# ANTIGENICITY OF POLYPEPTIDES (POLY ALPHA AMINO ACIDS)*

## XVI. Genetic Control of Immunogenicity of Synthetic Polypeptides in Mice

By PAUL PINCHUCK, Ph.D., and PAUL H. MAURER,‡ Ph.D.

*(From the Microbiology Department, New Jersey College of Medicine and Dentistry, Jersey City)*

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It has long been known that there is considerable variability in the ability of animals to produce an immune response to any given antigen. This is true, not only of the ability of different species to respond to a single purified material, but to the responses even among animals within a species. There is evidence that at least part of this variability is genetically determined; however, most antigens are sufficiently complex so that a direct demonstration of simple genetic transmission of the ability to respond has not been obtained.

Recent work with synthetic polypeptide antigens has shown that such materials can be antigenically simple (1). It has been demonstrated that the ability of poly-L-lysine to act as a hapten carrier for the 2,4-dinitrophenyl group in guinea pigs is transmitted as a simple Mendelian determinant (2–5). In our own studies, the response of Swiss mice (outbred) to a number of these polypeptides was demonstrated (6). It was shown that although none of these animals could respond to random copolymers of only 2 L-$\alpha$-amino acids, introduction of 4 to 5 mole per cent of a third amino acid made the polymers fairly good immunogens. The introduction of a small amount of alanine[1] into $G_{60}L_{40}$ produced the polymer $glu_{57}lys_{38}ala_5$ ($GLA_5$), which was immunogenic in about half the animals studied. Higher alanine contents (10 to 60 mole per cent) produced better antigens.

In the present study, the immunogenicity of some of these glu-lys-ala polymers in inbred strains of mice has been studied. Also, the pattern of transmission of the ability to respond to $GLA_5$ in the progeny of responders and

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[1] In this article, G, L, and A stand for the amino acids; glutamic acid, lysine, and alanine. Subscripts refer to mole per cent of the amino acid in the polymer.

non-responders of the previously studied outbred population has been determined. The results are in accord with a simple Mendelian determination of the ability to respond.

## *Materials and Methods*

The polymers used in this study are listed in Table I. The methods used for immunization, bleeding, and titrating of serum were those previously described (6).

TABLE I

*Polymers Tested for Immunogenicity*

| Ratio of amino acids in polymer | Nomenclature of polymer | Lot | Average molecular weight |
|---|---|---|---|
| $Glu_{57}lys_{38}ala_5$ | $GLA_5$ | M-22 | 50,000 |
| $Glu_{54}lys_{36}ala_{10}$ | $GLA_{10}$ | M-46 | 70,000 |
| $Glu_{36}lys_{24}ala_{40}$ | $GLA_{40}$ | M-45 | 55,000 |
| $Glu_{54}ala_{36}lys_{10}$ | $GAL_{10}$ | M-47B | 50,000 |

TABLE II

*Response of Inbred Mouse Strains to Terpolymers*

| Antigen..... | $Glu_{57}lys_{38}ala_5$ | | $Glu_{54}lys_{36}ala_{10}$ | | $Glu_{36}lys_{24}ala_{40}$ | | $Glu_{54}ala_{36}lys_{10}$ |
|---|---|---|---|---|---|---|---|
| Strain | Course II | Course III | Course II | Course III | Course II | Course III | Course III |
| $C_3H$/HeJ | 9/9* (12)‡ | 6/6 (46) | 8/8 (40) | 8/8 (149) | 14/14 (80) | 8/8 (650) | 5/5 (43) |
| $C_{57}$Bl/6J | 0/7 | 0/6 | 8/8 (17) | 8/8 (62) | 9/9 (200) | 4/4 (800) | 8/8 (79) |
| $C_{57}$Bl/10J | 0/10 | 0/10 | 10/10 (10) | 9/9 (17) | ND§ | ND | ND |
| BALB/cJ | 11/11 (12) | 11/11 (7) | 10/10 (17) | 8/8 (23) | 4/4 (170) | 12/12 (600) | ND |
| A/J | 0/4 | 0/3 | 6/6 (12) | 6/6 (22) | ND | ND | ND |
| CBA/J | 0/11 | 0/9 | 11/11 (9) | 11/11 (7) | ND | ND | ND |
| 129/J | ND | 9/9 (48) | 6/10 (4) | 8/8 (74) | ND | 9/9 (1200) | ND |

* Number responding/number tested.
‡ Values in parentheses refer to mean hemagglutination titer.
§ ND—Not done.

*Animals.*—The inbred strains of mice used were all obtained from the R. B. Jackson Laboratories, Bar Harbor, Maine. With the exception of the A/J strain, 15 to 20 gm females were used; the A/J animals were male. The mice used for the breeding were Swiss mice obtained from Cam Farms, Wayne, New Jersey. After being tested, as described in the preceding paper, the appropriate animals were caged, generally 1 male to 2 females. Females were separated shortly before term, and the litters were weaned at 28 days and separated by sex.

## RESULTS

*Inbred Strains.*—Table II presents the data on the animals of highly inbred strains. The data include both the number of animals responding and the mean hemagglutination titers of the sera. In all cases, the response is either 0 or 100

per cent. Three out of the 7 strains were able to respond to $GLA_5$. It was previously reported that 5 of these inbred strains, including the 3 which produced an immune response, were unable to make antibody to $G_{60}L_{40}$ and $G_{60}A_{40}$. All

TABLE III

*Immune Responses to $GLA_5$ by Offspring of Responder and Non-Responder Swiss Mice*

| Parents | | Progeny | | |
|---|---|---|---|---|
| Male | Female | No. of animals | Response | |
| | | | Course II | Course III |
| *A* Non-responders | | | | |
| $B_1$ | $B_2$ | ♀4 | 0/4* | 0/4 |
| | | ♂6 | 0/6 | 0/6 |
| | $B_4$ | ♀6 | 0/6 | 0/6 |
| | | ♂3 | 0/3 | 0/3 |
| *B*, Responders | | | | |
| $D_1$ | $D_2$ | ♀5 | 5/5 (19)‡ | 4/4 (23) |
| | | ♂4 | 4/4 (7) | 4/4 (78) |
| | $D_4$ | ♀4 | 4/4 (19) | 2/2 (40) |
| | | ♂4 | 4/4 (9) | 3/3 (70) |
| *C*, Responders | | | | |
| $C_1$ | $C_2$ | ♀5 | 4/5 (5) | 4/5 (56) |
| | | ♂4 | 2/4 (8) | 2/4 (96) |
| *D*, $F_1$ Non-responders | | Litter I | | |
| | | ♀1 | 0/1 | 0/1 |
| | | ♂4 | 0/4 | 0/3 |
| C-$F_1$6§ | C-$F_1$5§ | Litter II | | |
| | | ♀1 | 0/1 | 0/1 |
| | | ♂3 | 0/3 | 0/3 |

* Number responding/number tested.

‡ Values in parenthesis refer to mean hemagglutination titer of positive animals.

§ Negative-responding progeny of part *C*.

of the strains gave a 100 per cent response to $GLA_{10}$ and $GLA_{40}$. $C_3H$ and $C_{57}Bl/6$ mice were also tested against $GAL_{10}$, and produced a 100 per cent response.

*Swiss Mice.*—Antibody to $GLA_5$ was present in 47 per cent of the Swiss mice (6). Various matings were set up, as shown in Table III. The progeny of non-responders × non-responders were consistently non-responders; 0/19 animals in 2 litters being unable to respond (Table III, A). In Table III, B, the progeny

of male $D_1$ × 2 positively responding females ($D_2$, $D_4$) were all positive (17/17). Another pair of animals (♂ $C_1$ × ♀ $C_2$) produced 67 per cent responders (Table III, C). When 2 of these non-responding progeny were crossed (Table III, D, Fig. 1), their progeny in turn were consistently negative.

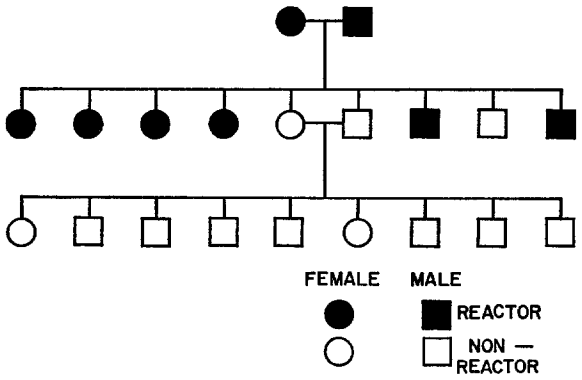


FIG. 1. Genetic transmission of ability to respond to the polymer $GLA_5$ in Swiss mice. The response of the parental and $F_1$ generations is given in Table III, C; the $F_2$ generation is given in Table III, D.

## DISCUSSION

The data presented provide clear-cut evidence for the control by a simple dominant Mendelian factor of the ability of mice to respond to the synthetic polypeptide antigen, $GLA_5$. Inbred strains of mice, which are virtually genetically identical, are either all responders or all non-responders, in contrast to the variable response among outbred Swiss mice. The reactivity of the progeny of these animals also supports the concept of Mendelian transmission of this trait. The progeny of non-responders are all non-responders (Table III, A), implying that the non-responders are (genetically) homozygous for this trait. Responders may be either homozygous or heterozygous. As examples of the former, there are such inbred strains as $C_3H$, BALB/c, and 129 or (presumably) the progeny of ♂ $D_1$ and ♀ $D_2$ and ♀ $D_4$ (Table III, B), where 19/19 animals were positive. An example of heterozygous responders is seen in the cross ♂ $C_1$ × ♀ $C_2$ (Table III, C), where 67 per cent of the animals were positive, and the $F_2$ generation produced by crossing non-responders was entirely negative (Table III, D). Three of the 7 inbred strains produced a 100 per cent response to $GLA_5$; that is, 43 per cent. It may be coincidental that 47 per cent of the Swiss mice could so respond, or it may be due to a similar frequency of the factor in the random bred populations from which the various inbred strains were derived. All 3 of the inbred strains which were responders are of the Asa 1

gamma globulin allotype (7), as is also the CBA/J strain. The two $C_{57}$ Bl strains are of the Asa 2 type, and A strain animals are Asa 4. It is not known if this apparent correlation is coincidental, or represents linkage to an allele of the complex Asa locus, which governs specificity of the $F_c$ fragment. Investigations are currently underway to determine if such linkage does, in fact, exist.

Past studies on the genetic control of the immune response have primarily focused either on resistance (or susceptibility) to some infectious agent or toxin (8), or on quantitative differences in the response to an antigen. Thus, Ipsen found that the minimum amount of tetanus toxoid required to induce a protective antibody response was different in various inbred strains of mice (9). Similarly, the levels of antibody produced to bovine albumin, pneumococcal polysaccharides, and heterologous (sheep) erythrocytes was characteristic for various inbred strains (10).

Populations which are not genetically homogeneous vary in the response to a variety of relatively purified antigens. In at least some cases, lines which breed true for the level of the response can be selected. Thus, a population of guinea pigs gave rise to animals with high and low levels of response to diphtheria toxoid (11).

A similar unpublished observation has been found by McDevitt, Humphrey, and Sela. Using the branched copolymer (T, G)-A-L, (comparable to our random, linear $glu_{36}lys_{24}ala_{35}tyr_5$), they found that the amounts of antigen bound by the serum of CBA mice was consistently low, whereas $C_{57}$ Bl animals produced a considerably higher response. The $F_1$ hybrids of these strains bound an intermediate amount of $I^{131}$-labeled polymer. Breeding studies indicated that although all of these animals could respond to this polymer, the level of antibody produced was inherited as a simple Mendelian factor.

The response of rabbits to tobacco mosaic virus is a trait with a high degree of hereditability (12). On the other hand, the wide level of response of these same animals to bovine serum albumin (BSA) was not a hereditable character; *i.e.*, environmental differences were predominant in this response. Some of the rabbits however, could not respond to massive doses of bovine serum albumin, failing to show either antibody or enhanced clearance of the antigen from the circulation (immune elimination). The progeny of these non-responders also were non-responders. Because the antigen used was relatively impure (7 bands in immunodiffusion against hyperimmune rabbit antiserum to BSA), the relative antigenic complexity of the determinants in BSA and the unfavorable characteristics of rabbits for breeding studies, this system does not seem to be as suitable for more detailed studies as do the experiments reported here.

Studies in this laboratory with guinea pigs of the NIH inbred strains 2 and 13 also show this pattern. The copolymer, $G_{60}L_{40}$, antigenic in 35 per cent of Hartley (random bred) animals, was antigenic in none of the strain 13 and all

of the strain 2 animals tested, whereas $GLA_{40}$ was 100 per cent immunogenic in both strains. The responses to a number of the polymers studied here were found by Ben-Efraim and Maurer to be strikingly different for these 2 strains, with strain 13 being of generally quite low reactivity (13), and the $F_1$ progeny of strains 2 x 13 reacting 100 per cent. Also, studies in our laboratory, using some of these polymers as hapten carriers in mice show that, the ability to induce antihapten antibodies is separate from the ability to induce formation of antipolymer antibodies. The initial metabolism of hapten-polylysine by guinea pigs *in vivo*, or by spleen extracts *in vitro*, was the same in animals genetically capable of responding to the conjugates as in those which could not (14). These studies imply that the initial handling, etc. of an antigen, while necessary, is not sufficient, and that the recognition mechanism is beyond the point of metabolism of the polymer. The results obtained in the paper suggest a genetic "recognition" system for antigens. Together, with the previously obtained data on immunogenicity of polymers in mice (6), these data suggest a possible mechanism of operation for the factor. Although copolymers are not antigenic in mice, introduction of even a few mole per cent of a third amino acid induces an immune response which is relatively independent of the nature of the third amino acid (*i.e.*, lys, ala, tyr, or phe). Likewise, copolymers, but not homopolymers, can act as haptene carriers in mice (15). Indeed, it is generally true that, given an appropriate carrier, almost any chemical grouping or 3-dimensional configuration can lead to production of specific antibodies. Thus, the postulated recognition system would not be at the level of synthesis of the immunoglobulin. It might well be at the level of complexing of antigen fragments with a particular cellular ribonucleic acid, which is known to occur. Experiments are currently underway in our laboratory to test this hypothesis.


## SUMMARY

The ability of mice to form antibodies against the random terpolymer $glu_{57}lys_{38}ala_5$ is controlled by a codominant Mendelian factor. Three of 7 inbred strains were 100 per cent responders; the others were completely negative. All of these strains could make antibody to related polymers with higher alanine content (10 and 40 mole per cent). Breeding studies using the progeny of Swiss mice indicated that a similar genetic factor was involved.


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