Proceedings of the National Academy of Sciences
Vol. 66, No. 4, pp. 1267–1274, August 1970

Genetic Control of the Immune Response of Guinea Pigs to Limiting Doses of Bovine Serum Albumin: Relationship to the Poly-L-Lysine Gene

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Communicated by Gerald M. Edelman, May 18, 1970

Abstract. The response of strain 2 guinea pigs to limiting doses of bovine serum albumin is under dominant genetic control linked with the poly-L-lysine gene. Inbred strains 2 and 13 guinea pigs make similar antibody responses to 10 μg bovine serum albumin, whereas strain 2 but not strain 13 animals produce significant amounts of antibodies in response to 0.1 μg. The relationship between the presence of the poly-L-lysine gene and the ability to respond to limiting doses of bovine serum albumin has also been investigated in random-bred Hartley strain guinea pigs.

In recent years immune responsiveness to selected synthetic polymers and copolymers of α-amino acids[1–6] and to some native proteins [7–9] has been shown to be controlled largely by individual autosomal dominant genes, referred to as "specific immune response genes."[6] The immune response of guinea pigs to several simple synthetic polyamino acid antigens (poly-L-lysine,[1] poly-L-arginine[9]), to a copolymer of L-glutamic acid and L-lysine, and to hapten conjugates of these polypeptides is dependent upon the presence of an autosomal dominant gene which is referred to as the poly-L-lysine (PLL)† gene.[2] The PLL gene is found in all inbred strain 2 guinea pigs as well as in a varying percentage of random-bred Hartley strain guinea pigs (responders) and is lacking in all inbred strain 13 guinea pigs (nonresponders). Hybrid (2 × 13)F_1 guinea pigs are all responders and are heteroxygous for the gene. In mice, the immune response to a series of branched synthetic polypeptides containing random sequences of L-glutamic acid and L-tyrosine, or L-histidine, or L-phenylalanine, on a backbone of D,L-alanine and L-lysine [(T,G)-A-L, (H-G)-A-L, (Phe,G)-A-L] has been shown to be largely determined by single dominant alleles at a single locus referred to as Ir-1 or by three closely linked genes.[6,10] Similarly, the immune response of mice to a random copolymer of L-glutamic acid and L-lysine containing 5% L-alanine is under the control of another dominant gene.[4]

Whereas specific immune response genes in mice have been shown not to be related to structural genes controlling allotypic markers of immunoglobulins,[6] a most interesting observation has been made concerning the relationship of these genes with loci controlling major transplantation antigens in mice and guinea pigs: (1) immune response genes at the Ir-1 locus in mice have been shown

to be closely linked with H^2 genotypes[11] and (2) the PLL gene was found to be similarly linked to the major locus controlling transplantation antigens in strain 2 guinea pigs.[12]

Identical genetic differences to those observed with synthetic polypeptides can be noted in the ability of inbred strains and individual random-bred animals to form immune responses to certain native proteins and their hapten conjugates, provided limiting doses of these antigens are used for immunization. Thus, Vaz and Levine[13] recently reported H^2-linked strain differences in the ability of mice to respond to very low doses of hapten protein conjugates; these differences disappeared when higher immunizing doses were used.

The present report demonstrates similar genetic differences in the immune responses of inbred strains 2 and 13 guinea pigs to limiting doses of a native protein antigen, bovine serum albumin (BSA), and establishes that immune responsiveness to low doses of BSA is linked with the presence of the PLL gene and therefore with the histocompatibility genotype of strain 2 guinea pigs. In random-bred Hartley animals, however, the situation is more complex: all PLL responder guinea pigs develop significant serum levels of antibodies in response to low immunizing doses of BSA, whereas PLL nonresponder guinea pigs can be divided into two distinct populations in this respect, both of which produce lower amounts of anti-BSA antibodies than PLL responder animals.

Materials and Methods. Experimental animals: Adult inbred strains 2 and 13 guinea pigs were obtained from the NIH Animal Production Section. They were mated to produce $(2 \times 13)F_1$ animals. Male F_1 guinea pigs were in turn mated with female strain 13 guinea pigs to produce $(2 \times 13)F_1 \times 13$ backcross offspring. Random-bred Hartley strain guinea pigs were obtained from the NIH Animal Production Section or were purchased from Camm Research, Wayne, N.J.

Materials: A poly-L-lysine HBr preparation with 110,000 average M.W. was purchased from Pilot Chemicals, Watertown, Mass. Bovine serum albumin was obtained from Armour Pharmaceutical, Kankekee, Ill. Keyhole limpet hemocyanin (KLH) was purchased from Pacific Biomarine Supply Co., Venice, Calif. Porcine insulin and ^3H porcine insulin were gifts from Dr. Pedro Cuatrecasas, NIH. Carrier-free ^{125}I was purchased from New England Nuclear, Boston, Mass. 1-fluoro-2,4-dinitrobenzene (DNFB) was purchased from Eastman Organic Chemical Co., Rochester, N.Y. ^3H-DNFB (1.67 Ci/mmol) was obtained from Nuclear-Chicago, Chicago, Ill. Complete Freund's adjuvant (CFA) was obtained from Difco Laboratories Inc., Detroit, Mich., and contained 0.5 mg/ml *M. butyricum*.

Preparation of conjugates: DNP_{32}-PLL and DNP_6-KLH were prepared by reacting PLL and KLH with 2,4-dinitrofluorobenzene under alkaline conditions as described previously.[14] The subscripts refer to the average number of DNP groups per molecule of PLL and per 100,000 mol wt units of KLH, respectively. ^3H-DNP-EACA was prepared by the reaction of ϵ-aminocaproic acid (EACA) with ^3H-DNFB.[15] ^{125}I BSA was prepared as described in ref. 16.

Immunization: Guinea pigs were injected in the four foot pads with 0.2 ml of the antigen solutions in 0.15 M NaCl and 1% normal guinea pig serum, emulsified with an equal volume of Freund's adjuvant. The doses of antigen injected are shown in the respective tables and figures.

The animals were bled from the retro-orbital plexus 3–4 weeks after immunization. Delayed hypersensitivity reactions were evaluated 24 hr after the intradermal injection of 10 μg of DNP-PLL in 0.1 ml of 0.15 M NaCl.

Assay of the antibody response: The serum concentrations of anti-BSA, anti-insulin, and anti-DNP antibodies were assayed by the Farr technique[17] in which the

binding of ^{125}I BSA, ^{3}H insulin, and ^{3}H-DNP-EACA respectively to the globulin fraction of the antisera was measured. For the anti-BSA and anti-DNP antibody assays, the globulin fraction was precipitated by 45% saturated $(NH_4)_2 SO_4$.[18] A sheep anti-guinea pig gamma globulin antibody was used to precipitate antibody-bound porcine insulin in the antiinsulin antibody assay.

The data are expressed as the percentage of binding of the radioactive ligands by the undiluted antisera and by $^1/_{10}$ or $^1/_{100}$ dilutions of the antisera.

PLL gene status of Hartley and (2 × 13)F_1 × 13 animals: To determine the PLL gene status of animals after their response to BSA and insulin, the animals were immunized intradermally with 0.1 mg DNP-PLL in CFA and tested 14 days later for delayed hypersensitivity to DNP-PLL. In addition, they were bled and the anti-DNP antibody concentration of their sera was determined as described above.[18]

Results. (1) The response of inbred strains of guinea pigs to BSA, DNP-KLH, and porcine insulin: The antibody responses of strains 2 and 13 guinea pigs 3–4 weeks after immunization with doses of BSA ranging from 100 to 0.1 μg are presented in Figure 1. The responses of the two inbred strains to 100 μg BSA appear very similar and to 10 μg BSA are identical. In contrast, when limiting doses of BSA are used, marked differences are observed. Strain 2 guinea pigs immunized with 1 or 0.1 μg BSA produced significant amounts of antibody, whereas strain 13 guinea pigs immunized with the same doses showed either very low levels of anti-BSA antibodies or no detectable response.

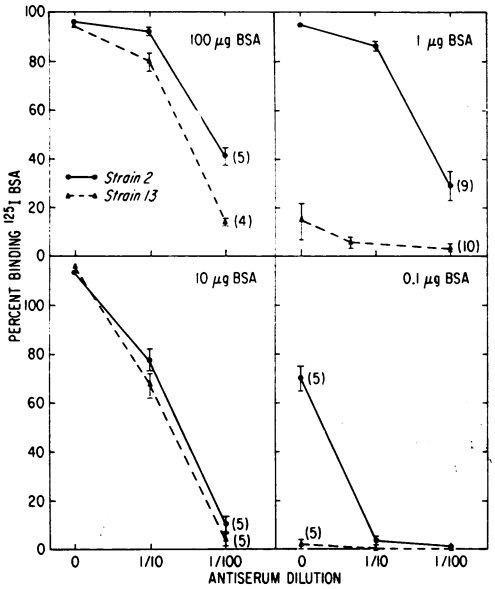

FIG. 1.—Anti-BSA antibodies in sera from strain 2 and strain 13 guinea pigs 3–4 weeks following immunization with various doses of BSA in CFA. Data are expressed as percentage of binding of 0.7 μg ^{125}I BSA by globulin fraction of 0.1 ml of antiserum dilutions. Number of animals per group is shown in parentheses; mean values and standard errors are presented.

The binding values for undiluted sera as shown after immunization with 10 μg BSA for strain 2 and 13 animals are represented incorrectly. The actual binding values are 93.4 and 94.4% respectively.

The genetic control of immune responsiveness of inbred guinea pigs to low doses of BSA was then investigated. The response to 0.1 μg BSA of the backcross offspring from the mating of (2 × 13)F_1 with strain 13 guinea pigs was evaluated and related to their inheritance of the PLL gene from their F_1 parent (Table 1). All four PLL positive animals made large amounts of anti-BSA antibodies. The three backcross animals which did not develop significant serum titers of anti-BSA antibodies lacked the PLL gene.

TABLE 1. *Anti-BSA antibody response in (2 × 13)F_1 × 13 backcross guinea pigs immunized with 0.1 γ BSA in CFA.**

Guinea pig no.	Serum Dilution 0	1:10	1:100	PLL gene status
1	94.7*	70.6	—†	+
2	96.3	82.5	14.1	+
3	93.2	42.4	4.4	+
4	94.5	57.6	—	+
5	—	—	—	−
6	—	—	—	−
7	8.5	—	—	−

* Serum anti-BSA antibody is expressed as % binding of 0.7 μg ^{125}I BSA by globulin fraction of 0.1 ml of antiserum dilutions.
† Indicates no significant binding.

To ascertain whether the observed genetic differences of strains 2 and 13 guinea pigs in their responses to limiting doses of BSA could be generalized to other protein antigens, a limited study was made of their responses to graded doses of a strong immunogen, DNP-KLH (Table 2) or of a weak antigen, porcine insulin (Table 3). The anti-DNP antibody responses of strains 2 and 13 guinea

TABLE 2. *Anti-DNP antibody response in strain 2 and strain 13 guinea pigs immunized with DNP-KLH in CFA.**

Immunizing antigen	Guinea pig no.	Strain 2 Serum Dilution 0	1:10	1:100	Guinea pig no.	Strain 13 Serum Dilution 0	1:10	1:100
μg	1	97.9*	99.3	78.4	1	90.0	72.0	47.6
10	2	97.9	95.0	82.4	2	90.7	82.3	75.4
					3	90.4	84.4	67.6
1	3	87.6	68.4	25.9	4	52.5	40.2	7.5
	4	85.7	62.0	22.3	5	56.9	37.3	—†
	5	90.0	77.2	30.6	6	36.2	17.5	—
	6	91.7	76.5	30.6				

* Serum anti-DNP antibody is expressed as percentage of binding of 0.1 ml 10^{-8} M ^{3}H-DNP-EACA by globulin fraction of 0.1 ml of antiserum dilutions.
† No significant binding.
0.1 μg DNP-KLH did not elicit significant responses in either strain 2 or strain 13 animals.

TABLE 3. *Antibody response of strain 2 and strain 13 guinea pigs immunized with various doses of porcine insulin in CFA.**

Immunizing antigen (μg)	Strain 2 Guinea pig no.	Binding ^{3}H insulin (%)	Strain 13 Guinea pig no.	Binding ^{3}H insulin (%)
100	1	29.3*	1	45.1
	2	16.0	2	53.2
	3	18.9	3	35.3
10	4	11.6	4	13.1
	5	11.9	5	14.5
	6	2.8	6	11.5
1	7	0	7	19
	8	8.3	8	19
	9	0	9	5.2
0.1	10	0	10	12.2
	11	13.7	11	7.8
	12	1.9	12	11.5

* Antiinsulin antibody is expressed as percentage of binding of 0.09 μg ^{3}H porcine insulin by γ-globulin fraction of 0.1 ml of antiserum. Binding value observed with normal serum ranged from 0 to 6%.

pigs immunized with 10 μg of DNP-KLH are not significantly different. However, when 1 μg of the conjugate is used, strain 2 antisera demonstrated greater binding for the DNP ligand than strain 13 antisera although the differences were not as marked as those observed in response to low doses of BSA (Fig. 1). The data in Table 3 demonstrate that strain 13 animals respond equally well if not better than strain 2 guinea pigs to porcine insulin.

(2) **The response of random-bred Hartley strain guinea pigs to low doses of BSA:** The data presented in section (1) have demonstrated genetically controlled differences in the response of inbred guinea pigs to low doses of BSA, and have shown that responsiveness in this dose range is associated with the presence of the PLL gene. As the PLL gene is also found in some but not all random-bred Hartley strain animals, it is relevant to inquire whether individual Hartley strain guinea pigs differ in their ability to respond to low doses of BSA and whether such differences are again related to the possession of the PLL gene.

Hartley strain guinea pigs were immunized with 1 or 0.1 μg of BSA; after their response had been evaluated, they were immunized with DNP-PLL and their PLL responder status was determined. The results of these experiments are presented in Figures 2 and 3. All Hartley strain PLL responder guinea pigs developed high antibody concentrations in their sera when immunized with 1 or 0.1 μg BSA. Hartley strain nonresponder guinea pigs distributed themselves into two distinct groups with respect to their ability to respond to limiting doses of BSA. This is clearly apparent when 0.1 μg BSA was used (Fig. 2). Type I PLL nonresponders are those which produced large amounts of anti-BSA antibody but significantly less as a group than PLL responder Hartley strain animals;

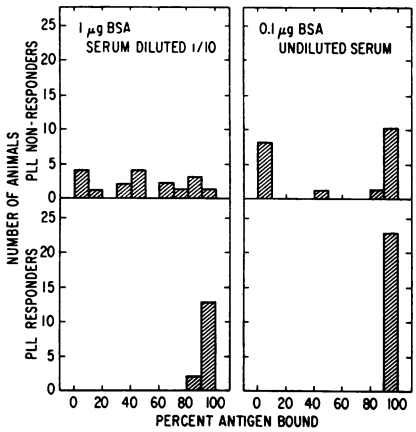

FIG. 2

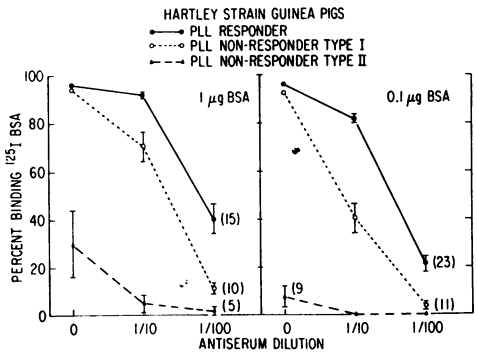

FIG. 3

FIGS. 2 and 3.—Relationship between the presence of the PLL gene in Hartley strain guinea pigs and their antibody response to limiting doses of BSA in CFA. Data are expressed as in Fig. 1. PLL responder guinea pigs all form large amounts of anti-BSA antibodies, whereas PLL nonresponder guinea pigs immunized with 0.1 μg BSA distribute themselves into two main groups: Type I with relatively high responses and Type II with very low anti-BSA antibody responses. The mean binding values and standard error for each of these groups are presented in Fig. 3.

Type II PLL nonresponders produced either very low or undetectable amounts of anti-BSA antibody just as strain 13 guinea pigs similarly immunized (Fig. 3).

Discussion. Differences in the antibody response to limiting doses of a native protein antigen, BSA, can be demonstrated between two inbred strains of guinea pigs; these differences disappear when higher immunizing doses are used. Responsiveness to low doses of BSA, a property of strain 2 but not of strain 13 guinea pigs, was shown to be inherited in the progeny of the mating of (2×13)-$F_1 \times 13$ backcross together with the PLL gene, and therefore with strain 2 histocompatibility genotype which is also linked to the PLL gene.

These observations raise several questions:

(1) The genetic difference in the ability to respond to relatively small amounts of BSA which distinguishes strain 2 from strain 13 animals may reflect their responses towards all foreign proteins or only towards some of these antigens. The studies of Vaz and Levine[13] have indeed demonstrated H^2-linked differences in the antibody response of inbred mouse strains to low amounts of several noncross-reacting hapten protein conjugates. However, our limited experience to date indicates that, while such differences in the responsiveness of strains 2 and 13 guinea pigs may extend to several noncross-reacting antigens, they are not equally observed with all immunogens. The anti-DNP responses of the two guinea pig inbred strains to DNP-KLH, in the low dose range, were not as markedly different as their antibody responses to BSA; the response to porcine insulin was similar in strains 2 and 13 guinea pigs.

(2) Are the genes which control, respectively, immune responsiveness to low doses of BSA in strain 2 guinea pigs, responsiveness to PLL antigens, and a major histocompatibility antigen in this strain identical or linked? An analysis of the response of random-bred Hartley strain guinea pigs to 0.1 μg BSA may clarify this issue. Random-bred Hartley strain PLL responder guinea pigs show the best response to limiting immunizing doses of BSA, whereas PLL nonresponder guinea pigs separate into two distinct populations, Type I responding well and Type II responding poorly or not at all. Moreover, experiments in progress in our laboratory by Drs. Martin and Ellman[19] have shown that an antiserum prepared in strain 13 guinea pigs against strain 2 tissues is cytotoxic for lymphocytes from Hartley strain PLL responder but not for lymphocytes from Hartley strain PLL nonresponder guinea pigs. The maintenance of linkage between these three genetic factors in random-bred animals where genetic recombination may occur is evidence that they are either identical or very closely linked.

(3) What is the nature of the process controlled by an autosomal dominant gene, linked to histocompatibility genotype, which permits an immune response to be made to small amounts of BSA? The interpretation of the differences in responsiveness to BSA would be considerably aided by an analysis of the precise specificity and affinity of the anti-BSA antibodies produced by strain 2, strain 13, and individual Hartley strain guinea pigs.

The data presented, however, indicate that there are at least three distinct pathways to initiate anti-BSA antibody responses in guinea pigs. The first one is exemplified by the ability of strain 13 guinea pigs to form normal amounts of anti-BSA antibodies when 10 or 100 μg BSA are injected. A second pathway is

illustrated by the response of PLL responder strain 2 and PLL positive Hartley strain guinea pigs to 0.1 μg of the antigen. A third one is demonstrated by the good response of Type I PLL nonresponder Hartley strain guinea pigs to low doses of BSA. Since the response of PLL gene positive guinea pigs to BSA is under genetic control linked to histocompatibility genotype, it is reasonable to consider that the pattern of responsiveness of the PLL nonresponder groups to this antigen may be susceptible to similar genetic analysis, and that strain 13 histocompatibility genotype could be associated with the poor response to limiting doses of BSA of Type II Hartley strain animals. These considerations further suggest that a limited number of genes are concerned with this early stage of immune recognition to even complex antigens.

The mechanism by which immune responses controlled by individual autosomal genes are induced to relatively simple polypeptide antigens or to more complex proteins such as BSA and the processes controlled by these genes are still matters for speculation; nevertheless, certain avenues of approach appear promising. As it seems extremely unlikely that specific immune response genes code for the structure of specific immunoglobulins, one must consider a hypothesis such as proposed recently by Jerne[20] for the generation of diversity in immunoglobulins by histocompatibility antigens, or alternatively postulate an additional level of cell-antigen interaction for the recognition of immunogenicity. The close linkage of immune response genes with histocompatibility genotypes should have considerable significance in this latter respect and suggests that if indeed both genetic factors are identical, histocompatibility antigens on classes of lymphocytes may affect the specific binding of antigens or the ability of these specific cells to be stimulated, since transfer experiments have established that lymphoid cells appear to be the cells where "immune response genes" are expressed.[21–23]

Note Added in Proof. Since this paper was submitted for publication five $(2 \times 13)F_1$ animals were immunized with 1 μg BSA. Their anti-BSA antibody response was indistinguishable from that observed in strain 2 animals. Also five additional $(2 \times 13)F_1 \times 13$ backcross animals were immunized with 1 μg BSA. Three of these five animals produced significant levels of anti-BSA antibodies and were PLL gene positive as judged by the response to active immunization with DNP-PLL. Two of these five animals failed to produce anti-BSA antibodies and were PLL gene negative.

We wish to thank Mrs. Clara Lee Horton for her valuable technical assistance.

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† Abbreviations used: PLL, poly-L-lysine; BSA, bovine serum albumin; KLH, keyhole limpet hemocyanin; DNFB, 1-fluoro-2,4-dinitrobenzene; CFA, complete Freund's adjuvant; DNP, dinitrophenyl; EACA, ε-aminocaproic acid.

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