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PREPARATION OF PROTEIN FROM MYCOBACTERIUM LEPRAE, SKIN TEST RESPONSES AND LYMPHOBLAST TRANSFORMATION IN VACCINATED ARMADILLOS

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In a previous publication (1) it was explained that several fundamental areas of leprosy research such as the postulated modes of transmission and the mechanism of resistance can be explored only with nonleprous individuals known to be maximally susceptible, and in the latter case in addition maximally resistant to infection with Mycobacterium leprae. For this reason one of the major objectives of our work with nine-banded armadillos involves attempts to develop valid tests for determining the degree of susceptibility to leprosy of any particular armadillo. The rationale of skin tests with heat-killed M. leprae for this purpose and some early findings were previously reported by Kirchheimer and Sanchez (2).

In our later experience susceptibility tests with heat-killed M. leprae based on cell responses and fate of these bacilli in the test site seem often difficult to interpret. We are trying to circumvent the difficulties by developing a skin test based on capacity of armadillos vaccinated with heat-killed leprosy bacilli to respond with delayed type of hypersensitivity to M. leprae-proteins and increased blast transformation of T-lymphocytes in the presence of M. leprae antigens. On theoretical grounds armadillos susceptible to a dose of leprosy bacilli to which most others are resistant should be among the ones unable to develop specific delayed type hypersensitivity or correlates like blast transformation. Leprosy bacilli were separated from infected armadillo liver by a method identical with the one described by Prabhakaran at this conference (K. Prabhakaran, E. B. Harris and W. F. Kirchheimer. Binding of ^{14}C-labeled DOPA by M. leprae in vitro).

For the preparation of the protein the dried powder of M. leprae was transferred to a rosett cell (Model 25) and suspended in 4 ml of water. The suspension was subjected to ultrasonic oscillation in a sonic oscillator, using the microtip (Sonifier Cell Disrupton, Model W 185 D, Heat Systems, Ultra Sonics, Inc., Plainview, New York, U.S.A.). The rosett cell was immersed in an ice bath. Sonic oscillation was carried out for a total period of 15 min. (15 sec. of oscillation alternating with 30. sec. rest periods). The bacilli are disrupted by this procedure. The suspension of disrupted organisms was centrifuged at 127,000 x G for 60 min. The sediment was suspended in 4 ml water and subjected to sonic oscillation again for 15 min. The material was centrifuged as before. The two supernatants were pooled and the sediment was discarded. Purification of the protein from the bacterial extract was done according to the method described by Landi (3). The amount of protein in the preparation was assayed by the method of Lowry et al (4).

Ten adult armadillos of either sex were vaccinated by intramuscular inoculation of 3.0×10^8 heat killed armadillo tissue-derived leprosy bacilli suspended in Freund's incomplete adjuvant. Forty-eight days later the armadillos received an intracutaneous inoculation of 4.0×10^7 heat-killed armadillo tissue-derived M. leprae (lepromin-A). Nodules up to 15 x 15 mm in size developed in all ten armadillos at the test sites within 24 hours. After 72 hours the nodules declined in size. These skin reactions at 24, 48 and 72 hours are shown in Table I.

Table 1. Skin Reactions in Armadillos to Lepromin A, Tested 48 Days after Vaccination with Heat-Killed M. leprae in Freund's Incomplete Adjuvant

Armadillo Number	Skin Induration in mm		
	24 hours	48 hours	72 hours
191	13 x 13	8 x 8	8 x 8
193	11 x 13	5 x 8	5 x 8
194	12 x 15	15 x 15	12 x 15
195	10 x 14	5 x 5	7 x 7
196	10 x 10	12 x 12	7 x 10
197	15 x 15	10 x 13	10 x 10
199	13 x 14	10 x 10	7 x 10
200	10 x 12	8 x 10	8 x 8
201	11 x 12	8 x 10	4 x 4
202	11 x 11	5 x 5	8 x 8

The cutaneous inoculation sites were biopsied after 28 days. Sections showed that tissue at the test sites consisted of macrophages and giant cells. Part of the tissue was necrotic and bacilli if seen looked disintegrated.

Eight months after the initial vaccination six of the ten armadillos were skin tested in the genital region with 29 and 170 microgram of M. leprae protein. None of these armadillos reacted to 250 US units TU. The skin reactions to the M. leprae-protein and blast transformation of cultured lymphocytes in the presence of M. leprae are summarized in Table 2. The six vaccinated armadillos had erythematous skin responses to M. leprae-protein and five of them had significantly increased blast transformation of lymphocytes. The latter findings are based on measurements of uptake of radioactive thymidine. Fourteen not vaccinated armadillos failed to react to 170 microgram of M. leprae-protein and to 250 US units TU.

Table 2. Skin-Reactions of Vaccinated Armadillos to M. leprae-Protein and Blast Transformation of Their Lymphocytes in Presence of M. leprae

Armadillo Number	M. leprae-Protein Erythemas in mm at 24, 48, 72 hours						T/C*
	29 Microgram			170 Microgram			
191	--			4 x 4	2 x 2	4 x 4	1.08
193	4 x 4	0	0	5 x 5	5 x 5	3 x 3	2.36
194	5 x 5	3 x 3		--	--	--	13.69
195	7 x 8	3 x 3	0	4 x 4	8 x 8	13 x 13 (necrotic)	8.61
199	6 x 6	2 x 2	0	0	0	6 x 6	31.48
201	5 x 5	3 x 3	0	3 x 3	3 x 3	3 x 3	8.49

*T/C = Trial over Control; T/C 1.45 is significant stimulation.

The skin reactivities of human beings with positive and negative Fernandez, Mitsuda and tuberculin reactions is being assessed by Bedi in India.

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