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Interactions Between Two Antigens Governed by the Poly-L-lysine Gene: Failure of One to Inhibit Competitively an *in Vitro* Response to the Other[1] (37847)

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In the guinea pig, the capacity to respond immunologically to poly-L-lysine (PLL) and its hapten conjugates is governed by the PLL gene, an autosomal dominant gene closely linked to the major histocompatibility locus of the species. Animals possessing the gene (responders) uniformly respond to immunization with PLL in standard adjuvants—such as Freund's complete adjuvant containing 0.5 mg ml^{-1} *Mycobacterium butyricum*—by developing cutaneous delayed hypersensitivity, its *in vitro* correlates such as antigen-induced thymidine incorporation into cultured lymphoid cells, and a moderate though readily detectable level of circulating antibody. In contrast, animals homozygously lacking the gene make no detectable immune response to routine immunization with PLL, although an antibody response without evidence of cellular hypersensitivity can be elicited by immunization with a very strong adjuvant —such as Freund's complete adjuvant containing 10 mg ml^{-1} *Mycobacterium tuberculosis* H37Rv—or with PLL complexed with an immunogenic carrier protein such as methylated bovine serum albumin (1–3).

All guinea pigs of the inbred strain 2 are responders to PLL while all of strain 13 are nonresponders. Of random-bred Hartley animals a varying proportion, depending on their genetic background, respond. Among random-bred animals responsiveness to PLL is associated with possession of the serologically detectable strain 2 major histocompatibility specificity (3), although recently a small proportion of such animals has been detected which either possess the strain 2 specificity without PLL responsiveness or the reverse (M. E. Dorf, personal communication). Thus the PLL gene in guinea pigs, like the several specific immune-response genes subsequently identified in the mouse (Ir-1 and others), appears to follow the pattern seen in a number of species of genes linked to the major histocompatibility locus (H-linked Ir genes) which govern recognition of antigenic determinants and responses mediated by thymus-derived cells (4). Indeed, the antigen-recognition function of some genetically controlled immune responses not previously thought to be related to histocompatibility loci now appears to be associated with the major histocompatibility locus of the species, although other genes may govern the amount or affinity or both of antibody synthesized (5).

So far as is reported in the literature or is the case to our knowledge, all guinea pigs responding to PLL and they alone respond to the random copolymer of L-glutamic acid and L-lysine (GL). Such animals also respond to protamine, poly-L-ornithine, poly-L-homarginine, poly-L-arginine, and their hapten conjugates (6, 7) as

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well as to such oligopeptides as oligolysines with a 2,4-dinitrophenyl (DNP) group conjugated either at the α or at the carboxy-terminal ϵ position or oligolysyltrialanyl-ϵ-DNP-lysine peptides (8, 9). It is commonly accepted that all these responses, although individually specific, are governed by the PLL gene, but enough animals have not been tested to exclude rigorously the possibility that these several responses are governed by distinct but closely linked genes. Other genetic loci governing the capacity to respond to random copolymers of glutamic acid and alanine and of glutamic acid and tyrosine (10–12) and to limiting doses of foreign serum albumins (13, 14) have been found to be linked to the major histocompatibility and PLL loci in the guinea pig.

The gene products of the H-linked Ir genes have not been identified, and in view of the difficulty in detecting immunoglobulin on the surfaces of thymus-derived cells it has been tempting to speculate that these genes may code for a T-cell receptor for antigen. Such an hypothesis is especially attractive in view of the numerous studies (e.g., 15, 16) demonstrating different patterns of cross-reactivities among related antigens in eliciting T-cell-mediated responses as compared with their affinities for circulating antibody. Now if the Ir gene product is a molecule which binds to antigen and if responsiveness to two or more antigens is governed by a single Ir gene, as appears to be the case with the PLL gene, the binding of one such antigen to the Ir gene product might be susceptible to competitive inhibition by another such antigen, with resulting impairment of the response to the first antigen. We have investigated this possibility using two antigens, DNP-PLL and GL, responsiveness to both of which is associated with possession of the PLL gene. Antigen-stimulated incorporation of radiolabeled thymidine by cultured lymph node cells from previously immunized animals is used as an index of T-cell activation by antigen. These experiments were designed to determine whether the presence in the cultures of GL in excess would inhibit the response to DNP-PLL of lymph node cells from animals possessing the PLL gene and previously immunized with DNP-PLL.

Materials and Methods. Animals. The experimental animals were adult guinea pigs of both sexes of the random-bred NIH Hartley strain bred at the Harvard School of Public Health.

Reagents. Poly-L-lysine hydrobromide, mol wt 100,000 (New England Nuclear Corp., Boston, MA), was treated with 2,4-dinitrofluorobenzene (Eastman Organic Chemicals, Rochester, NY) as described (17), and the resulting conjugate was found by micro-Kjeldahl nitrogen determination and measurement of optical absorbance at 360 nm (using ϵ_{360} = 17,400) to contain 12 DNP groups/100,000 daltons. Dinitrophenylated guinea pig albumin (DNP-GPA) was generously furnished by Dr. David H. Katz and contained 30 DNP groups/molecule. Poly-L-glutamic acid–poly-L-lysine hydrobromide (6:4) (GL), mol wt 40,000, was obtained from New England Nuclear Corp., bovine gamma globulin (BGG) from Armour, Kankakee, IL, and dextran sulfate sodium salt, mol wt 18,000, sulfur 14.38%, from Mann Research Laboratories, New York, NY.

Immunization. Six animals were immunized with DNP-GPA and 10 with DNP-PLL. In each case 0.4 ml of an emulsion of 0.2 ml 0.15 *M* NaCl containing 0.1 mg antigen with 0.2 ml Freund's complete adjuvant containing 0.5 mg/ml *M. butyricum* (Difco Laboratories, Detroit, MI) was injected in the footpads. Responders to PLL were identified by intracutaneous injection of 10 μg DNP-PLL in 0.1 ml 0.15 *M* NaCl 5 wk after immunization; 8 of the 10 animals developed areas of induration more than 5 mm the next day and were identified as responders.

Lymph node cell cultures. Animals were killed under ether anesthesia and the regional lymph nodes draining the sites of immunization were dissected free and dispersed into a single-cell suspension in Eagle's minimal essential medium (Microbiological Associates, Bethesda, MD) sup-

plemented with L-glutamine 2 m*M*, penicillin 100 units/ml, streptomycin 100 μg/ml, and heat-inactivated fetal calf serum 10% (TCM). The cell suspension was washed twice in TCM and resuspended to a concentration of 10^7 viable cells/ml. To 1 ml of TCM containing antigen and other substances under study, 0.5 ml of cell suspension was added in 12 × 75 mm plastic culture tubes (Falcon Plastics, Oxnard, CA) and the tubes loosely capped and incubated at 37° in a moist atmosphere containing 5% CO_2. After 48 hr 1 μCi ^{3}H-thymidine (New England Nuclear) in 0.1 ml TCM was added to each tube and the cultures incubated 24 hr more. The cultures were then harvested on 25 mm Millipore filters (HAWP 025, Millipore Corp., Bedford, MA), previously wetted with phosphate-buffered saline (0.15 *M* NaCl, 0.005 *M* phosphate, pH 7.4) (PBS) containing 1 m*M* thymidine, washed with 5 ml PBS containing 1 m*M* thymidine, two 5 ml portions of 5% trichloroacetic acid containing 1 m*M* thymidine, and 5 ml 95% ethanol. The filters were placed in counting vials, 10 ml of scintillation fluid (Aquasol, New England Nuclear) were added, and the samples were counted in a Beckman liquid scintillation counter. Results are expressed as ratios of counts incorporated by cultures incubated in the presence of antigen to counts incorporated by cultures from the same animal incubated without antigen.

Results. To determine whether the presence of GL would affect *in vitro* antigen-stimulated thymidine incorporation in an unrelated antigen system, lymph node cells from two animals immunized 4 and 5 wk before with DNP-GPA were cultured with varying concentrations of the immunizing antigen and, for each dose of DNP-GPA, with two concentrations of GL. Similar cultures containing BGG in place of GL were set up as well in order to distinguish any effect peculiar to GL from nonspecific effects due to the presence of peptides or proteins in general. The results, presented in Table I, show moderate enhancement by GL and BGG alike of thymidine incorporation in cultures stimulated by antigen at a concentration of 1 μg/ml and a slight diminution of the response to stimulation by antigen at a concentration of 100 μg/ml.

Five animals immunized 5–10 wk earlier with DNP-PLL and identified as responders by skin-testing 4 wk after immunization were used to investigate the effect of GL on antigen-stimulated incorporation of thymidine in a system under control of the PLL gene. Cultures containing BGG in place of GL were included to distinguish nonspecific effects from effects due to the presumed common genetic control of responses to DNP-PLL and to GL. The results are presented in Table II. In cultures incubated with DNP-PLL alone, there was significant stimulation of thymidine incorporation by antigen at a concentration of 1 μg/ml but marked depression at a concentration of 10 μg/ml. GL at concentrations of either 0.1 or 1.0 mg/ml had little effect on base line incorporation or the

TABLE I. Effect of GL or BGG on Antigen-Stimulated Thymidine Incorporation by Lymph Node Cells from Animals Immunized with DNP-GPA.[a]

Other antigens	Dose (mg/ml)	Conc of DNP-GPA (μg/ml)		
		0	1	100
None		1.00 ± 0.05 (4)	7.79 ± 1.55 (4)	16.78 ± 2.16 (3)
GL	0.1		6.74 ± 1.15 (4)	7.90 ± 4.52 (4)
	1.0		10.25 ± 3.30 (4)	11.61 ± 6.61 (4)
BGG	0.1		8.93 ± 2.28 (4)	13.17 ± 5.03 (3)
	1.0		9.65 ± 2.59 (4)	7.92 ± 4.55 (4)

[a] Ratios of counts per minute of ^{3}H-thymidine incorporated by cultures incubated with DNP-GPA or other antigens or both to counts per minute incorporated by like cultures from the same animal incubated without antigen: mean ± standard error (number of cultures).

TABLE II. Effect of GL or BGG on Antigen-Stimulated Thymidine Incorporation by Lymph Node Cells from Animals Immunized with DNP-PLL.[a]

Other material	Dose (mg/ml)	Conc of DNP-PLL (μg/ml) 0	1	10
None		1.00 ± 0.10 (20)	4.95 ± 0.38 (20)	0.06 ± 0.00 (20)
GL	0.1	1.16 ± 0.08 (20)	5.26 ± 0.52 (20)	6.90 ± 0.81 (20)
	1.0	0.90 ± 0.11 (20)	4.15 ± 0.42 (20)	6.87 ± 0.72 (20)
BGG	0.1	0.86 ± 0.10 (12)	3.92 ± 0.29 (12)	0.10 ± 0.02 (12)
	1.0	0.57 ± 0.11 (12)	2.74 ± 0.38 (12)	0.07 ± 0.01 (12)
Dextran sulfate	10 μg/ml	1.08 ± 0.10 (8)	5.66 ± 0.82 (8)	0.13 ± 0.02 (8)
	100 μg/ml	0.61 ± 0.05 (8)	3.25 ± 0.89 (8)	6.82 ± 1.04 (8)

[a] Ratios of counts per minute of ^{3}H-thymidine incorporated by cultures incubated with antigen or other material or both to counts per minute incorporated by like cultures from the same animal incubated without antigen or other substance: mean ± standard error (number of cultures).

reponse to DNP-PLL at 1 μg/ml but reversed the depression caused by DNP-PLL at 10 μg/ml. BGG had no significant effect at either dose save for a small suppression of background incorporation. As at the physiologic pH maintained in the cultures DNP-PLL bears a high density of positive charge while GL bears a net negative charge owing to the excess of glutamyl over lysyl residues, it was hypothesized that the impairment of thymidine incorporation by moderate concentrations of DNP-PLL might result from this high density of charge and that its reversal by GL might result in some fashion from neutralization by the net negative charge of GL of the positive charge of DNP-PLL. In the last two animals tested, this hypothesis was tested by using dextran sulfate, which like GL is negatively charged at neutral pH. The protective effect of the higher dose of dextran sulfate (100 μg/ml) was seen to approximate that of GL.

Discussion. The failure of excess concentration of GL to inhibit the activation by DNP-PLL of T-cells from animals immunized with DNP-PLL demonstrates that the reaction of immune cells to DNP-PLL which is conferred by the PLL gene, is not at some stage mediated by a common receptor susceptible to competitive inhibition. Alternative hypotheses—that the PLL Ir gene product is a preexisting common receptor modified by somatic mutation, that a specific receptor is selected from a set coded for by PLL gene, or that the responses to GL and to DNP-PLL are governed by distinct, linked genes, to name but a few—could be multiplied at will, but our experiments do not support one hypothesis over the others.

The incidental observation that impairment of response by supraoptimal concentrations of DNP-PLL is reversed by both dextran sulfate and by GL suggests that the impairment is due to the high density of positive charge on the DNP-PLL molecule and that it could very probably be reversed by any soluble, nontoxic polyanion. However, the mechanism of polycation toxicity and of its reversal by a polyanion remains obscure.

Summary. Poly-L-glutamic acid-poly-L-lysine (GL) and dinitrophenyl-poly-L-lysine (DNP-PLL) are two antigens to which the capacity of guinea pigs to make cell-mediated immune responses appears to be governed in common by a single, dominant, histocompatibility-linked gene, the poly-L-lysine (PLL) gene, although the responses to the two antigens are individually antigen-specific. The hypothesis that this common genetic control might be associated with the involvement at some stage in the antigen-recognition process of a receptor common to both antigens and susceptible to competitive inhibition was tested by incubating, in the presence of DNP-PLL alone and of DNP-PLL together with excess GL, cultures of lymph node cells from animals possessing

the PLL gene and previously immunized with DNP-PLL. The presence of GL did not inhibit the response to DNP-PLL (as judged by incorporation of radioactive thymidine), thus providing no evidence for the hypothesized common receptor for the two antigens.

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