

HISTOCOMPATIBILITY TYPE AND IMMUNE RESPONSIVENESS IN RANDOM BRED HARTLEY STRAIN GUINEA PIGS

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The ability of guinea pigs to respond immunologically to poly-L-lysine (PLL),¹ to a random copolymer of L-glutamic acid and L-lysine (GL), and to hapten conjugates of these materials is largely determined by the presence of a single autosomal dominant "immune response gene," designated the PLL gene. Inbred strain 2 guinea pigs are homozygous for the PLL gene, whereas inbred strain 13 guinea pigs lack the gene. A varying proportion of commercially available random bred Hartley strain guinea pigs possess the PLL gene. Animals homozygous or heterozygous for the PLL gene are termed "responders" and animals lacking the gene, "nonresponders," with respect to immunization with either PLL or GL hapten conjugates (1-4).

In a recent study (2 × 13)F₁ responder guinea pigs were backcrossed with strain 13 nonresponder animals and the progeny tested for both their inheritance of the PLL gene and the genes determining the expression of major strain 2 histocompatibility antigens. A cytotoxic strain 13 antistrain 2 isoantiserum capable of causing significant lysis of ⁵¹Cr-labeled (2 × 13)F₁ lymphocytes was employed in this study. All nine backcross guinea pigs inheriting the PLL gene from the (2 × 13)F₁ parent were shown to have also inherited the strain 2 histocompatibility antigens recognized by the isoantiserum. These histocompatibility antigens were lacking in eight backcross progeny not possessing the PLL gene. Further, in mixed lymphocyte reactions, only lymphocytes from PLL responder backcross guinea pigs were capable of stimulating DNA synthesis in strain 13 lymphocytes (5). These findings indicated that the PLL gene was "linked" to a locus controlling a major histocompatibility antigen. They paralleled the earlier observation of McDevitt and Chinitz that in mice, the "specific immune response locus," IR-1, is linked to the H₂ histocompatibility locus (6).

These results are equally consistent with the notion that, rather than just being linked to, the immune response genes are in fact identical with genes coding for certain histocompatibility antigens (7). If this were so, outbred animals possessing a particular immune response gene should express a common histocompatibility antigen. Further, this particular antigen should not be present on tissues of nonresponder animals. Thus, in guinea pigs, cells from all Hartley strain responder animals should have a histo-

¹ Abbreviations used in this paper: PLL, poly-L-lysine; GL, random copolymer of L-glutamic acid and L-lysine; DNP-PLL, 2,4-dinitrophenyl-PLL. NIH, National Institutes of Health.

compatibility antigen in common with strain 2 animals and this antigen should be absent from cells of all nonresponder guinea pigs. The following experiments were performed to investigate this possibility.

Materials and Methods

Experimental Animals.—55 Hartley strain guinea pigs were obtained from either the National Institutes of Health (NIH) Animal Production Unit (33 animals) or CAMM Animal Research Laboratory, Wayne, N. J. (22 animals). 10 NIH Multipurpose and 5 Abyssinian strain guinea pigs (gift of Dr. J. Battisto) were also included in this study. Strains 2 and 13 guinea pigs were obtained from the NIH Animal Production Unit; these animals were mated to produce (2×13)F₁ hybrid animals.

Materials.—A poly-L-lysine HBr preparation with 110,000 avg mol wt was purchased from Pilot Chemicals, Inc., Watertown, Mass. The PLL was reacted with 2,4-dinitrofluorobenzene as described (1) and 2,4-dinitrophenyl-PLL (DNP₁₂ PLL) was prepared. The subscript refers to the number of DNP groups per molecule. Complete Freund's adjuvant was obtained from Difco Laboratories, Detroit, Mich. and contained 0.5 mg/ml *Micrococcus butyricum*. ⁵¹Cr, 185 μCi/μg, was obtained from Amersham-Searle, Chicago, Ill. Fetal calf serum was purchased from North American Biologicals, Inc., Miami, Fla. Lyophilized guinea pig serum, as a source of complement, was purchased from Baltimore Biological Laboratories, Cockeysville, Md. Goat anti-rabbit gamma globulin was obtained from Dr. Rose Mage and was conjugated with fluorescein isothiocyanate as previously described (8).

Identification of Guinea Pigs Possessing the PLL Gene.—All guinea pigs were tested for the presence of the PLL gene by their response to immunization with DNP-PLL as described elsewhere (1). 28 Hartley animals responded to this immunization with both delayed hypersensitivity and the production of high amounts of anti-DNP antibody; they were thus shown to possess the PLL gene. The remaining 27 Hartley strain guinea pigs and all the NIH Multipurpose and Abyssinian strain animals tested failed to make such a response.

Preparation of Isoantiserum against Histocompatibility Antigens of Strain 2 and Strain 13 Guinea Pigs.—Lung, liver, spleen, and lymph node tissues from strain 2 guinea pigs were gently ground in a glass hand homogenizer with twice their volume of saline. The tissue suspension was emulsified with complete Freund's adjuvant and a strain 13 guinea pig was immunized with 0.2 ml of the emulsion in each footpad. The animal was then injected every wk for 10 wk with 1 ml of the strain 2 tissue mixture in saline, divided into five intradermal doses. The guinea pig was bled weekly for a month and these sera pooled (batch A). The animal was bled again 1 wk later (batch B).

A Hartley guinea pig was similarly immunized with strain 13 mixed-tissue homogenates to produce an anti-strain 13 isoantiserum. The antiserum was absorbed with strain 2 lymphocytes to remove antibodies cross-reactive with strain 2 histocompatibility antigens.

Detection of Strain 2 Histocompatibility Antigens by ⁵¹Cr Cytotoxicity Test.—Inguinal and popliteal lymph nodes were removed from the guinea pigs and gently teased over a 80 gauge mesh stainless steel sieve to yield single cell suspensions. The medium used throughout was Hanks' balanced salt solution supplemented with 10% fetal calf serum. The cells were washed once and resuspended to a concentration of 5×10^7 cells per ml. 50 μCi of ⁵¹Cr per ml were added to each of the cell suspensions and the mixtures were incubated at 37°C for 30 min. The cells were then washed four times at 4°C and finally resuspended to a concentration of 10^7 cells per ml. A volume of 0.1 ml of cell suspension was added to 0.1 ml of diluted isoantiserum. Unless otherwise stated the dilution of the isoantiserum was 1:5. After 15 min at room temperature, 0.1 ml of a 1:2 dilution of reconstituted lyophilized guinea pig serum, pH 7.2, (complement source) was added to each tube. The mixture was left at room temperature for a

further 2 hr and 2 ml of medium were then added. The tubes were centrifuged and a 0.2 ml sample was measured and compared with the radioactivity present in an equal volume of the supernatants from tubes containing cells incubated in medium plus complement only, and with that present in tubes containing cells which were frozen and thawed four times. The percentage of maximum ^{51}Cr released specifically from the lymphocytes of each guinea pig was calculated as follows:

$$\frac{\text{Radioactivity released by antiserum dilution} - \text{Radioactivity released in absence of antiserum}}{\text{Radioactivity released by frozen-thawed cells} - \text{Radioactivity released in absence of antiserum}} \times 100$$

Each cell suspension was tested in quadruplicate.

TABLE I
Percentage of Maximum ^{51}Cr Release from Guinea Pig Lymphocytes Exposed to a 1:5 Dilution of Strain 13 Anti-Strain 2 Isoantiserum in the Presence of Complement

Batch of isoantiserum	PLL responder				PLL nonresponder					
	$(2 \times 13)\text{F}_1$		Hartley*		Hartley*		NIH Multipurpose		Abyssinian	
	No. of animals	% \pm SE max. rel.	No. of animals	% \pm SE max. rel.	No. of animals	% max. rel.	No. of animals	% max. rel.	No. of animals	% max. rel.
A	5	54.6 ± 4.1	5	48.0 ± 2.9	8	0.6				
B	4	24.2 ± 3.6	23	14.7 ± 1.2	19	0.0	10	0.3	5	0.0
Total No. of Animals and mean % max. rel.	9	41.1	28	20.7	27	0.2	10	0.3	5	0.0

* 18 responder and 14 nonresponder Hartley strain animals were obtained from NIH, while 10 responder and 13 nonresponder animals were obtained from CAMM Animal Research Laboratory.

Strain 2 Hepatoma Cell Preparation.—Strain 2 hepatoma cells, propagated in the ascites form, were obtained through the courtesy of Dr. Stanley Wepsic, National Institutes of Health, Bethesda, Md. The origin and properties of these cells have been described previously (9).

Determination of the Percentage of Lymphocyte Contamination within the Hepatoma Cell Preparation.—The hepatoma cell preparation used in the absorption study was washed twice with Eagle's medium containing 1% normal rabbit serum. A guinea pig lymph node cell suspension was also prepared. Both cell types were smeared onto glass slides, fixed in 95% alcohol, and air-dried. The slides were washed in phosphate buffered saline and exposed to a 1:50 dilution of rabbit anti-guinea pig lymphocyte serum, the preparation and characteristics of which have been described previously (10). The slides were left at room temperature for 30 min, washed, and exposed to fluoresceinated goat anti-rabbit globulin solution for a further 30 min. The slides were washed and examined under a Zeiss fluorescence microscope. 2000

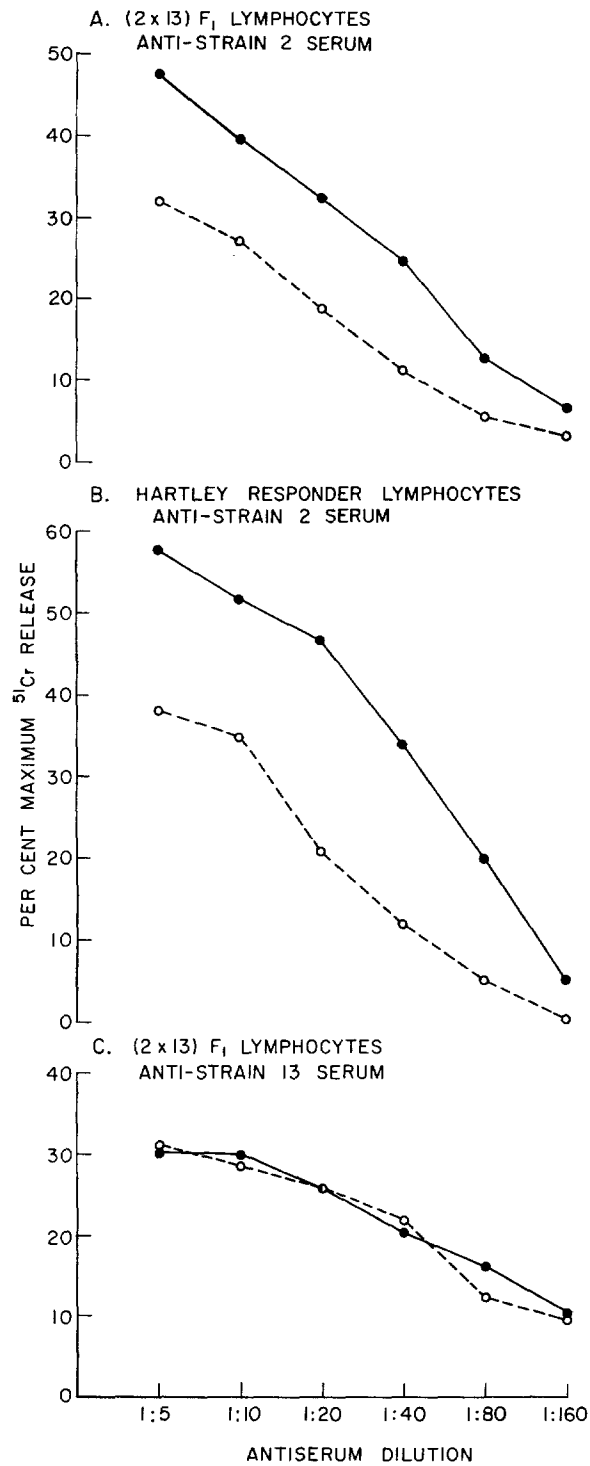


FIG. 1.

cells in both the hepatoma ascites fluid smear and normal lymph node cell smears were examined. The percentages of cells showing bright fluorescence at their periphery were determined. To vindicate this technique, known numbers of lymphocytes were added to the hepatoma cell preparation. The added lymphocytes could be detected with a high degree of accuracy within the hepatoma cell preparation.

RESULTS

Lymph node lymphocytes from PLL responder and nonresponder guinea pigs were tested for the presence of the histocompatibility antigen(s) recognized by strain 13 anti-strain 2 isoantiserum using the ^{51}Cr release cytotoxicity assay method (Table I). Significant ^{51}Cr release was observed from lymphocytes of all animals possessing the PLL gene, whereas no significant ^{51}Cr -release occurred from lymphocytes from nonresponder guinea pigs. The percentage of lysis observed with cells from responder Hartley strain animals was slightly less than that achieved when the same dilution of the isoantisera were tested against lymphocytes from $(2 \times 13)\text{F}_1$ animals.

The question must be considered whether the antigen(s) common to Hartley responder and strain 2 guinea pigs, detected by the isoantisera used in this study, was (were) restricted to lymphocytes or represented a general tissue histocompatibility antigen. We therefore investigated if the antigen was present on nonlymphoid cells. It was not detected on erythrocytes since strain 2 erythrocytes were not agglutinated by the isoantiserum. It was, however, apparently present on strain 2 ascites hepatoma cells. Thus when 0.1 ml of the isoantiserum (batch A) was incubated with 50×10^6 hepatoma cells for 60 min at 37°C , significant reduction in lytic activity against ^{51}Cr -labeled lymphocytes from both $(2 \times 13)\text{F}_1$ and Hartley responder animals occurred (Fig. 1 *a, b*). This absorption was specific for strain 2 antigen since comparable exposure of an anti-strain 13 isoantiserum to strain 2 hepatoma cells did not reduce its lytic activity against $(2 \times 13)\text{F}_1$ cells (Fig. 1 *c*). In further experiments, to determine whether the lytic activity could be completely absorbed from the anti-strain 2 isoantiserum, a 1:10 dilution of the isoantiserum was exposed to varying numbers of either strain 2 lymphocytes or strain 2 hepatoma cells for 60 min at 37°C . The remaining activity against $(2 \times 13)\text{F}_1$ cells was determined and compared with that of the unabsorbed sera. As shown in Fig. 2, complete absorption was achieved by either 9×10^7 hepatoma cells or 4.5×10^6 lymphocytes. The level of lymphocyte contamination in the hepatoma cell preparation was determined using a rabbit anti-guinea pig-lymphocytes serum followed by exposure to fluoresceinated goat anti-rabbit

FIG. 1. Percentage of maximum ^{51}Cr release achieved by varying dilutions of either anti-strain 2 or anti-strain 13 isoantiserum, absorbed (○-----○) or not absorbed (●——●) with 50×10^6 hepatoma cells, and tested against lymphocytes from either $(2 \times 13)\text{F}_1$ or Hartley responder lymphocytes.

globulin. The percentage of contamination was found to be 0.5% (Table II). Therefore contaminating lymphocytes could not explain the capacity of the hepatoma cell preparation to absorb anti-strain 2 histocompatibility activity from the isoantiserum.

DISCUSSION

The data presented demonstrate that an antiserum prepared in a strain 13 guinea pig against strain 2 histocompatibility antigens is able to detect the

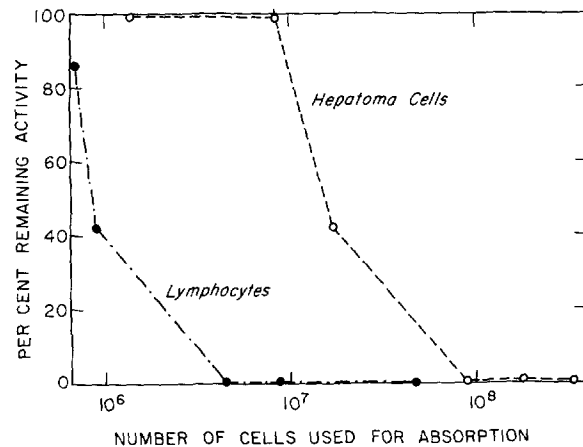


FIG. 2. Percentage of remaining lytic activity of anti-strain 2 isoantiserum absorbed with varying numbers of strain 2 lymphocytes or hepatoma cells.

TABLE II

Percentage of Cells Staining with Fluoresceinated Goat Anti-Rabbit Globulin Solution, Following Exposure to Rabbit Anti-Guinea Pig Lymphocyte Serum

Source of cells	Percentage of cells stained
Lymph node	89%
Hepatoma-induced ascites	0.5%

PLL responder guinea pigs in a population of random bred Hartley strain guinea pigs. Thus only lymphocytes from Hartley responder guinea pigs were lysed when exposed to a strain 13 anti-strain 2 isoantiserum. It can therefore be concluded that Hartley guinea pigs with the PLL gene possess a histocompatibility antigen which is either identical, or strongly cross-reacting with, a major strain 2 histocompatibility antigen. It is further concluded that although the antigen is found in a higher concentration on lymphocytes it is also present on hepatoma cells and therefore probably represents a general tissue histocompatibility antigen.

Although strain 2 and Hartley responder guinea pigs share at least one histocompatibility antigen they are not histocompatible. Thus an inoculum of 2×10^6 strain 2 leukemia cells (11) that was uniformly lethal for strain 2 and $(2 \times 13)F_1$ guinea pigs failed to kill Hartley responder guinea pigs.² Further, antisera cytotoxic to strain 2 lymphocytes could be raised in a Hartley responder recipient even though these animals share a common histocompatibility antigen. The anti-strain 2 isoantisera raised in Hartley responder guinea pigs showed considerable cross-reaction against strain 13 lymphocytes.² These findings were expected since there has been no known relationship between strain 2 inbred guinea pigs and the outbred Hartley animals, whereas strain 2 and strain 13 are related in that a single male guinea pig was used to initiate both strains (footnote 3 and reference 12).

The persistent association between responder status and a histocompatibility antigen in both $([2 \times 13] \times 13)$ backcrossed and outbred Hartley strain guinea pigs, strongly suggests that a single genetic locus determines both characteristics. Thus, if two closely linked loci had accounted for the results observed with the backcrossed animals, one would have expected "crossing over" to have occasionally occurred during the random matings among Hartley strain animals over the many years these animals have been bred. No evidence of such a crossing over was observed in a sample of 55 Hartley animals obtained from two different breeders. Our findings therefore seem to implicate histocompatibility antigens as important determinants of immune responsiveness. The mechanism by which they convey immune responsiveness remains to be determined.

SUMMARY

Outbred Hartley strain guinea pigs capable of responding immunologically to 2,4-dinitrophenylated poly-L-lysine were shown to display a histocompatibility specificity in common with inbred strain 2 guinea pigs. This histocompatibility specificity was not detected in guinea pigs unable to respond immunologically to DNP-PLL. The result suggests that the poly-L-lysine specific immune response gene is very closely linked or even identical with a gene determining a major histocompatibility antigen in guinea pigs.

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