STUDIES ON ARTIFICIAL ANTIGENS

III. The Genetic Control of the Immune Response to Hapten–Poly-L-Lysine Conjugates in Guinea Pigs*

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Previous studies on the genetic transmission of the capacity for an immune response have shown that there is a statistically significant relation between the abilities of parents and of offspring to respond to a given antigen (1–3). Mendelian genetic patterns were not observed, however, possibly because of the structural complexity of the immunizing antigens employed. Poly-L-α-amino acids and hapten conjugates of synthetic polypeptides are antigens of comparative structural simplicity. Such materials are accordingly suitable for studies of the genetic transmission of the capacity for immune responses.

In previous reports, it has been shown that only 10 to 40 per cent of random-bred Hartley guinea pigs have the capacity to respond immunologically to 2,4-dinitrophenyl (DNP) conjugates of poly-L-lysine (PLL), whereas 100 per cent of these guinea pigs can respond to DNP conjugates of homologous or foreign proteins (4, 5). Only those guinea pigs capable of responding immunologically to DNP-PLL could respond also to PLL conjugates of 3 other immunogenic haptens (5). These findings suggest that the capacity of an individual guinea pig to become hypersensitive to hapten-PLL conjugates, depends on its ability to properly metabolize the PLL carrier (5).

In the present work, breeding experiments were carried out in order to study the genetic transmission in guinea pigs of the capacity to become immunized by hapten-PLL conjugates. The results obtained indicate that this trait is transmitted genetically as a unigenic Mendelian dominant.

EXPERIMENTAL

Guinea Pigs.—Random-bred Hartley strain guinea pigs were obtained from Camm Research Inc., Black Oak Ridge, New Jersey. Strain 2 and strain 13 guinea pigs were obtained from the Laboratory of Immunology of the National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, through the courtesy of Dr. M. Brandriss.

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Antigens.—PLL_{316}·HBR with an average degree of polymerization of 316 lysine residues per molecule (from intrinsic viscosity measurements, manufacturer's analysis) was purchased from Pilot Laboratories, Waltham, Massachuset. DNP-PLL conjugates and benzylpenicilloyl-PLL conjugates (BPO-PLL) were prepared, purified, and analysed by methods given previously (5). The following preparations were used: DNP_{20}-PLL_{316}, DNP_{24}-PLL_{316}, and BPO_{24}-PLL_{316} (the subscripts refer to average numbers of haptenic groups per molecule of conjugate). DNP_{41}–bovine serum albumin (BSA) was prepared by methods given previously (6).

Immunization.—0.4 ml of an emulsion containing 100 μg of the conjugate (as PLL base) and 0.2 ml of complete Freunds adjuvant (Difco Laboratories, Inc., Detroit) was injected into its hind foot-pads.

Skin Tests.—0.1 ml of saline solutions containing 10 μg of the hapten-PLL conjugates was injected intradermally. The test sites were observed at 2 to 3 hours and at 24 hours. The immediate (Arthus) reactions were graded according to the severity of the edema and hemorrhage (6). The average diameter of the area of erythema and induration of the delayed reactions were recorded. In controls (guinea pigs injected with adjuvants alone), and in non-responding animals, the test dose of hapten-PLL conjugates gave a 4 to 6 mm area of pale edema at 2 to 3 hours, and a 3 to 7 mm area of pale induration without necrosis at 24 hours.

Serum Antibody Determinations.—Passive cutaneous anaphylaxis (PCA) was done by method of Ovary (7) using 250 μg of DNP_{41}-BSA as the challenging dose. Passive hemolysis of DNP_{41}-BSA–coated tanned sheep red blood cells (SRBC) was carried out on sera inactivated at 56°C for 1 hour and absorbed twice with SRBC (8).

Breeding Experiments.—67 guinea pigs were immunized with either BPO_{24}-PLL_{316}, or with DNP_{20}-PLL_{316}. There were 42 per cent responders. Breeding pairs of responders or of non-responders were set up. Offspring were immunized with DNP_{24}-PLL_{316} when they had reached weights of 300 to 400 gm, and their immune responses were observed.

TABLE I

Antigenicity of DNP_{24}-PLL_{316} in Offspring of Responder Parents and Non-Responder Parents

Parents	Offspring	
Responders* (8 breeding pairs)	Responders* 18; 82 per cent (6♂, 12♀)	Non-responders‡ 4; 18 per cent (1♂, 3♀)
Non-responders‡ (9 breeding pairs)	0	26 (14♂, 12♀)

* Responders refer to guinea pigs who showed an immune response to DNP-PLL, evidenced by skin reactivity to DNP_{24}-PLL_{316} and PCA or passive hemolysis with their sera with DNP_{41}-BSA.

‡ Non-responders are animals which were negative to all these 3 tests.

RESULTS

Antigenicity of DNP_{24}-PLL_{316} in Offspring of Responder Parents and Non-Responder Parents.—Table I shows that 82 per cent of the offspring of 8 breeding pairs of responder parents showed an immune response to DNP_{24}-PLL_{316}. Responders showed both Arthus and delayed allergic skin reactions to the

immunizing conjugates, and their sera contained both skin-sensitizing and hemolytic antibodies specific for the DNP haptenic group (Table II). None of 26 offspring of 9 breeding pairs of non-responder parents showed a demonstrable immune response. The animals showed negative skin reactions, and antihapten antibodies could not be demonstrated in their sera by the methods described above (Table II).

TABLE II

Immune Responses of Offspring from 2 Responder Breeding Pairs and 1 Non-Responder Breeding Pair

Families	Offspring	Skin tests		Serum tests	
		Arthus*	Delayed* reaction	PCA‡	Passive hemolysis§
III	1♀	Neg.	Neg.	Neg.‖	Neg.‖
	2♂	1+	17	1/1000	1/40
	3♀	1+	15	1/500	1/80
VIII	4♀	2+	25	1/500	1/320
	5♀	3+	23	1/500	1/320
	6♂	2+	25	1/250	1/1280
XLII	7♀	Neg.	Neg.	Neg.‖	Neg.‖
	8♂	Neg.	Neg.	Neg.	Neg.
	9♂	Neg.	Neg.	Neg.	Neg.
	10♂	Neg.	Neg.	Neg.	Neg.

* 10 μg DNP_{24}-PLL_{316}. Positive delayed reactions showed necrosis. Numbers are average diameter of reactions in millimeters.

‡ Challenging antigen, 250 μg DNP_{41}-BSA. Values refer to lowest serum dilution showing positive reactions in 3 guinea pigs.

§ Performed with tanned SRBC coated with DNP_{41}-BSA.

‖ Negative with 1/10 dilution of sera.

Antigenicity of Hapten-PLL Conjugates in Inbred Guinea Pigs.—Table III shows that 100 per cent of 40 strain 2 guinea pigs immunized consecutively with BPO_{24}-PLL_{316} and with DNP_{20}-PLL_{316} developed immune responses to these conjugates. In contrast, none of 11 strain 13 guinea pigs immunized with these conjugates showed evidence of an immune response to these conjugates.

DISCUSSION

The foregoing results show that 100 per cent of the offspring of non-responder parents were not capable of immune responses to DNP_{24}-PLL_{316}, whereas 82 per cent of the offspring of responder parents were capable of an immune response to DNP_{24}-PLL_{316} (Tables I and II). These data indicate that the

genetic transmission of this trait (*i.e.* the ability to respond immunologically to hapten-PLL conjugates), is transmitted genetically as a unigenic autosomal Mendelian dominant trait. Consistent with this view is the finding that inbred guinea pig strains 2 and 13 (which resulted from repeated full sibling breedings) were phenotypically (and probably also genotypically) homozygous with respect to this trait. Confirmation of this view would require study of the offspring of matings of homozygous and heterozygous responders with non-responders. Such experiments are in progress.

The results of previous experiments suggest that the capacity to respond immunologically to hapten-PLL conjugates depends on the ability of an individual guinea pig to metabolize the PLL carrier in the precise ways required

TABLE III

Antigenicity of Hapten-PLL Conjugates in Inbred Guinea Pigs

Guinea pig strain	No. of pigs immunized	Guinea pigs becoming hypersensitive,* immunizing conjugates	
		BPO_{24}-PLL_{316}	DNP_{20}-PLL_{316}
		per cent	*per cent*
Random-bred albino Hartley	40	10 to 40	10 to 40
Strain 2	40	100	100
Strain 13	11	0	0

* Animals becoming hypersensitive (responders) showed both Arthus and delayed allergic skin reactions and their sera contained detectible antihapten antibodies by PCA or ring precipitin test. Non-responders showed negative skin reactions, and their sera were negative for antihapten antibodies by PCA and by ring precipitin test.

to induce the immune response (5). The inference that this trait may be genetically controlled by a single gene would indicate that a single metabolic step is involved. In the light of what is already known about the induction of antibody formation and the metabolism of antigens (9–11), such a step might be the enzymatic degradation of the PLL carrier, or the subsequent coupling of antigenic fragments with low molecular weight RNA. Further information on the nature of this trait may be obtained by studies of the metabolism of suitably labeled hapten-PLL conjugates in responder and non-responder guinea pigs.

SUMMARY

The genetic transmission of the capacity to develop an immune response to hapten-polylysine conjugates was studied in guinea pigs.

82 per cent of the 22 offspring of 8 pairs of responder (guinea pigs which are capable of an immune response) parents were also responders, whereas, none of the 26 offspring of 9 pairs of non-responder parents were responders.

None of 11 strain 13 guinea pigs and 100 per cent of 40 strain 2 guinea pigs were responders.

These findings are consistent with the view that the capacity to respond immunologically to hapten-polylysine conjugates is genetically transmitted as a unigenic Mendelian dominant.

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