

SPECIFIC IMMUNE RESPONSE GENES OF THE GUINEA PIG
IV. DEMONSTRATION IN RANDOM-BRED GUINEA PIGS THAT RESPONSIVENESS
TO A COPOLYMER OF L-GLUTAMIC ACID AND L-TYROSINE IS PREDICATED
UPON THE POSSESSION OF A DISTINCT STRAIN 13
HISTOCOMPATIBILITY SPECIFICITY*

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The immune responsiveness of guinea pigs to the synthetic antigen GT,¹ a random linear copolymer of L-glutamic acid and L-tyrosine, is determined by the presence of a unigenic autosomal dominant mendelian trait designated the "GT gene." This gene is expressed in all inbred strain 13 guinea pigs and in a variable percentage of random-bred Hartley guinea pigs, but it is totally lacking in inbred strain 2 guinea pigs. Those animals having the GT gene respond to GT immunization with both cellular and humoral immunity and are thus termed "responders." "Nonresponders" lack the gene and make no detectable immune response after GT immunization (1, 2).

The backcross progeny resulting from mating GT responder (2×13)F₁ guinea pigs to nonresponder strain 2 animals segregate in a 1:1 ratio of responders to nonresponders. Among these backcross animals, linkage between the GT gene and the genetic locus coding for major strain 13 histocompatibility antigens was demonstrated by showing the presence of these strain 13 histocompatibility specificities only on the cells from GT responders (2).

The linkage between specific immune response genes and histocompatibility genotypes has been demonstrated previously in both guinea pigs and mice and may be considered a general phenomenon. The GT gene is similar in behavior to the poly-L-lysine (PLL) gene which is linked to the genes coding for major strain 2 histocompatibility specificities in guinea pigs (3, 4) and to the genes at the *Ir-1* locus in mice

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¹ Abbreviations used in this paper: GT, poly- α -(L-glutamic acid [50%], L-tyrosine [50%]); PLL, poly-L-lysine.

which are linked to the *H-2* histocompatibility locus (5). In all of these studies no animals were found in which expression of the immune response gene and the appropriate histocompatibility genotype was not associated. The possibility must be seriously considered that immune response genes may be identical with genes coding for certain histocompatibility specificities. In previous experiments, the presence of major strain 2 histocompatibility specificities on cells of 94 random-bred guinea pigs from various sources was always associated with the possession of the PLL gene (4). The inseparability of the PLL gene and the genes coding for major strain 2 histocompatibility specificities in these random-bred guinea pigs, among whom detectable "crossing-over" would have been expected after the many years of random breeding, may be considered strong evidence for a single genetic locus controlling both characteristics.

In the present study we report on the relationship between immune responsiveness to GT and the expression of strain 13 histocompatibility specificities among random-bred guinea pigs. We demonstrate the presence of two distinct major histocompatibility specificities in these animals detected by strain 2 anti-strain 13 isoantisera. One of these specificities is linked to the GT gene and the other is not.

Materials and Methods

Animals.—Hartley guinea pigs, an albino random-bred strain, and NIH multipurpose guinea pigs, a pigmented random-bred strain, were obtained from the Animal Production Unit, National Institutes of Health, Bethesda, Md. All animals used in this study weighed between 250 and 350 g when immunized.

Reagents.—Poly- α -(L-glutamic acid [50%], L-tyrosine [50%]), GT, mol wt 14,500, was obtained from Miles Laboratories, Inc., Elkhart, Ind. Complete Freund's adjuvant containing 0.5 μ g/ml *Mycobacterium butyricum* was obtained from Difco Laboratories, Detroit, Mich. ^{51}Cr , 185 $\mu\text{Ci}/\mu\text{g}$, was obtained from Amersham-Searle, Chicago, Ill.

Determination of Responder Status.—A solution of GT in saline was emulsified with an equal volume of complete Freund's adjuvant. The concentration was adjusted so that each guinea pig received 500 μ g of GT in 0.4 ml of emulsion distributed evenly in the four footpads. 3 wk after immunization, the animals were tested with 50 μ g of GT in 0.1 ml saline injected intradermally. The skin tests, examined 24 hr later, were considered positive if the erythema and induration significantly exceeded the largest nonspecific irritation observed in non-immunized controls. There were no equivocal reactions. Those animals demonstrating positive skin tests were considered responders. This correlated perfectly with their capacity to form anti-GT antibodies demonstrable by the Farr-type assay described previously (1).

Preparation of Antihistocompatibility Antisera.—Strain 2 anti-strain 13 antisera were produced as described previously (3). Briefly, strain 2 guinea pigs were immunized with homogenates of lymph node and spleen cells from strain 13 animals emulsified in complete Freund's adjuvant. Subsequently the animals were boosted every 2 wk with intradermal injections of homogenates of strain 13 lymphoid cells emulsified with incomplete adjuvant. The sera used in these studies were obtained 8-10 wk after the initial immunization.

The sera were heat inactivated at 56°C for 30 min. For absorption studies, the heat-inactivated sera were diluted fivefold and mixed with spleen and lymph node cells obtained from the appropriate animals, in a ratio of 3 ml diluted serum to 1 ml packed cells. The mixture was incubated at 37°C for 30 min after which the cells were removed by centrifugation.

This absorption procedure was repeated until the antiserum was no longer cytotoxic to an aliquot of the cell population used for the absorption.

⁵¹Cr Cytotoxicity Test.—Cells from the popliteal or axillary lymph nodes of each animal were labeled with ⁵¹Cr as described previously (4) and tested for susceptibility to cytolysis with the strain 2 anti-strain 13 antisera in the presence of complement. One million ⁵¹Cr-labeled lymph node cells were mixed with a 1/15 final dilution of the antiserum and a 1/6 final dilution of lyophilized guinea pig serum as a source of complement in a total volume of 0.3 ml of L-15 medium containing 10% fetal calf serum. After incubation of the mixture at 37°C for 60 min, 0.7 ml of the cold medium was added to each sample and the tubes centrifuged at 2500 rpm for 10 min in the cold. The radioactivity in 0.5 ml of the supernatant was counted and compared with the radioactivity present in an equal volume of supernatants from samples containing cells incubated in medium with complement only, and with that present in tubes containing cells frozen and thawed four times. The percentage of maximum ⁵¹Cr released

TABLE I
Linkage between the GT Gene and the Locus Coding for One of the Major Strain 13 Histocompatibility Specificities in Hartley Guinea Pigs

GT status	No. of animals	Per cent release ⁵¹ Cr*	
		Unabsorbed serum	Absorbed serum
		%	
Responder	25	56.1‡ (42.8–75.7)	43.3 (27.8–60.9)
Nonresponder type I	8	0.84 (0–2.6)	0 (0–0)
Nonresponder type II	4	35.8 (18.5–43.0)	2.4 (0–5.8)

* The per cent ⁵¹Cr released by a 1:5 dilution of a strain 2 anti-strain 13 isoantiserum (unabsorbed serum) or by the same serum absorbed with spleen and lymph node cells from one of the type II GT nonresponders. Details of the absorption procedure and the assay are presented in Materials and Methods.

‡ Value expressed is the mean. The range is in parentheses.

specifically from the cells of each animal was calculated as follows:

Per cent release =

$$100 \times \left(\frac{\text{cpm released by antiserum} - \text{cpm released in absence of antiserum}}{\text{cpm released by frozen-thawed cells} - \text{cpm released in absence of antiserum}} \right)$$

RESULTS

When lymph node cells from Hartley guinea pigs were tested for the presence of strain 13 histocompatibility specificities, the cells from all GT responders were lysed by strain 2 anti-strain 13 antisera (Table I). Two-thirds of the GT nonresponders, designated as “type I,” did not have detectable strain 13 histocompatibility antigens on their cells. However, the cells from 4 of 12 Hartley GT nonresponders, designated as “type II,” were lysed by the anti-strain 13

antiserum. The mean per cent ^{51}Cr released from the cells of the four type II GT nonresponders was only 35.8% compared to a mean of 56.1% ^{51}Cr released from the cells of GT responders, indicating that these type II nonresponder guinea pigs lacked some strain 13 specificity present in GT responders. Absorption of the anti-strain 13 antiserum with cells obtained from a type II GT nonresponder animal rendered the antiserum noncytotoxic to cells obtained from all GT nonresponders. Thus, the mean per cent ^{51}Cr released by the antiserum from cells of the four type II nonresponders expressing some strain 13 histocompatibility specificity decreased from 35.8 to 2.4% after the absorption. However, the absorbed antiserum retained its specific cytolytic activity against cells from all GT responder animals. The mean per cent ^{51}Cr released from the cells of GT responders decreased slightly from 56.1 to 43.3%. Thus there are

TABLE II
Linkage between the GT Gene and the Locus Coding for One of the Major Strain 13 Histocompatibility Specificities in NIH Multipurpose Guinea Pigs

GT status	No. of animals	Per cent release $^{51}\text{Cr}^*$	
		Unabsorbed serum	Absorbed serum
		%	
Responder	11	46.9‡ (43.1-54.8)	28.1 (18.0-37.3)
Nonresponder	5	42.8 (39.8-48.1)	3.2 (0-7.2)

* The per cent ^{51}Cr released by a 1:5 dilution of a strain 2 anti-strain 13 isoantiserum (unabsorbed serum) or by a 1:5 dilution of the same serum absorbed with spleen and lymph node cells obtained from GT nonresponders as described in Materials and Methods.

‡ Value expressed is the mean. The range is in parentheses.

at least two major histocompatibility specificities expressed on cells of Hartley guinea pigs that are recognized by the strain 2 anti-strain 13 antiserum. The genetic locus controlling the expression of only one of the strain 13 histocompatibility specificities is linked to the GT gene, while the other is not.

The results of a similar experiment using NIH multipurpose guinea pigs were a little different. The lymph node cells from all NIH multipurpose guinea pigs tested, both GT responders and nonresponders, were lysed by strain 2 anti-strain 13 isoantisera in the presence of complement (Table II). There was no significant difference in the percentage release of ^{51}Cr between the responders and nonresponders. Absorption of the antiserum with spleen and lymph node cells obtained from GT nonresponder animals rendered it noncytotoxic to cells from all other GT nonresponders. Thus, absorption of the anti-strain 13 antiserum decreased the mean per cent ^{51}Cr that it released from cells of GT nonresponder guinea pigs from 42.8 to 3.2%. However, the absorbed antiserum

retained its cytolytic activity against cells obtained from all GT responder animals, although the per cent ^{51}Cr released was decreased. Absorption of the serum caused the mean per cent ^{51}Cr released to drop from 46.9 to 28.1%. Absorption of the anti-strain 13 antiserum with spleen and lymph node cells from GT responder animals renders the antiserum noncytotoxic to cells from both GT responders and nonresponders as well as to cells from strain 13 guinea pigs. Thus, it appears that on the cells of NIH multipurpose guinea pigs at least two histocompatibility specificities are expressed that are recognized by the strain 2 anti-strain 13 antiserum. As in random-bred Hartley guinea pigs, the gene coding for one of those specificities is linked to the GT gene.

DISCUSSION

Using two isoantisera prepared in inbred strain 2 guinea pigs directed against the histocompatibility antigens present on the cells of strain 13 guinea pigs, we have demonstrated that at least two different histocompatibility specificities that are identical with or highly cross-reactive with major strain 13 antigens are expressed on the cells of random-bred guinea pigs. Both anti-13 isoantisera behaved identically and detected both specificities. One of those specificities is found only on cells of GT responder guinea pig. Thus, the genetic locus controlling the expression of that specificity could not be dissociated from the GT gene. Among the 53 random-bred animals tested, no animals were found in which the two genes did not occur together. The relationship between the GT gene and the strain 13 histocompatibility locus to which it is linked, like the relationship between the PLL gene and the strain 2 histocompatibility locus to which it is linked (4), is very intimate. The absolute association of immune responsiveness with a particular histocompatibility specificity in both the GT and PLL systems strongly suggests that a single genetic locus controls the expression of both characteristics.

The expression of each of the strain 13 histocompatibility specificities is most probably controlled by separate genetic loci. However, from our study of the backcross progeny from the mating of GT responder (2×13) F_1 guinea pigs with nonresponder strain 2 animals, we know that all the strain 13 histocompatibility specificities detectable by strain 2 isoantisera are inherited together with GT responsiveness (2). The cells of the GT nonresponder backcross animals were not susceptible to cytolysis by the strain 2 anti-strain 13 antisera used in this study. Thus it would appear that in the guinea pig as in man and in the mouse there are two separate genetic subloci controlling the expression of major histocompatibility specificities which are both located on the same chromosome. This interpretation is given strong support by the finding that in some random-bred Hartley and NIH multipurpose guinea pigs, only one of the two strain 13 specificities is detected. This situation which is not infrequent could, by analogy with the major histocompatibility loci of mouse

and man, have resulted from crossing over between the two postulated guinea pig subloci. However, our data do not rule out the possibility that the two specificities recognized by the anti-strain 13 antisera are directed at different antigenic determinants on the same molecule. A single genetic locus controlling both GT responsiveness and the expression of strain 13 histocompatibility specificities could conceivably have mutated so that the expression of GT responsiveness and of only one of the histocompatibility specificities is lost. However, this is the less likely of the two possibilities.

The two species in which the genetics of histocompatibility specificities has been most extensively studied has been the human and the mouse. In both the *HL-A* system of man and the *H-2* system of mice, there are two major histocompatibility subloci located on the same chromosome. In the mouse the immune response genes at the *Ir-1* locus have been mapped within the *K* region of the *H-2* locus, and the possibility has not yet been ruled out that they are identical with factors controlling individual specificities in this region.² The demonstration in strain 13 guinea pigs of two major histocompatibility specificities whose controlling genes are on the same chromosome indicates that the genetics of histocompatibility antigens in guinea pigs may be analogous to the *HL-A* and *H-2* systems. Furthermore, the finding that the GT gene is associated with only one of these specificities is very analogous to the demonstrated association of *Ir-1* with the *K* region of the *H-2* locus, illustrating another characteristic shared by guinea pig and mouse histocompatibly linked immune response genes.

SUMMARY

The lymph node cells from all L-glutamic acid and L-tyrosine (GT) responder random-bred guinea pigs were susceptible to lysis by strain 2 anti-strain 13 isoantisera in the presence of complement. These same antisera were cytolytic for lymph node cells of only some of the GT nonresponder animals. However, after absorption with cells, from a nonresponder guinea pig, susceptible to lysis, the anti-strain 13 antisera were no longer able to lyse cells from any GT nonresponder guinea pigs while retaining a large measure of their cytolytic activity for cells of all GT responder guinea pigs. Thus, at least two major strain 13 histocompatibility specificities are expressed on the cells of random-bred guinea pigs. The genetic locus controlling the expression of only one of those strain 13 histocompatibility specificities is linked to the GT immune response gene.

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