

GENETIC CONTROL OF CUTANEOUS BASOPHIL
HYPERSENSITIVITY

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A well-established expression of cell-mediated immunity has been separated from classical tuberculin type delayed hypersensitivity. Originally called "Jones Mote" sensitivity (1), it has been renamed cutaneous basophil hypersensitivity (CBH) (2) to emphasize the large numbers of basophilic leukocytes that infiltrate the dermis in the skin lesions. Both conditions can be distinguished as well by differences in immunogenic (3) and tolerogenic (4) requirements, as well as persistence of sensitivity.

Both expressions of cell-mediated, delayed-in-onset type hypersensitivity reactions (DH and CBH) have been thought to be mediated by sensitized thymus-derived (T) lymphocytes, but recent studies implicate antigen specific B cells as either effectors of CBH (5) or view CBH as a product of a classical delayed reaction modified by suppressor B cells (6).

To further investigate the role of T and B cells in the mediation of CBH, we took advantage of artificial antigens, the response to which is known to be controlled by histocompatibility-linked immune response genes (7). In the guinea pig, the development of delayed hypersensitivity as well as the production of antibody to the synthetic polymers poly-L-lysine (PLL), poly-L-glutamic-lysine (GL), and their dinitrophenylated (DNP) derivatives has been shown to be a Mendelian dominant trait governed by a single gene (8). Strain 2 and a fraction of outbred (responder) Hartley guinea pigs have the capacity to respond to these antigens, whereas strain 13 animals and nonresponder Hartleys do not.

Nevertheless, genetically nonresponder animals have the capacity to produce antibody to DNP-PLL when this is complexed to an immunogenic carrier, thus indicating that their B cells do indeed recognize the hapten, whenever this is bound to a carrier molecule that is amenable to T-cell recognition (9).

These findings lend support to the contention that the phenotypic expression of responder status must reside, at least operationally, in T cells.

In this paper we present evidence that control of the development of CBH is also linked to the same histocompatibility genes, thus indicating that this reaction also operates through antigen-specific effector T cells. We sensitized responder and nonresponder Hartley guinea pigs with DNP-GL in incomplete Freund's Adjuvant (IFA) and tested them for the appearance of CBH. Then shortly thereafter we checked their ability to produce delayed hypersensitivity to noncross-reacting DNP-PLL given in complete Freund's Adjuvant (CFA). Typical CBH and DH were obtained in responder animals, whereas nonresponders failed to give positive reactions of either type.

Materials and Methods

Animals. Female Hartley PLL responder and nonresponder guinea pigs, 300 g, were purchased from Camm Research Institute, Wayne, N. J.

Antigens. PLL, Mol Wt 40,000, was obtained from Sigma Chemical Company, St. Louis, Mo. A random linear copolymer of GL, Mol Wt 40,000, in the proportions of 60:40 was a kind gift of Dr. B. Benacerraf. 2,4-dinitrobenzenesulfonic acid, sodium salt (DNP SO₃H), was purchased from Eastman Organic Chemicals, Rochester, New York.

Dinitrophenylation of PLL and GL. The method indicated by Williams and Chase (10) was employed. Briefly, 50 mg of PLL, 50 mg of K₂CO₃, and 14 mg of DNP SO₃H were dissolved in 3.2 ml of distilled water and stirred for 1 h, shielded from light with aluminum foil. The solution was then dialysed for several days against multiple changes of water and read in a spectrophotometer at 360 nm. The degree of dinitrophenylation of the polymer was calculated to be 7.6 mol of DNP per PLL, using the molar absorptivity of DNP-lysine at 360 nm of 17,530. To prepare DNP-GL identical proportions of the reagents were used, but the reaction was allowed to continue overnight. 5 mol of DNP per mol of GL was calculated employing the same method.

Immunization and Skin Tests

FOR CBH. Groups of three responder and nonresponder guinea pigs were immunized in the foot pads with 100 and 10 μ g of DNP-GL in IFA. 1 wk later the animals were shaved and depilated on one flank and skin tested with 1, 10, and 100 μ g of DNP-GL in 0.1 ml of saline. Gross reactions were read 24 h thereafter, and the erythema was measured in millimeters.

FOR DH. All animals previously tested for CBH plus two normal controls were immunized 1 day later with 100 μ g of DNP-PLL in CFA in the foot pads. 3 wk later they were shaved and depilated on the opposite flanks and were skin tested with 1 μ g DNP-PLL in 0.1 ml of saline, since larger doses gave local irritation and necrosis. Gross reactions were read and again 24 h thereafter, and the degree of induration and erythema was measured in millimeters.

Biopsies and Histology. Skin sites tested with DNP-GL and DNP-PLL were biopsied with sterile instruments under anesthesia with ether. The wounds were sealed with 9-mm "autoclips" (Clay Adams, Inc., Parippany, N. J.) and wiped with 70% ethanol. The biopsies were cut in pieces of no more than 1-mm thickness with a razor blade on a plate of dental wax and fixed in glutaraldehyde-paraformaldehyde for 5 h. Then they were placed in 0.1 M cacodylate buffer until postfixed in 2% OsO₄, dehydrated, and embedded in Epon. 1- μ m sections were cut with a Porter-Blum MT 1 microtome and stained with 10% Giemsa in 2% sodium borate.

Sections were observed under oil with a light microscope with the aid of an ocular micrometer. Total cellularity and the percentage basophils in the upper dermis were calculated as reported before (11).

Results

CBH to DNP-GL.

GROSS REACTIONS. As can be seen in Table I, typical erythematous, but nonindurated, CBH reactions were obtained at 24 h in all but one responder animal, but none of the nonresponder animals. There was little difference in skin reactivity in animals immunized with 10 μ g or 100 μ g of antigen. Largest reactions were seen with a skin test dose of 100 μ g DNP-GL, and these averaged about 25 mm of erythema.

HISTOLOGY. The cellular composition of the skin sites can be seen in Table II, where values are expressed as means of cell counts with standard errors. It is apparent that there is a considerable difference between responder and nonresponder animals in terms of percentage basophils and also, but to a lesser degree, in total cellularity. In responder animals the microscopic picture was in all cases that of a typical CBH (12). As has been usual in CBH, the best reactions were generally achieved at skin sites of animals immunized with smaller (10 μ g)

TABLE I
Cutaneous Basophil Hypersensitivity and Delayed Hypersensitivity Reactions to Synthetic Polymers in Responder And Nonresponder Guinea Pigs

	CBH to DNP - GL						DH to DNP - PLL			
	Immunizing dose	Adjuvant used	Number animal	Average reaction to skin test with:			Immunizing dose	Adjuvant used	Number animal	Average reaction to skin test with 1 μ g
				100 μ g	10 μ g	1 μ g				
Responders	100 μ g	IFA	2	29* - ‡	11-	0-	100 μ g	CFA	5	17+
	10	IFA	3	0- 22-	0- 20-	0- 7-	100	CFA	1	0- 13+
Nonresponders	100	IFA	2	0-	0-	0-	100	CFA	5	0-
	10	IFA	3	0-	0-	0-	100	CFA	1	0-

* Millimeter of erythema.

‡ Degree of induration: -, none; +, marked.

TABLE II
Cellular Composition of CBH and DH Reactions to Synthetic Polymers in Responder and Nonresponder Guinea Pigs

Status	Antigen and adjuvant	Dose used for:		Number animals	Total cellularity	Cells in upper dermis				
		Immunizing	Skin testing			M	B	N	E	MC
		μ g				%	%	%	%	%
R	DNP-GL/IFA	100	10	2	162 \pm 1.5*	75	16 \pm 12.5	1.5	4.5	4
R	DNP-GL/IFA	100	100	2	157 \pm 1.0	71	28 \pm 8.5	<1	2	<1
R	DNP-GL/IFA	10	10	3	148 \pm 15.7	68	25 \pm 8.9	1	2.7	3
R	DNP-GL/IFA	10	100	3	232 \pm 25.1	52	42 \pm 8.9	<1	<1	4.7
NR	DNP-GL/IFA	100	10	2	156 \pm 16	85	2 \pm 1.0	7	2	4.5
NR	DNP-GL/IFA	100	100	2	126 \pm 4.5	90	3.5 \pm 0.5	3	<1	3
NR	DNP-GL/IFA	10	10	3	121 \pm 15	98	1.3 \pm 0.9	<1	<1	<1
NR	DNP-GL/IFA	10	100	3	150 \pm 19	91	5 \pm 1.0	<1	2.3	1.7
R	DNP-PLL/CFA	100	1	1	359	91	2	2	1	4
NR	DNP-PLL/CFA	100	1	1	122	92	1	8	<1	<1

R, responders; NR, nonresponder; M, mononuclear cells; B, basophils; N, neutrophils; E, eosinophils; MC, mast cells.

* Means \pm S.E.

and skin tested with larger (100 μ g) doses of antigen, but significant reactions were obtained in all other instances as well. Mean basophil counts in responder animals immunized with IFA varied from 16-42%, while similarly treated nonresponders never showed more than 5% basophils. No significant differences were detected in the number of eosinophils, neutrophils, and mast cells between both groups.

DH to DNP-PLL

GROSS REACTIONS. Gross reactions to 1 μ g of DNP-PLL were erythematous and indurated in retested and control responder animals, as can be seen in Table I. One of the animals in this group that failed to give a positive CBH skin test to DNP-GL also failed to give a positive DH response to DNP-PLL and was

therefore considered to be a nonresponder. Retested and control nonresponder animals always gave negative skin tests.

HISTOLOGY. Table II shows for comparison that the cellular composition of reactions in a responder animal immunized with CFA was that of a classical delayed hypersensitivity reaction. The total cellularity was much higher than in CBH, with mononuclear cell predominating and relatively few basophils. Practically no cellular response was elicited under the same conditions in nonresponder animals, the existing mononuclear cellularity representing background levels.

Discussion

The results in this paper indicate that CBH to DNP-GL, as well as subsequent DH to noncross-reacting DNP-PLL, can only be obtained in guinea pigs which possess the genetic ability to respond to these antigens. None of these delayed-onset reactions could be obtained in nonresponder animals.

The reactions observed in responder guinea pigs were grossly and histologically typical for either type of cell-mediated reaction, with the exception of one animal labeled responder by the supplier which was on further testing determined to be a nonresponder, since it failed to give a positive DH to the antigen it was selected for.

These results provide further evidence that CBH is dependent on and mediated by T cells, since the defect of nonresponder guinea pigs in the PLL system seems to be manifested in their T and not B cells (13).

Although it seems likely that the ability to develop DH and CBH to synthetic polymers is under the same genetic control and mediated by sensitized T cells, these experiments in fact do not suggest whether they depend on a single T cell, possibly triggered in different ways, or on two distinct T-cell subpopulations, both of which exhibit the phenotypic expression of the genetic immune response status.

Summary

CBH to DNP-GL could be elicited only in responder guinea pigs which possess the genetic ability to develop classic delayed type hypersensitivity to DNP-PLL, the response to which is governed by the same gene. Since the defect in nonresponder animals seems to reside at the level of their T cells and not B cells, these results lend support to the contention that CBH, as well as DH, is dependent on and probably mediated by T cells.

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