

SPECIFIC IMMUNE RESPONSE GENES OF THE GUINEA PIG

II. RELATIONSHIP BETWEEN THE POLY-L-LYSINE GENE AND THE GENES CONTROLLING IMMUNE RESPONSIVENESS TO COPOLYMERS OF L-GLUTAMIC ACID AND L-ALANINE AND L-GLUTAMIC ACID AND L-TYROSINE IN RANDOM-BRED HARTLEY GUINEA PIGS*

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(Received for publication 12 April 1971)

The use of synthetic polypeptides as antigens has led to several demonstrations of genetic control of specific immune responses, first in guinea pigs (1) and subsequently in mice (2, 3) and rats (4). Until now, however, only in mice has a group of these "immune response genes" been uncovered, thereby permitting some degree of genetic mapping. McDevitt and his colleagues, studying the immunogenicity of three branched multichain synthetic polypeptides containing a backbone of poly-L-lysine (PLL)¹ with side chains of poly-D,L-alanine to which are attached random sequences of L-glutamic acid and either L-tyrosine, L-histidine, or L-phenylalanine, (T,G)-A-L, (H,G)-A-L, or (Phe,G)-A-L, have shown that immune responses to each of these antigens are controlled by separate autosomal dominant genes (5, 6). They have further demonstrated that these genes are closely linked to the *H-2* locus of inbred mice and appear in a region designated as the *Ir-1* locus (7, 8).

In the first paper in this series (9) we demonstrate the existence of autosomal dominant genes governing the immune responses of guinea pigs to random

* Supported in part by a U.S. Public Health Service Research grant AI 09920.

† Recipient of U.S. Public Health Service Special Postdoctoral Fellowship 7F03 AI42841 from the National Institute of Allergy and Infectious Diseases.

¹ Abbreviations used in this paper: DNP, dinitrophenyl; GA, random linear copolymer of L-glutamic acid with L-alanine; GT, random linear copolymer of L-glutamic acid with L-tyrosine; (H,G)-A-L, poly-L-lysine with side chains of poly-D,L-alanine to which are attached random sequences of L-glutamic acid and L-histidine; PBS, 0.015 M phosphate buffer, pH 7.5, containing 0.15 M NaCl; (Phe,G)-A-L, poly-L-lysine with side chains of poly-D,L-alanine to which are attached random sequences of L-glutamic acid and L-phenylalanine; PLL, poly-L-lysine; (T,G)-A-L, poly-L-lysine with side chains of poly-D,L-alanine to which are attached random sequences of L-glutamic acid and L-tyrosine.

linear copolymers of L-glutamic acid with either L-alanine (GA) or L-tyrosine (GT). These genes are expressed in random-bred as well as inbred guinea pig strains. Although the identification of these genes is greatly facilitated by the use of inbred strains, random-bred animals may be more useful for studies of intergenetic relationships. Since most of these animals are not homozygous, various crossovers may have occurred over the years that have been propagated in the colony, thus permitting the demonstration of genetic differences that could not readily be observed in inbred strains. In the present paper we report on our studies in random-bred Hartley guinea pigs of the relationship of the GA and GT genes to each other and to the "PLL gene," a single autosomal dominant gene controlling the immune responses of guinea pigs to PLL and its hapten conjugates (1, 10, 11). We have attempted to determine whether or not these immune response genes are part of a common linkage group, as are the genes functioning at the *Ir-1* locus in mice. Our data demonstrate that the genes controlling immune responsiveness to GA, GT, and PLL are not inherited independently. The GA and PLL immune response genes appear linked; that is, they tend to segregate together. Conversely, the presence of immune responsiveness to either GA or PLL tends to exclude the presence of GT responsiveness.

Materials and Methods

Polymers.—Poly- α -(L-glutamic acid [50%], L-tyrosine [50%]), GT, mol wt 14,500, was obtained from Miles Laboratories, Inc., Elkhart, Ind. Poly- α -(L-glutamic acid [60%], L-alanine [40%]), GA, mol wt 43,000, and PLL, HBr, mol wt 110,000, were obtained from Pilot Chemicals Inc., division of New England Nuclear Corp., Boston, Mass. The PLL was reacted with 2,4-dinitrofluorobenzene as previously described (12) to form DNP₃₃-PLL. The subscript refers to the average number of dinitrophenyl (DNP) groups per molecule of PLL.

Animals.—Hartley strain guinea pigs were obtained from the N.I.H. Animal Production Unit (N.I.H. Hartleys) and from Camm Research Institute, Inc., Wayne, N.J. (Camm Hartleys). All animals used in these studies weighed between 250 and 450 g.

Immunization of Guinea Pigs.—Each guinea pig was immunized simultaneously with two antigens in separate sites. Solutions of the synthetic antigens in 0.015 M phosphate buffer, pH 7.5, containing 0.15 M NaCl (PBS) were emulsified in an equal volume of complete Freund's adjuvant containing 0.5 mg/ml *Mycobacterium butyricum* (Difco Laboratories, Detroit, Mich.). Concentrations were adjusted so that each animal was injected with 100 μ g of each antigen in 0.2 ml of the emulsion. The 0.2 ml was divided between a front and rear footpad. Each guinea pig thus received 100 μ g of each of two antigens in a total of 0.4 ml of emulsion.

Skin Tests.—3 wk after immunization the guinea pigs were challenged with intradermal injections of the immunizing antigens. Animals immunized with GA or GT were tested with 50 μ g of the appropriate antigen dissolved in 0.1 ml normal saline. DNP-PLL skin tests were performed with 10 μ g of this antigen dissolved in 0.1 ml normal saline. The skin test sites were examined 24 hr later and graded positive (induration and erythema greater than 8 mm in diameter) or negative. Those guinea pigs displaying cellular immunity to an antigen are called "responders" to that antigen. The sera obtained 3 wk after immunization were tested for antibody directed against the immunizing antigens using the assays described previously (9). There is an excellent correlation between responder status and the presence of circulating

antibody. As we shall demonstrate in a subsequent report² there is no detectable cross-reactivity of either delayed hypersensitivity or antibody responses between any of the antigens used in this study.

RESULTS

Simultaneous immunization of N.I.H. Hartley guinea pigs with GA and DNP-PLL reveals that the ability to respond to GA is inherited along with the PLL gene in this strain. All of the PLL responders are also GA responders, and all of the PLL "nonresponders" do not respond to GA (Table I). In Camm Hartley guinea pigs, however, the nonidentity of the genes controlling GA and PLL

TABLE I
Distribution of GA and PLL Responsiveness in Random-Bred Hartley Guinea Pigs Simultaneously Immunized with GA and DNP-PLL

	Camm*		N.I.H.*	
	GA+‡	GA-	GA+‡	GA-
PLL+	24 (14)§	1 (11)	18 (16)§	0 (2)
PLL-	7 (17)	22 (12)	0 (2)	2 (0)

* Indicates the source of these Hartley strain guinea pigs (see Materials and Methods).

‡ Plus sign indicates responder, minus sign, nonresponder, to the antigen

§ Numbers in parentheses represent the expected results if the respective immune response genes segregated independently.

responsiveness is demonstrated. Although most of the Camm Hartleys immunized with GA and DNP-PLL are either responders or nonresponders to both antigens, about 15% of these animals respond to either GA or PLL but not both (Table I).

While the existence of these animals proves that the genes controlling the ability to respond to GA and to PLL are not identical, the frequency of these singly responsive animals is much lower than would be predicted if the two genes segregate independently. The expected distribution of GA and PLL responsiveness, assuming independent segregation of their respective immune response genes, is shown in the parentheses in Table I. The values are based on the 46% PLL and 57% GA response rates observed in these animals. The observed distribution of GA and PLL responsiveness in Camm Hartley guinea pigs is clearly not the result of independent segregation. Rather, it indicates

² Bluestein, H. G., I. Green, P. H. Maurer, and B. Benacerraf. Specific immune response genes of the guinea pig. III. Influence of the GA and GT immune response genes on the specificity of cellular and humoral immune responses to a terpolymer of L-glutamic acid, L-alanine, and L-tyrosine (GAT). Manuscript in preparation.

that the genes controlling immune responsiveness to GA and PLL tend to segregate together.

The association of GA and PLL responsiveness among Camm Hartley guinea pigs is graphically illustrated in Fig. 1. Approximately 57% of the unselected population respond to GA. However, if these animals are grouped according to their PLL responsiveness as many as 90% of PLL responders and only 24%

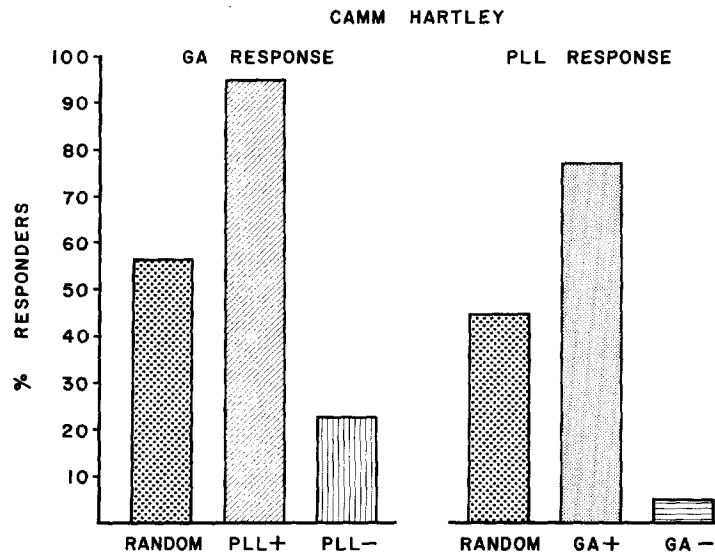


Fig. 1. Frequency of responsiveness to GA as a function of PLL responsiveness, and frequency of PLL responsiveness as a function of GA responsiveness in random-bred Camm Hartley guinea pigs simultaneously immunized with DNP-PLL and GA (see Materials and Methods). Columns labeled "Random" indicate the frequency of responders in the unselected population. PLL+ and GA+ signify responders to those antigens; PLL- and GA-, non-responders.

of PLL nonresponders respond to GA. Similarly, although PLL responsiveness was observed in 46% of these Camm Hartley guinea pigs, 77% of GA responders and only 4% of GA nonresponders respond to PLL. Fig. 1 also graphically demonstrates a significantly higher frequency of occurrence of GA responder-PLL nonresponder guinea pigs than of the converse PLL responder-GA non-responder animals.

The relationship between GT and DNP-PLL responsiveness in Hartley guinea pigs simultaneously immunized with those two antigens is shown in Table II. The observed phenotypic distribution, especially among Camm Hartley guinea pigs, is inconsistent with independent segregation of the GT and PLL immune response genes. Rather, there is a marked tendency for GT and PLL responsiveness to segregate away from each other. Thus, only 14%

of the Camm Hartleys responded alike to GT and PLL (i.e. were either responders or nonresponders to both antigens), compared to the 41% like re-

TABLE II
Distribution of GT and PLL Responsiveness in Random-Bred Hartley Guinea Pigs Simultaneously Immunized with GT and DNP-PLL

	Camm*		N.I.H.*	
	GT+‡	GT-	GT+‡	GT-
PLL+	2 (6)§	7 (3)	9 (11)§	12 (10)
PLL-	18 (14)	2 (6)	4 (2)	0 (2)

* Indicates the source of these Hartley strain guinea pigs (see Materials and Methods).

‡ Plus sign indicates responder, minus sign, nonresponder, to the antigen.

§ Numbers in parentheses represent the expected results if the respective immune response genes segregated independently.

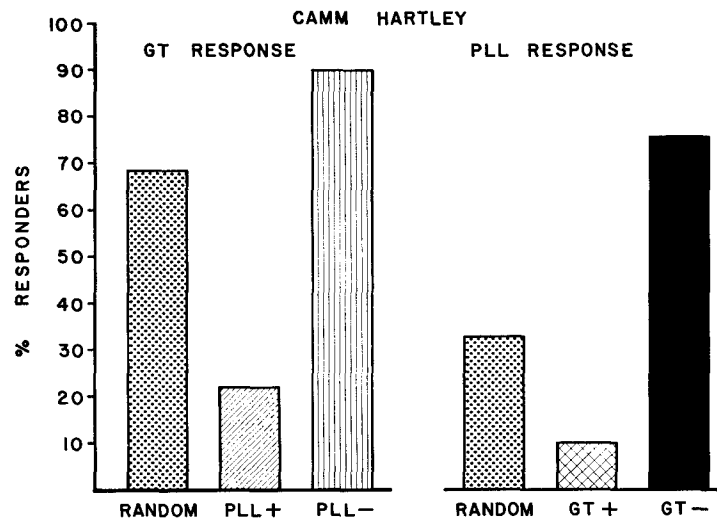


Fig. 2. Frequency of responsiveness to GT as a function of PLL responsiveness, and frequency of PLL responsiveness as a function of GT responsiveness in random-bred Camm Hartley guinea pigs simultaneously immunized with DNP-PLL and GT (see Materials and Methods). Columns labeled "Random" indicate the frequency of responders in the unselected population. PLL+ and GT+ signify responders to those antigens; PLL- and GT-, nonresponders.

sponders one would expect if the GT and PLL genes were inherited independently.

The tendency toward mutual exclusion of GT and PLL responsiveness among Camm Hartley guinea pigs is graphically demonstrated in Fig. 2. GT responsive-

ness occurs in only 22% of PLL responders compared to 90% of PLL non-responders. Among the unselected Camm Hartley population, 69% respond to GT. If one looks at PLL responsiveness as a function of GT responder status, a similar relationship is apparent. Only 10% of GT responders also respond to PLL, while 78% of GT nonresponders are PLL responders. In comparison, 31% of the unselected group of animals respond to PLL.

Since, as we have shown, responsiveness to GA and PLL are linked in most random-bred Hartley guinea pigs, one would expect that the distribution of GA and GT responsiveness among Hartley guinea pigs should be similar to that seen with PLL and GT. When Camm Hartley guinea pigs are simultaneously immunized with GA and GT, the number of double responders and non-

TABLE III
Distribution of GA and GT Responsiveness in Random-Bred Camm Hartley Guinea Pigs Simultaneously Immunized with GA and GT

	GA+*	GA-
GT+	17 (24)‡	17 (10)
GT-	25 (18)	1 (8)

* Plus sign indicates responder, minus sign, non-responder, to the antigen.

‡ Numbers in parentheses represent the expected results if the respective immune response genes segregated independently.

responders to these antigens is significantly lower than would be expected assuming independent segregation of their respective immune response genes (Table III). The number of guinea pigs responding differently to GA and GT, on the other hand, is significantly higher than would be predicted. These results are clearly not consistent with an interpretation of independent segregation of the GA and GT immune response genes. They demonstrate that the presence of GT responsiveness tends to exclude the presence of GA, as well as PLL, responsiveness

A comparison of the relationship between PLL responder status and the ability to respond immunologically to either GA or GT is shown in Fig. 3. In the unselected Camm Hartley population, the response rates to GA and GT are similar, 57 and 69%, respectively. In contrast, among PLL responders 96% also respond to GA while only 22% are also GT responders. Conversely, among PLL nonresponders there are only 24% GA responders while 90% respond to GT. It is readily apparent that neither the GA nor GT immune response genes are inherited independently from the PLL gene. However, while the GA and PLL genes appear linked, the GT and PLL immune response genes tend to be mutually exclusive

DISCUSSION

In guinea pigs, three different genetic traits have been identified which control the ability to develop both cellular and humoral immune responses to specific synthetic linear polypeptide antigens. Immune responsiveness to PLL, GA, and GT is each determined by distinct autosomal dominant genes. Although these immune response genes are clearly separate, they are not inherited independently.

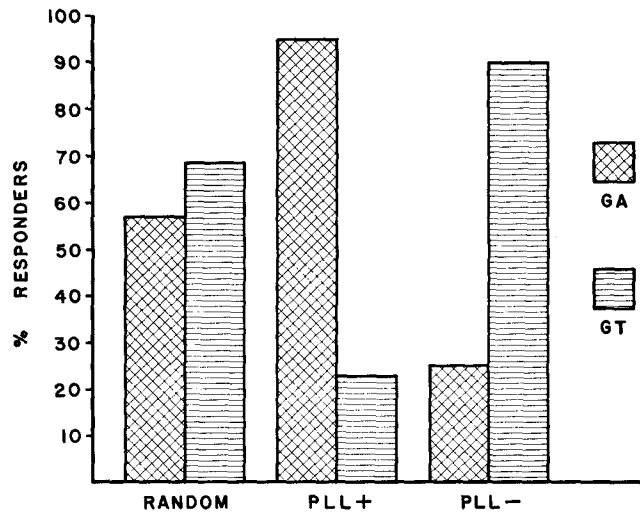


Fig. 3. Frequency of GA and GT responsiveness as a function of responder status to PLL in random-bred Camm Hartley guinea pigs simultaneously immunized with DNP-PLL and either GA or GT (see Materials and Methods). Columns labelled "Random" indicate the frequency of responders in the unselected population. PLL+ indicates responders and PLL-, nonresponders to that antigen.

Responsiveness to GA and PLL appears to be inherited identically in the inbred guinea pig strains and their hybrid offspring. Thus, as has been demonstrated (9), both GA and PLL are immunogenic in all strain 2 but no strain 13 guinea pigs and in all $(2 \times 13)F_1$ hybrids. In addition, as we will demonstrate in a subsequent report,³ GA and PLL responsiveness are inherited together in the $(2 \times 13)F_1 \times 13$ backcross progeny. PLL responsiveness is also inherited together with a major strain 2 histocompatibility antigen in these backcross animals. This indicates that there exists in strain 2 guinea pigs a single chro-

³ Bluestein, H. G., L. Ellman, I. Green, and B. Benacerraf. Specific immune response genes of the guinea pig. IV. Linkage between the GA and GT immune response genes and histocompatibility genotypes in inbred guinea pigs. Manuscript in preparation.

mosome containing the GA and PLL immune response genes and a major strain 2 histocompatibility locus.

Among random-bred Hartley guinea pigs, however, the genes controlling responsiveness to GA and PLL are "linked" but not identical. Thus, whereas these genes are inherited together in most of the guinea pigs we have tested, among Camm Hartley animals having a low PLL gene frequency there is a small percentage of animals which respond to GA but not PLL or to PLL but not GA. The existence of these animals, which probably result from "crossing over" between the PLL and GA genes, demonstrates the nonidentity of these genes.

It is puzzling that GA responder-PLL nonresponder animals occur much more frequently than do PLL responder-GA nonresponder animals. One would normally expect both types of crossover animals to occur with equal frequency. It is unlikely that our results would occur due to chance since a similar observation was made several years ago (13). Among the possible explanations for this phenomenon are: (a) that there may be a preferred site for crossing over which is located within the PLL gene cistron, thus destroying the integrity of PLL gene; (b) that the absence of the PLL gene in some of the GA responders may not be due to crossing over but rather result from a nonfunctional mutation of the PLL gene that is propagated within this colony; and (c) that some of the crossovers permitting PLL responder-GA nonresponder animals may have eliminated some genetic material associated with the GA genome which may be necessary for normal viability. We are unable to distinguish among these alternatives at present.

The inheritance of the ability to respond to GT is also not independent of the PLL and GA immune response genes. Unlike the linkage between the PLL and GA genes, however, responsiveness to GT tends to segregate away from PLL and GA responsiveness. In the two inbred strains, strain 2 guinea pigs respond to GA and PLL but not GT, while strain 13 animals respond to GT but not GA or PLL. Among Hartley guinea pigs most of the animals respond to GT and not GA and PLL, or vice versa. Thus, the ability to respond to GT appears to be inherited as an allele or pseudoallele to the GA and PLL immune response genes in random-bred animals. Furthermore, the chromosome strand, or chromatid, containing the GT gene apparently does not carry the GA or PLL genes, nor does the chromatid carrying the GA and PLL genes contain the GT gene.

Recent data to be presented in a subsequent report³ demonstrate that GT responsiveness in backcross progeny of nonresponder strain 2 \times (2 \times 13)F₁ hybrids segregates as a unigenic autosomal dominant trait and is inherited together with a major strain 13 histocompatibility antigen. Thus, like the strain 2 animals, strain 13 guinea pigs possess a chromosome that contains both an

immune response gene and a major histocompatibility locus. It appears from this study with random-bred guinea pigs that the Hartley animals possess chromosomes similar to those present in both strain 2 and strain 13 inbred guinea pigs containing both immune response genes and genes coding for histocompatibility antigens. The chromatids of such a chromosome might, like the strain 13 chromosome, contain both the "GT gene" and a strain 13 histocompatibility locus; or, like the strain 2 chromosome, it might contain the GA and PLL genes and a strain 2 histocompatibility locus. The very close linkage between the PLL gene and strain 2 histocompatibility antigens observed in Hartley guinea pigs (14) certainly supports this hypothesis. The chromosome strands resembling the strain 2 and strain 13 chromosomes, respectively, are probably not the only chromatids that can make up this chromosome in the random-bred population. There may be others containing immune response genes and histocompatibility loci not yet identified. The existence of animals unresponsive to all three antigens, GA, GT, and PLL, indicates that there is at least one alternative form of the chromosome in the Hartley colony. If the interpretation is correct, the progeny from Hartley animals responding to GA, GT, and PLL, mated with nonresponders for all three antigens should segregate as 50% GT responders and 50% GA and PLL responders with no offspring responding as the parents. In addition, the GT responders should have strain 13 histocompatibility antigens on their lymphocytes while the GA and PLL responders should have strain 2 histocompatibility antigens on their cells. These experiments are now underway in our laboratory.

The two most thoroughly investigated systems demonstrating gene control of specific immune responsiveness have been the immune response genes in guinea pigs and the genes comprising the *Ir-1* locus in mice. It is highly significant that these two systems appear similar in most aspects studied thus far. In both, responsiveness to specific synthetic polypeptide antigens is controlled by single autosomal dominant genes which exert their functions in lymphoid cells; and both the guinea pig immune response genes and the *Ir-1* genes are linked to major histocompatibility loci. In this study, we observe a further important parallel between these two systems. We demonstrate that in guinea pigs as in mice, there exists a group of closely related immune response genes each controlling immune responsiveness to different synthetic polypeptide antigens of limited heterogeneity.

SUMMARY

The ability of guinea pigs to make immune responses to GA, a linear random copolymer of L-glutamic acid and L-alanine, GT, a random linear copolymer of L-glutamic acid and L-tyrosine, and PLL, a linear homopolymer of L-lysine, is controlled by different autosomal dominant genes specific for each of those

polymers. We have investigated the relationship between the PLL gene and the GA and GT immune response genes by simultaneously immunizing random-bred Hartley strain guinea pigs with GA and PLL, GT and PLL, or GA and GT.

In most Hartley guinea pigs the ability to respond immunologically to GA and to PLL is inherited together; that is, most animals responding to GA respond to PLL and vice versa. However, a few animals respond to either GA or to PLL but not both, demonstrating that the GA and PLL immune response genes are not identical but linked in most Hartley animals. Conversely, when simultaneously immunized with GT and PLL, most Hartley guinea pigs respond to either PLL or GT but not both, indicating that GT and PLL responsiveness tends to segregate away from each other. Thus, the GT and PLL immune response genes also are not inherited independently but, rather, behave as alleles or pseudoalleles. Similar results are observed when Hartley guinea pigs are simultaneously immunized with GA and GT. The ability to respond to GA segregates away from the ability to respond to GT.

Our studies demonstrated that the specific immune response genes thus far identified in guinea pigs controlling the ability to respond to GA, GT, and PLL, respectively, are found on the same chromosome. In most Hartley animals, the GA and PLL immune response genes are often linked, i.e. occur on the same chromosome strand, and tend to behave as alleles or pseudoalleles to the GT immune response gene.

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