Genetic Control in Guinea Pigs of Immune Response to Conjugates of Haptens and Poly-L-Lysine

Abstract. *Random-bred Hartley strain guinea pigs which do not respond immunologically to conjugates of hapten and poly-L-lysine mere mated with heterozygous guinea pigs which do. These responders were considered heterozygous for this trait since their mating resulted in at least one nonresponder offspring. Of 31 offspring from 10 breeding pairs (nonresponder × heterozygous responder) 14 were responders. There was no evidence that this trait is sex-linked. This finding confirms the view that, in guinea pigs, development of an immune response to the aforementioned conjugates is a genetically transmitted autosomal, unigenic Mendelian dominant trait.*

Approximately 25 percent of random-bred Hartley strain guinea pigs develop an immune response to conjugates composed of hapten lightly coupled to poly-L-lysine (*PLL*) (*1, 2*). Guinea pigs immunized with these PLL conjugates of several different haptens either synthesized antibodies specific for each hapten, or failed to show an immune response to any of them (*3*). This response had the character of an "all-or-none" response. The guinea pigs showing an immune response (responders) showed strong hypersensitivity and a high concentration of antibody to hapten in their serums, whereas the nonresponders showed no evidence of an immune response when given sensitizing injections in complete adjuvant followed by repeated booster injections (*1–3*). In addition, two highly inbred guinea pig strains were either 100 percent responders (strain 2) or 100 percent nonresponders (strain 13) (*4*). These observations suggested that the capacity of guinea pigs to become hypersensitive to conjugates of hapten and poly-L-lysine is under simple genetic control.

In order to determine the nature of the genetic control, the offspring of pairs of responders and of pairs of nonresponders were studied. None of 26 offspring from 19 breeding pairs of nonresponders showed an immune response to hapten-PLL conjugates, whereas 18 out of 22 offspring from responder guinea pigs showed an immune response to hapten-PLL conjugates (*4*). These data are consistent with the view that in guinea pigs the ability to acquire hypersensitivity to hapten-PLL conjugates is transmitted as a unigenic Mendelian dominant trait. In order to confirm this view, we have studied the offspring of the mating of heterozygous responders to nonresponders. If this trait is indeed transmitted as a unigenic Mendelian dominant, 50 percent of the offspring of these matings should be responders. We now report the results of such breeding experiments.

Random-bred guinea pigs were divided into groups of responders and nonresponders according to their immune response to a dinitrophenyl-PLL conjugate (DNP_{24}-PLL_{316}) (*5*) in complete Freund's adjuvant (*3*). Heterozygous responders are phenotypic responders which, when mated, produce at least one nonresponder offspring. Nonresponders are considered homozygous since mated pairs of nonresponders produce only nonresponder offspring (*4*). We used six breeding pairs consisting of nonresponder mothers and heterozygous responder fathers, and four breeding pairs consisting of heterozygous responder mothers and nonresponder fathers. The 31 offspring of these matings were immunized (when they reached a body weight of 300–350 g) with 0.1 mg of DNP_{24}-PLL_{316} in complete adjuvant. They were skin-tested with the immunizing conjugate on the 14th day after immunization, bled on the 21st day, and tested for systemic anaphylaxis on the 24th day, as described (*3*). The various assays of immune responses are shown for two representative families (Table 1). Table 2 shows that 14 of 31 offspring (45.3 percent) of the matings, nonresponders × heterozygous responders, were capable of an immune response to the hapten-polylysine conjugate. This distribution (45.3 : 54.7) is not significantly different from the 50 : 50 distribution expected for the case of a unigenic Mendelian dominant trait ($\chi_{(1)}^2 = 0.29$; $p > 0.5$). In these and in the previous breeding experiments (*4*), there was no evidence that this trait is sex-linked. Our experiments thus confirm that the ability of guinea pigs to develop an immune response to hapten-polylysine conjugates is transmitted genetically as an autosomal, unigenic Mendelian dominant trait. As to the nature of this trait, the immune response is a complex phenomenon involving many steps controlled by different genes, such as the metabolism of antigen (*6*), formation of the inducer, and the synthesis of specific polypeptide chains.

Table 1. Immune responses of offspring of two representative families.* HR, hetero-responder. NR, nonresponder.

Skin tests†		Serum tests		Systemic anaphylaxis§
Arthus	Delayed	PCA‡	Precipitin¶	
Family I, NR ♀ × HR ♂				
Neg‖	Neg	Neg	Neg	Neg
2+	4+	Pos	Pos	Died
Family VII, HR ♀ × NR ♂				
Neg	Neg	Neg	Neg	Neg
Neg	Neg	Neg	Neg	Neg
3+	4+	Pos	Pos	Died

* Immunized with 0.1 mg DNP_{24}-PLL_{316} in complete adjuvant. † Tested by intradermal injection of 10 μg DNP_{24}-PLL_{316}. ‡ Serum dilutions of 1:50 (responder) and 1:10 (nonresponder) were analyzed by passive cutaneous anaphylaxis (PCA); 0.5 mg of DNP–guinea pig serum albumin ($DNP_{24}GPA$) was used for challenge. ¶ Ring precipitin tests with DNP_{24}-GPA (0.1 mg/ml) as antigen. § Challenged with 0.5 mg of $DNP_{24}GPA$ intravenously. ‖ Pos, positive; Neg, negative.

Previous studies with the hapten-PLL system have shown: (i) Nonresponder animals who cannot respond to hapten-PLL conjugates can respond immunologically to the same haptens coupled to protein (*3*). (ii) Guinea pigs can respond immunologically either to PLL conjugates of all immunogenic haptens or to none (*1*). (iii) Both responder and nonresponder guinea pigs appear to degrade hapten-PLL conjugates equally (*6*). (iv) Exhaustive acylation with

Table 2. Percentage of offspring of the mating (nonresponders × heterozygous responders) who are immune responders to hapten-polylysine conjugates. The offspring were immunized with 0.1 mg of DNP_{24}-PLL_{316} in complete adjuvant. Responders gave positive allergic reactions to the immunizing antigen and their serums showed antibodies to DNP. Nonresponders showed no evidence of an immune response to DNP-PLL.

Family number	Offspring	
	Responders	Non-responders
NR ♀ × HR ♂		
I	1	1
II	3	1
III	2	2
IV	1	1
V	2	2
VI	0	3
HR ♀ × NR ♂		
VII	1	2
VIII	2	1
IX	1	1
X	1	3
Total	14 (45.3%)	17 (54.7%)

succinic anhydride of the free ε-amino groups of immunogenic hapten-PLL conjugates destroys their antigenicity for all guinea pigs (*2*). These observations suggest that this trait involves an essential, lysine-specific, single metabolic operation upon the antigen in the pathway to form the inducer.

BERNARD B. LEVINE
BARUJ BENACERRAF
New York University School of Medicine, New York

References and Notes

1. F. S. Kantor, A. Ojeda, B. Benacerraf, *J. Exptl. Med.* **117**, 55 (1963).
2. B. B. Levine, *Proc. Soc. Exptl. Biol. Med.* **116**, 1127 (1964).
3. ———, A. Ojeda, B. Benacerraf, *Nature* **200**, 544 (1963).
4. ———, *J. Exptl. Med.* **118**, 953 (1963).
5. Subscripts refer to average numbers of hapten and lysine residues per molecule of conjugate.
6. B. B. Levine and B. Benacerraf, *J. Exptl. Med.* **120**, 965 (1964).
7. We thank Miss V. Levytska for assistance. Supported by the Health Research Council of the City of New York under contracts I-138, I-240, and U-1297, and by PHS grant E-2094.

6 November 1964

Blood of a Cockroach: Unusual Cellular Behavior

Abstract. *In blood smears of a cockroach a small cell occurs which is frequently paired with a larger anucleate cytoplasmic body. The larger body in such pairs is free of nucleic acid, but contains polysaccharides, as demonstrated by the periodic acid–Schiff reaction. At one extreme of a series of these associations, the cell is completely distinct from the cytoplasmic body. At the other extreme the nucleus of the cell is within and apparently a part of the larger body. A graded series of pairs suggests a mechanism resembling phagocytosis in which the cytoplasmic body either gains or regains cell status by retaining the nucleus of the ingested member.*

Cells in the hemolymph of the adult cockroach *Gromphadorhina portentosa* Schaum, from Madagascar, seem to fit classifications established from hematologic studies of other insects (*1*). However, an unusual feature of this hemolymph is that it contains numerous, membrane-limited, anucleate cytoplasmic structures which are relatively large and crescent-shaped. Each structure is intimately associated with a single small cell, but the degree of association varies among the pairs. A comparison of many pairs indicates that the cytoplasmic body is phagocytic, and that the nucleus of the ingested small cell may survive and, perhaps, function in the "alien" cytoplasm. When the general realm of living things is considered, survival of an anucleate cytoplasm is in itself a rare phenomenon. Of course, limited cytoplasmic endurance following nuclear extrusion is well recognized from determinations of the life span of the mammalian erythrocyte. It is also known that amoebae carry on some vital activities, including food ingestion, for a period up to 15 days after enucleation (*2*). Energy production persists for considerably longer periods in anucleate fragments of newt eggs (*3*), sea urchin eggs (*4*), and *Acetabularia* (*5*).

More than 300 preparations of roach blood have been examined. Blood was obtained in all instances from the dorsal surface of the insect's abdomen following inter-tergal puncture. Instant clot formation allowed easy manipulation of the sample with forceps. Standard and histochemical procedures were applied to whole as well as sectioned material. Thin preparations, necessary for optimum resolution by phase-contrast microscopy, were made by compressing a clot between a slide and a No. 0 coverglass. The degree of compression required in no way altered the appearance of cells; this was confirmed by examining blood samples prepared by other techniques, but few such samples were suitable for photography. For example, small clots were examined without the application of a coverglass. Larger clots were dissociated by opening the tips of the forceps while dragging the clot over a short distance of the slide. Such preparations have thick and thin portions, and hemocytes remain positioned at every angle. Hence, the cytological phenomenon reported cannot be considered an artifact induced by compression with the coverglass. That the forces of coagulation itself do not contribute to inter- and intracellular disorganization was confirmed by examining samples of hemolymph withdrawn from insects previously injected with heparin. Cocaine hydrochloride and potassium oxalate were additional anticoagulants applied to check the effect of heparin. It is perhaps noteworthy that whatever direct or indirect effect *Gromphadorhina* hemocytes may have had on clot formation, no cell lysis occurred, whereas lysis does occur in the hemocytes of some other insects when clotting takes place (*6*).

Therefore, the numerous paired structures consisting of a single small cell and a larger body resembling a cell but lacking a nucleus, and seen in varying degrees of intimate relationship, do not represent nuclear extrusion resulting from either clot formation or handling. Furthermore, nuclear egestion, in contrast to phagocytosis, is discounted on the basis of the evidence presented in Fig. 1. In this figure, two anucleate cytoplasmic bodies (Fig. 1*a*) appear to have been engaged in ingesting a single cell, an event observed only twice. The binding force of opposed cytoplasmic extensions on the cell in Fig. 1*b* also suggests ingestion rather than extrusion. Asymmetric cytoplasmic

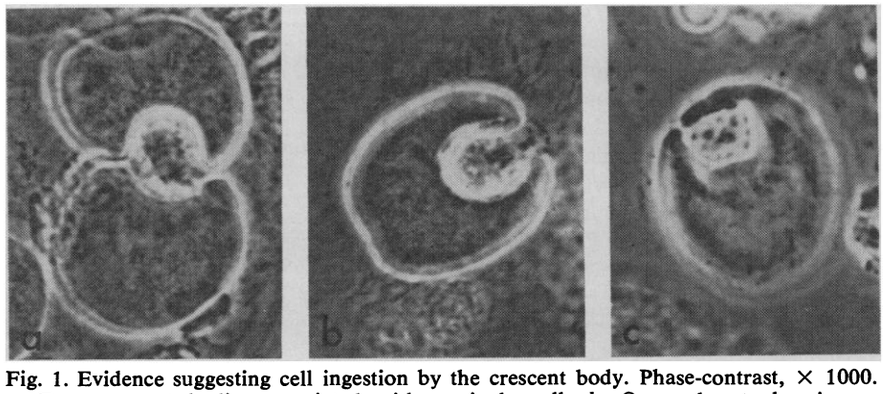

Fig. 1. Evidence suggesting cell ingestion by the crescent body. Phase-contrast, × 1000. *a*, Two crescent bodies associated with a single cell. *b*, Opposed cytoplasmic extensions of the crescent body appear to have exerted a binding force on the related cell. *c*, One extension of the crescent body exceeded the "growth" of the opposing member in the final stage of cell ingestion.