The Induction of Immediate and Delayed Sensitivity in the Marmoset (34802)

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Because of its small size, the marmoset is potentially a very useful primate for laboratory investigation; however, very little data are available on the immunologic responses of these animals. Consequently, the following data are reported which provide information on the ability of these animals to elicit delayed hypersensitivity reactions to tuberculin and to dinitrofluorobenzene (DNFB) as well as an antibody response to these substances and a conjugate prepared by reacting DNFB and protein.

Materials and Methods. For sensitization, each of two groups of three marmosets (*Saguinus mystax*) weighing 453–633 g were injected in the nuchal area with 0.5 ml of either of two emulsions; the first contained 0.25 ml of Arlacel A-Drakeol 6VR (35:65), 0.25 ml of saline, 2 mg of heat-killed BCG, and 1 mg of dinitrofluorobenzene (DNFB), the second was similar except that 1 mg of a dinitrophenyl–bovine serum albumin conjugate with a molar ratio of 33 dinitrophenyl groups per mole of albumin (DNP_{33} BSA) was substituted for the DNFB. These mixtures were designed to induce tuberculin sensitivity, delayed contact sensitivity to DNFB, and antibody with DNP-hapten specificity. On Days 13 and 27 after immunization, tuberculin tests were carried out with PPD at 1.0 μg (5 TU) per 0.1 ml and contact sensitivity tests by the application of DNFB (0.4, 0.2, 0.1 m*M*) in 5 μl of acetone as previously described (1, 2).

Gel-diffusion studies were carried out on microscope slides employing a plastic template (3), and in 60-mm plastic petri dishes using a Feinberg gel cutter (4). Sera collected from the femoral vein of the marmosets on Days 16 and 21 were diffused against DNP_{33}BSA and against a nondialyzable tuberculin culture filtrate, fraction D (5). Passive cutaneous anaphylaxis was accomplished by an intradermal injection into 350-g Hartley strain guinea pigs of 0.1 ml of a 1:10 dilution of marmoset serum and 4–5 hr later an intracardial injection of 1 ml of a solution containing 1 mg of DNP_{33}BSA and 1% of Evans blue dye. In the gel-diffusion and PCA tests a BSA solution was used as a control. Hemagglutination was carried out using sera diluted at least 10-fold and DNP-sensitized red cells using the method of Levine and Levytska (6) modified by the use of formalinized sheep erythrocytes.

Results. The results of induction of delayed hypersensitivity and of antibody are given in Table I. The BCG component of the emulsion caused five of the six marmosets to become tuberculin sensitive as demonstrated by testing with PPD at the 1-μg level; 1 of the sensitized animals responded to 0.1 μg. The DNFB component caused two of three marmosets to become sensitive by Day 27 and, interestingly, one of three animals developed delayed contact sensitivity to DNFB after the injection of DNP_{33}BSA.

Marmosets giving delayed reactions showed no reactivity at 6 hr but well-developed reactivity at 24 hr. At the first tuberculin test (13 days after sensitization) the reactions seemed better developed at 48 than at 24 hr; this did not seem true at 27 days. The tuberculin reactions were not exactly like human or guinea pig reactions. The reaction sites were not so indurated as typical human reactions, and the erythema was somewhat more diffuse than that usually ob-

TABLE I. Immunologic Response of Marmosets.

	Marmoset number											
	1		2		3		4		5		6	
	Immunizing emulsion											
	DNFB + BCG						$DNP_{33}BSA$ + BCG					
	Delayed hypersensitivity response											
Days postinj.:	13	27	13	27	13	27	13	27	13	27	13	27
Skin test sub.												
PPD 1.0 μg	12[a]	117	36	165	0	0	240	176	40	110	180	210
0.1 μg	0	0	9	16	0	0	0	0	0	0	0	0
DNFB 0.2 μmole	—	—	+	+	—	+	—	+	—	—	—	—
DNFB 0.04 μmole	—	—	—	—	—	—	—	—	—	—	—	—
	Antibody response to $DNP_{33}BSA$											
Days postinj.:	16	21	16	21	16	21	16	21	16	21	16	21
PCA	—	—	—	—	—	—	+	+	+	±	—	—
Hemagglutination	—	—	—	—	—	—	200[b]	—	200	—	—	—
Gel diffusion	—	—	—	—	—	—	+	+	—	—	—	—

[a] The product of two diameters of erythema in mm^2.

[b] The reciprocal of the dilution.

served in guinea pigs.

Precipitating antibody was produced in only one animal (No. 4) against $DNP_{33}BSA$ as determined by gel diffusion. This animal and one other (No. 5) also showed antibody by hemagglutination and by PCA in guinea pigs. None of the sera contained precipitating antibody against tuberculin as determined by gel diffusion. In all tests in which BSA was used as a control, negative results were obtained showing that the gel-diffusion and PCA tests were hapten specific.

Discussion. The marmoset was capable of showing delayed hypersensitivity to tuberculin with a degree of reactivity similar to that usually observed in BCG-sensitive guinea pigs and humans (7). In this respect the marmoset does not differ markedly from other nonhuman primates (8). No attempt was made in this study to carry out the tuberculin test by injection into the upper eyelid (9) since the animals seemed to respond readily to intradermal injection.

DNFB and such related compounds as DNCB (dinitrochlorobenzene) usually induce delayed hypersensitivity in almost all guinea pigs and humans tested (10, 11). While two of three marmosets did develop delayed contact hypersensitivity, they did so only at the relatively large dose of 0.2 μmoles, about 100 times the dose required to elicit a similar reaction in guinea pigs (Godfrey, H. P., and Baer, H., manuscript in preparation). It was demonstrated previously that sensitive rhesus monkeys will react to 0.3 μmole of DNFB, but no attempt was made at that time to find the minimum reactive dose (2).

Marmosets injected with DNFB did not produce detectable antibody although almost all guinea pigs responded to this substance with the production of abundant precipitating, PCA, and hemagglutinating antibody (Godfrey, H. P., and Baer, H., manuscript in preparation).

The $DNP_{33}BSA$ preparation induced antibody production in two of three marmosets and this was somewhat transient since it was not detected after postimmunization Day 21.

It must be concluded, therefore, that by the dose and method used for immunization, the marmosets (in comparison to guinea

pigs) responded well only to the tuberculin test. Other routes, doses, and vehicles could not be tested due to the limited supply of these animals.

Summary. An attempt was made to evaluate the immunological competence of the marmoset (*S. mystax*). Delayed hypersensitivity was induced to tuberculin and dinitrofluorobenzene. Only two of six animals produced measurable antibody, one marmoset produced precipitating antibody, and these two animals produced antibodies detectable by PCA and hemagglutination.

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