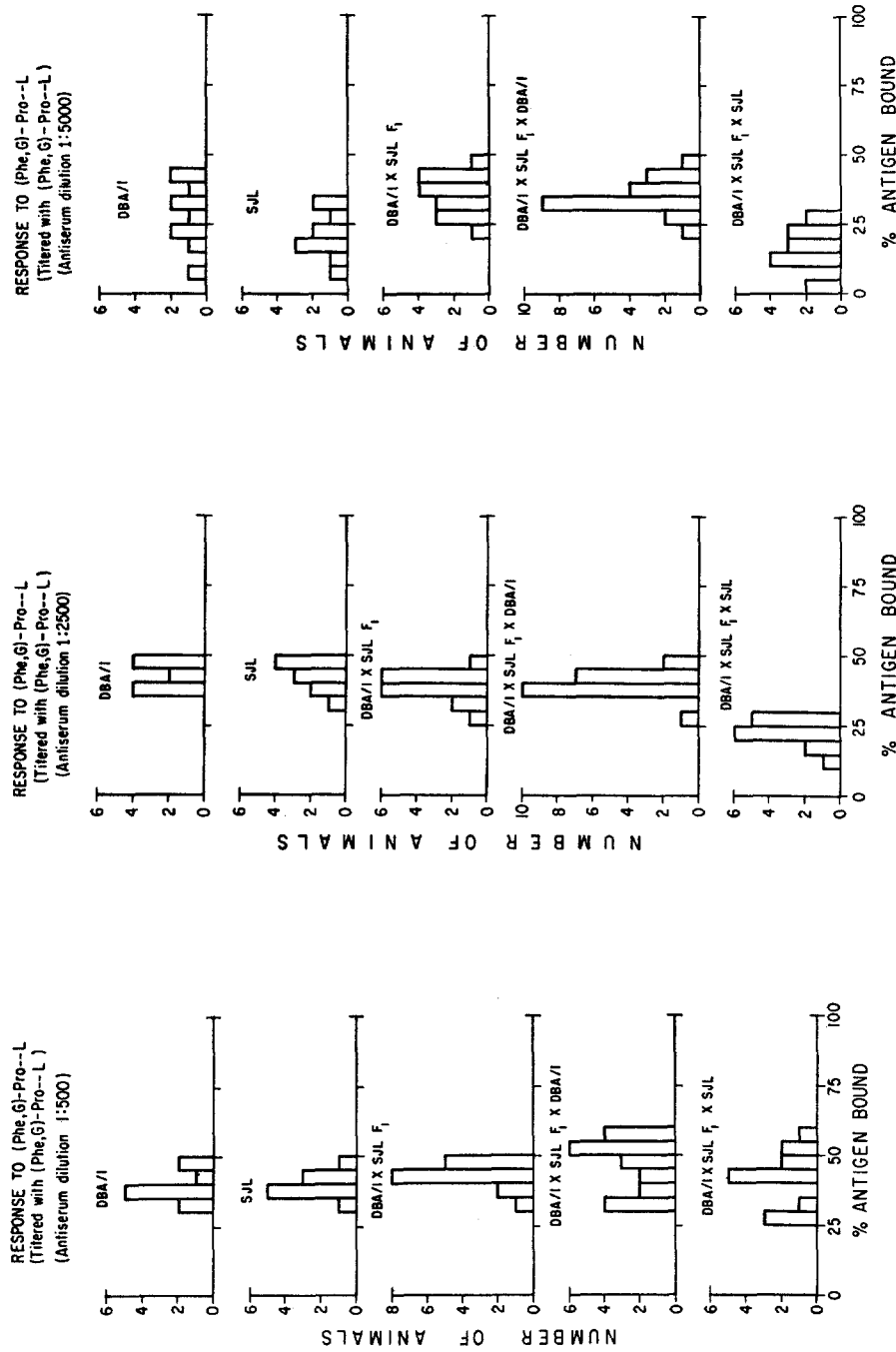


FIG. 3. The immune response of mice to (Phe,G)-Pro-L titered with tritium-labeled (Phe,G)-Pro-L. The specific activity of (Phe,G)-Pro-L-(Ac)-³H was 0.114 μg/μg. (Phe,G)-Pro-L tends to aggregate in dilute solutions (13), and 60-70% of the labeled antigen precipitates spontaneously after the centrifugation required for the antibody assay. Only 64% of the antigen remaining in the supernatant is precipitable by antibody. The antisera were titered at 1:500, 1:2500, and 1:5000 dilutions.

closely linked. Therefore, it is possible to determine whether or not the ability to respond well to (T,G)-Pro-L is linked to the structural genes for the Fc fragments of the SJL high-responder strain. This can be done by testing antibody response and allotype in a segregating population—the (DBA/1 × SJL) F_1 × DBA/1, where, if linkage exists, the high-responding mice should be Ig-1^b positive and low responders should lack this allele. Immunodiffusion

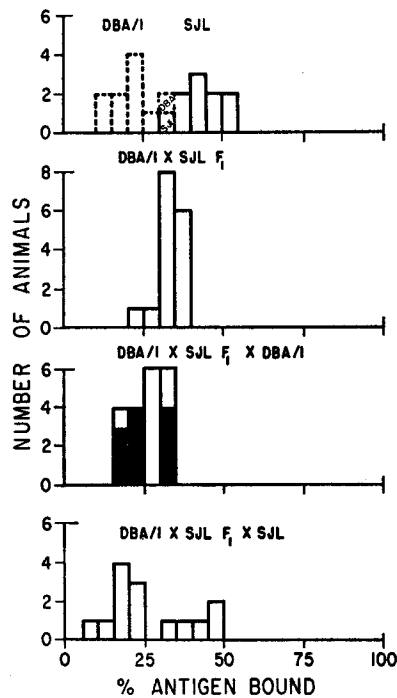


FIG. 4. The immune response of mice immunized with (Phe,G)-Pro-L and titered with (T,G)-Pro-L-¹²⁵I at 1:500 dilution. In the third graph from the top, the solid bars indicate those animals carrying the H-2^a allele donated by the SJL grandparent.

experiments in agar gel performed with antisera taken from the (DBA/1 × SJL) F_1 × DBA/1 backcross mice and anti-Ig-1^b antisera show no linkage between the ability to respond well to (T,G)-Pro-L and the Ig-1^b allotype donated by the SJL grandparent, the high responders to (T,G)-Pro-L (Fig. 2). Similar results were obtained previously for the response to (T,G)-A-L, where no linkage was found between response to (T,G)-A-L and the γG_{2a} allotype of the responding animal (27).

Immune Response to (Phe,G)-Pro-L.—Fig. 3 illustrates the response of DBA/1, SJL, the F_1 , and the two backcross populations to (Phe,G)-Pro-L,

titered with the tritium-labeled homologous antigen at three antiserum dilutions. The results are striking in that they show at 1:500 dilution of antisera a nearly equal response to this antigen in all five groups (Fig. 3). At 1:2500 dilution of antisera, the percentage of antigen bound in all the groups is similar except for (DBA/1 \times SJL) F_1 \times SJL sera, which show a decrease in the capacity to bind the homologous antigen (Fig. 3). At 1:5000 dilution, the capacity of antisera taken from DBA/1 and SJL mice to bind the homologous antigen is lower (Fig. 3). Antisera from (DBA/1 \times SJL) F_1 and the backcross F_1 \times DBA/1 begin to show a lower binding capacity only at 1:250,000 dilution of antisera. However, even at a 1:250,000 dilution, the differences in the antigen-binding capacity between the five groups are not great.

When the *same* anti-(Phe,G)-Pro-L sera are tested for their ability to bind (T,G)-Pro-L (Fig. 4), segregation of ability to bind (T,G)-Pro-L is apparent. SJL anti-(Phe,G)-Pro-L binds (T,G)-Pro-L better than DBA/1 anti-(Phe,G)-Pro-L, although the differences at this dilution of antisera (1:500) are not great. (DBA/1 \times SJL) F_1 mice are intermediate responders, and F_1 \times DBA/1 mice show a slight segregation into low and intermediate responders. In this situation, as in the case when F_1 \times DBA/1 mice were immunized with (T,G)-Pro-L, there is no linkage of anti-(T,G)-Pro-L binding capacity with the H-2^a allele. In the third graph from the top, the solid bars indicate those animals carrying the H-2^a allele donated by the SJL grandparent, and it is clear that the H-2^a allele is found in the lowest and highest responders. F_1 \times SJL backcross mice sera (bottom graph) are expected to possess intermediate or high capacity to bind (T,G)-Pro-L, but, in this case as well, F_1 \times SJL anti-(Phe,G)-Pro-L sera range in their ability to bind (T,G)-Pro-L from *low* to high.

Fig. 5 illustrates the genetic segregation seen when anti-(Phe,G)-Pro-L antisera are tested for their ability to bind (Phe,G)-A-L. The antisera were titered at 1:500 and 1:50,000 dilutions; the segregation is seen more clearly at the higher dilution of antisera. The capacity of DBA/1 antisera to bind (Phe,G)-A-L is very high. SJL anti-(Phe,G)-Pro-L sera bind (Phe,G)-A-L very weakly. Anti-(Phe,G)-Pro-L sera from F_1 \times SJL backcross mice segregate in their ability to bind (Phe,G)-A-L, and the capacity to bind (Phe,G)-A-L is clearly linked to the H-2 locus in the F_1 \times SJL backcross. In the bottom graphs (Fig. 5), the unshaded bars indicate the animals carrying the H-2^a allele donated by the DBA/1 grandparent. The H-2^a allele is found only in mice whose sera have a high capacity to bind (Phe,G)-A-L. Identical results in this cross were obtained when (Phe,G)-A-L was used as the immunogen (17). The capacity to bind (Phe,G)-A-L-(Ac)-³H is very high for antisera taken from high responders, and only at 1:250,000 dilution of antisera is there a decrease in the percentage of antigen bound (about 50% instead of 70% at the lower dilutions).

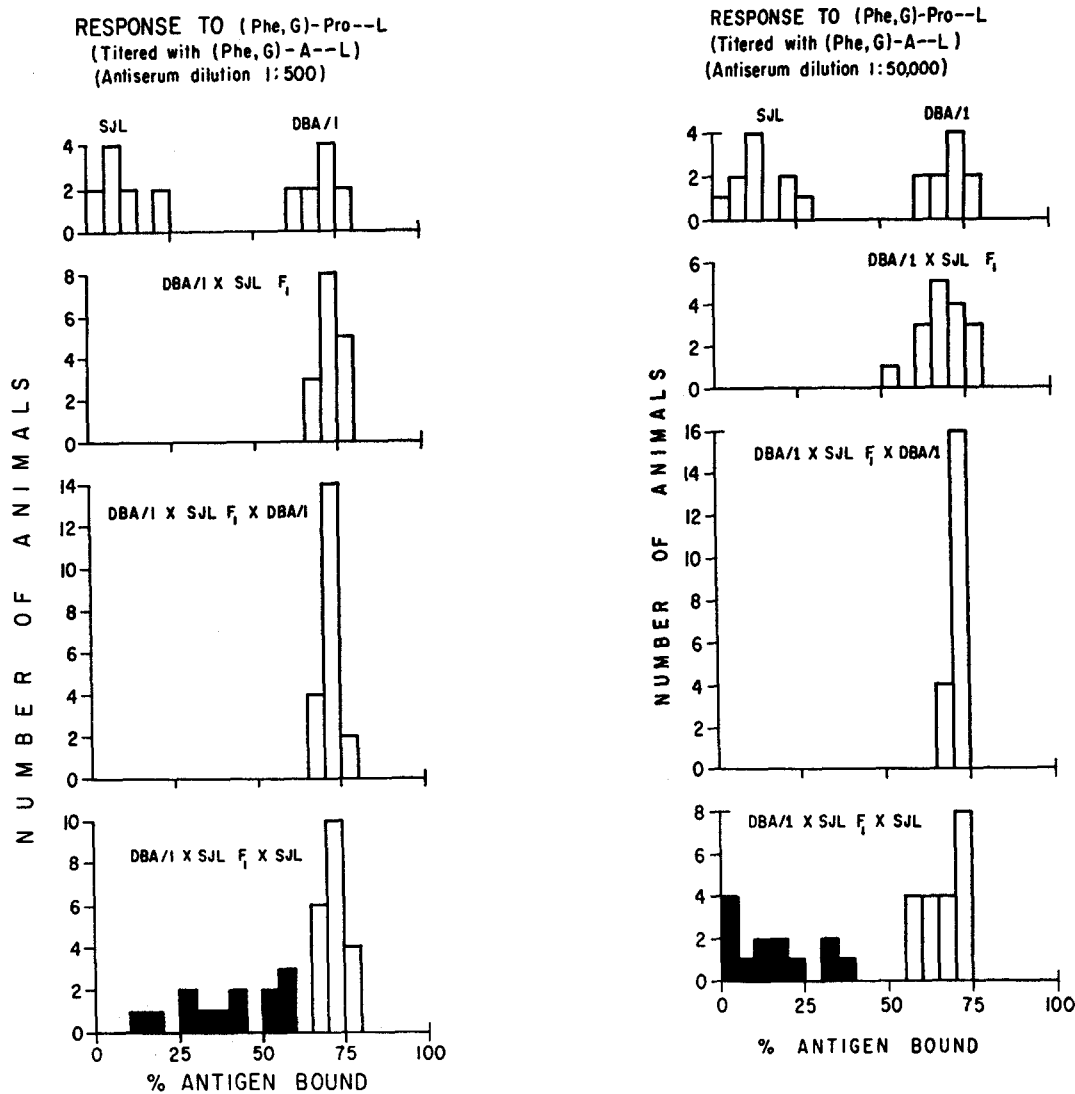


FIG. 5. The immune response of mice immunized with (Phe,G)-Pro-L and titered with tritium-labeled (Phe,G)-A-L at 1:500 and 1:50,000 dilution. The specific activity of (Phe,G)-A-L-(Ac)-³H was 0.17 $\mu\text{C}/\mu\text{g}$, and this preparation was 80% precipitable by excess specific antibody. In the bottom graph, the unshaded bars indicate the animals carrying the H-2^a allele donated by the DBA/1 grandparent.

Immune Response to BSA.—In order to find out whether the low ability of $F_1 \times$ SJL anti-(T,G)-Pro-L and anti-(Phe,G)-Pro-L sera to bind (T,G)-Pro-L- ^{125}I is a general characteristic of $F_1 \times$ SJL mice, the five groups of mice [SJL, DBA/1, (DBA/1 \times SJL) F_1 , $F_1 \times$ DBA/1, and $F_1 \times$ SJL] were immunized with BSA. Antisera were tested by the same antigen-binding capacity

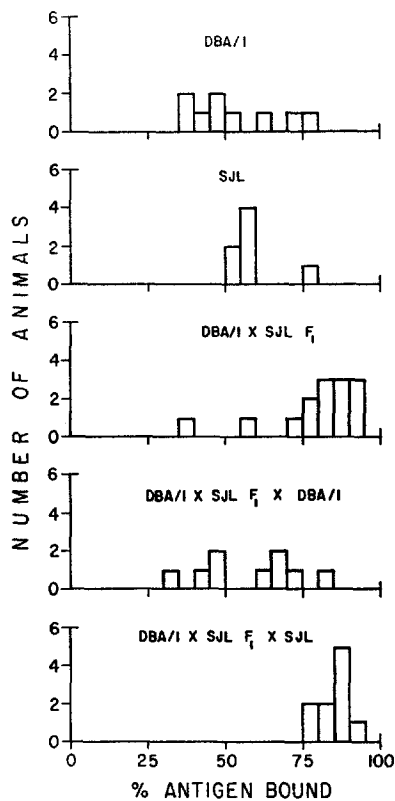


FIG. 6. The immune response of mice immunized with BSA and titered with BSA- ^{125}I at 1:5000 dilution. The specific activity of the BSA- ^{125}I was $10.4 \mu\text{c}/\mu\text{g}$, and 98% of this preparation was precipitable by excess specific antibody.

assay used for all antigens (Materials and Methods), except that in this case 0.5% gelatin was used as a diluent instead of 1% BSA (13, 16). All five groups are high responders to BSA, including $F_1 \times$ SJL. Fig. 6 illustrates the immune response to BSA at a 1:5000 dilution of antisera, a dilution at which the percentage antigen bound values begins to decrease. Even at this dilution, the antigen-binding capacity of $F_1 \times$ SJL antisera is high, and this group appears to include the best responders to BSA.

Inhibition Experiments.—In order to find out whether anti-(Phe, G)-Pro-L antibodies are divided into two separate populations, one capable of binding (T, G)-Pro-L and the other capable of binding (Phe, G)-A-L, or if antibodies to (Phe, G)-Pro-L are all one group with cross-reacting specificities, inhibition tests were performed with (DBA/1 × SJL)_{F1} anti-(Phe, G)-Pro-L antisera, which can bind both of the above-mentioned polypeptides. The inhibition experiments were performed at a dilution of antisera in which the percentage antigen bound value is 30–55%, i.e. a 1:500 dilution for testing binding of (T, G)-Pro-L-¹²⁵I and a 1:250,000 dilution to test capacity to bind (Phe, G)-A-L-(Ac)-³H. Addition of a 100-fold excess of (T, G)-Pro-L to individual (DBA/1 × SJL)_{F1} anti-(Phe, G)-Pro-L sera had no effect on the ability of these sera to bind (Phe, G)-A-L-(Ac)-³H. The average percentage of labeled (Phe, G)-A-L bound after addition of (T, G)-Pro-L was 55. Without addition of (T, G)-Pro-L, the average percentage of labeled (Phe, G)-A-L bound by the same antisera at the same dilution was 51. After addition of a 100-fold excess of nonradioactive (Phe, G)-A-L to (DBA/1 × SJL)_{F1} anti-(Phe, G)-Pro-L antisera, the average percentage of (T, G)-Pro-L-¹²⁵I bound was 37. A similar percentage of antigen was bound (33%) in the absence of (Phe, G)-A-L. (In control studies, addition of unlabeled antigen reduced the binding of the same antigen, labeled, by 70–100%.)

Quantitative Precipitin Tests.—Quantitative precipitin curves were performed using pooled _{F1} anti-(Phe, G)-Pro-L antisera with the homologous (Phe, G)-Pro-L antigen, and with the cross-reacting antigens (T, G)-Pro-L and (Phe, G)-A-L, to provide more quantitative data concerning the amount of antibodies to (Phe, G)-Pro-L detected by the cross-reacting antigens. The amount of precipitating antibody with the homologous antigen (Phe, G)-Pro-L was 770 μg/ml. 390 μg/ml of antibody was precipitated with the cross-reacting polypeptide (Phe, G)-A-L, and 325 μg/ml antibody was precipitated with (T, G)-Pro-L. 82% of the (Phe, G)-Pro-L and 79% of the (Phe, G)-A-L added were precipitated in the equivalence zone, but only 23% of the (T, G)-Pro-L added was precipitated at this point. Addition of an excess of rabbit anti-mouse γ-globulin to the supernatant, taken off the precipitate, did not precipitate a significant amount of labeled antigen, indicating that the major amount of antibody in the serum was precipitated at the equivalence zone.

DISCUSSION

The antibody response to (T, G)-Pro-L in the DBA/1 × SJL cross shows a genetic segregation similar to that seen for anti-(T, G)-A-L response in the CBA × C57 cross (14, 16), except that in the _{F1} × DBA/1 population there is a complete absence of linkage of high anti-(T, G)-Pro-L response with the H-2^a allele donated by the high-responding SJL parent. Ability to respond to (T, G)-Pro-L is not linked to immunoglobulin allotype, as is true for ability

to respond to (T,G)-A-L. However, there is one anomalous result. The $F_1 \times$ SJL population would be expected to be intermediate and high responders to (T,G)-Pro-L, but they are actually intermediate or low responders to this antigen. A similar result is seen when $F_1 \times$ SJL mice are immunized with (Phe,G)-Pro-L and titered either with the immunizing antigen or with (T,G)-Pro-L. However, $F_1 \times$ SJL mice immunized with bovine serum albumin respond as well as or better than the SJL or DBA/1 parents, indicating that there is no nonspecific immune defect in this subline. There is no simple genetic explanation for this anomalous response in the $F_1 \times$ SJL population. It is compatible with the presence of several modifying genetic factors which influence ability to respond in a complex manner.

Barring the anomalous response of $F_1 \times$ SJL backcross mice, the genetic control of ability to respond to (T,G)-Pro-L appears to be similar to the genetic control of the ability to respond to (T,G)-A-L (14), except that in the former case there is no linkage to the H-2 locus. The recent finding of Gasser (28) that the ability of domestic inbred mice to respond to the Ea-1 erythrocyte antigens found only in wild mice is a genetically controlled trait, which is linked to agouti in the Vth linkage group, and which maps near the H-3 and H-6 loci, raises the possibility that there may be a close relationship between histocompatibility antigens and ability to respond to a variety of exogenous antigens (16, 28). The nature of this relationship remains unclear. The locus controlling the response to the polypeptides built on A-L has been designated Ir-1 (immune response-1) (29). The gene(s) controlling the response to Ea-1 has been called Ir-2 (28). Careful mapping studies will be required to determine whether Ir-1 and Ir-2 are identical with the H-2 and H-3 or H-6 loci, respectively, or distinct from these loci but closely linked to them. Evidence has already been presented (16) suggesting that the linkage of Ir-1 and H-2 is not due to a cross-reaction between (T,G)-A-L and H-2 isoantigens, and Gasser (28) has reported similar evidence for Ir-2. We have tentatively designated the genetic factor(s) controlling ability to respond to (T,G)-Pro-L as Ir-3. Studies are in progress to establish the presence or absence of linkage to other histocompatibility loci.

The demonstration that ability to respond to (T,G)-Pro-L (Ir-3) is not linked to immunoglobulin allotype is similar to the demonstration that ability to respond to (T,G)-A-L (Ir-1) is also not linked to immunoglobulin allotype. These observations gain added significance from the recent demonstration by Todd and Mandy (30) that the a1, a2 allotype markers which are found in the N-terminal 35 amino acids of the rabbit γ G heavy chain (31) are linked to the a11 and a12 allotype markers which are located at position 219 in the rabbit γ G heavy chain. If this finding, that allotype markers in the N-terminal (variable) portion of the γ G heavy chain are linked to other allotype markers in the constant region of the γ G heavy chain, is true for species other than the rabbit and is also true for the variable and constant region genes for light chains, then the present results, that Ir-1 and Ir-3 are not linked to allotype markers known to be

localized to the Fc regions of mouse heavy chain genes, indicate that these traits are not correlated with any known immunoglobulin structural genes.¹ Barring separate variable region genes which are inserted between the structural genes coding for the N-terminal 35 amino acid residues and the structural gene coding for the Fc fragment, it would appear that Ir-1 and Ir-3 are not the phenotypic expression of separate variable region genes.

The tentative conclusions mentioned above require definite proof, particularly because of the demonstration that the antibody response to (Phe,G)-Pro-L is controlled by genetic factors which appear to affect the specificity of the antibodies produced. Thus, DBA/1, SJL, (DBA/1 × SJL)F₁, and the two backcross generations, F₁ × DBA/1 and F₁ × SJL, are good responders to the homologous antigen (Phe,G)-Pro-L (Fig. 3). However, when the *same* antisera are tested for their ability to bind (T,G)-Pro-L, segregation of ability to bind (T,G)-Pro-L is apparent (Fig. 4). SJL anti-(Phe,G)-Pro-L sera bind (T,G)-Pro-L better than anti-(Phe,G)-Pro-L sera from DBA/1, and F₁ × DBA/1 antisera are segregated in their ability to bind this cross-reacting polypeptide. In this case, as is found for F₁ × DBA/1 anti-(T,G)-Pro-L, there is no linkage between the capacity to bind (T,G)-Pro-L and the H-2^a allele donated by the high-responder SJL grandparents. On the other hand, F₁ × SJL anti-(Phe,G)-Pro-L sera are segregated in their ability to bind (Phe,G)-A-L (Fig. 5). In this case, the ability to bind (Phe,G)-A-L is closely linked to the H-2^a locus donated by the DBA/1 grandparent of the F₁ × SJL cross. These results are identical with the findings obtained with this cross when (Phe,G)-A-L is used as the immunogen (17). The results obtained by studying the immune response to (Phe,G)-Pro-L show definite genetic control of the specificity of antibodies produced against the same polypeptide antigen in two genetically different strains of mice. This genetic control is probably the result of interaction between two gene loci, each affecting the response to (Phe,G)-Pro-L. DBA/1 mice produce antibodies to the (Phe,G) part of the polypeptide, while SJL mice make antibodies to the (Pro-L) part of the same antigen. These results indicate that there are different loci controlling the immune response to antigenic determinants of different amino acid composition. Similar evidence that the specificity of antibodies to the same antigen produced in different animals is under genetic control has been reported by Arquilla et al. (9-11) and by Pinchuck and Maurer (12), as described above (see Introduction).

When F₁ anti-(Phe,G)-Pro-L antisera are tested for their ability to bind either (T,G)-Pro-L or (Phe,G)-A-L in the presence of a 100-fold excess of the other antigen, no inhibition of binding is found, indicating that the two populations of antibodies are separate and distinct. This finding is borne out by precipitin curves of anti-(Phe,G)-Pro-L antisera from (DBA/1 × SJL)F₁

¹ In studies with Dr. P. G. H. Gell and Dr. A. S. Kelus (unpublished observations), no association was found between high or low response to (T,G)-A-L in Himalayan and Sandy-lop rabbits (14) and the a-1,2,3 (heavy chain) or b-4,5,6 (light chain) allotype loci.

mice. Pooled F_1 anti-(Phe, G)-Pro-L contained 770 $\mu\text{g}/\text{ml}$ of antibody precipitable by (Phe, G)-Pro-L, 325 $\mu\text{g}/\text{ml}$ of antibody precipitable by (T, G)-Pro-L, and 390 $\mu\text{g}/\text{ml}$ of antibody precipitable by (Phe, G)-A-L. These results imply that (DBA/1 \times SJL) F_1 mice produce two entirely separate antibody populations against (Phe, G)-Pro-L.

A complete understanding of these genetic controls requires that we know (a) the exact cell type which transfers ability to respond, i.e. whether a lymphocyte or some other cell type; (b) whether this cell is the precursor of the antibody-producing cell itself; and (c) whether the gene affects antibody specificity. We do not yet know the answer to the first two questions raised, although the data presently available² indicate that ability to respond is transferable with highly purified peripheral blood lymphocytes. The results presented in this study indicate that the genetic control of the ability to respond to a particular determinant affects the specificity of the antibodies produced, suggesting that the recognition of an antigenic determinant is genetically controlled, or that these genes in some way affect the structure of the antibody-combining site.

SUMMARY

The immune response to a synthetic polypeptide built on multichain polyproline, poly-L-(Tyr, Glu)-poly-L-Pro-poly-L-Lys [(T, G)-Pro-L], in the offspring of a cross between DBA/1 and SJL mice is under a genetic control superficially similar to the one operating for the immune response to a similar synthetic polypeptide built on multichain polyalanine, poly-L-(Tyr, Glu)-poly-D, L-Ala-poly-L-Lys [(T, G)-A-L], in the offspring of a cross between CBA and C57 mice. In both cases, the genetic control is a quantitative trait in which the major gene(s) is (are) dominant and the trait is not linked to any of the known structural genes coding for mouse immunoglobulin heavy chains. However, the genetic control of response to (T, G)-Pro-L, designated immune response-3 (Ir-3), is qualitatively different from the one operating for (T, G)-A-L [immune response-1 (Ir-1)] in that it is not linked to the histocompatibility-2 (H-2) locus.

A study of the immune response to a related polypeptide built on multichain polyproline, poly-L-(Phe, Glu)-poly-L-Pro-poly-L-Lys [(Phe, G)-Pro-L], in the DBA/1 \times SJL cross has shown a genetic control of antibody specificity. F_1 \times DBA/1 backcross anti-(Phe, G)-Pro-L sera segregate in their ability to bind (T, G)-Pro-L, and there is no linkage of anti-(T, G)-Pro-L binding capacity with the H-2^a allele of the SJL grandparent. F_1 \times SJL anti-(Phe, G)-Pro-L sera segregate in their capacity to bind poly-L-(Phe, Glu)-poly-D, L-Ala-poly-L-Lys [(Phe, G)-A-L] and the ability to bind (Phe, G)-A-L is clearly linked to the H-2^a allele from the DBA/1 grandparent. Thus, in mice all

² Tyan, M. L., and H. O. McDevitt. Unpublished observations.

responding well to a given antigen [(Phe, G)-Pro--L], the specificity of the antibodies produced [i.e., anti-(Phe,G) or anti-prolyl] is genetically determined. Cross-inhibition of binding in (DBA/1 \times SJL) F_1 anti-(Phe, G)-Pro--L antisera indicates that the anti-(Phe,G) and anti-prolyl specificities are a function of two separate and largely non-crossreacting antibody populations.

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