

## Effect of Specific Vaccine on Cell-Mediated Immunity of Armadillos Against *M. leprae*<sup>1</sup>

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As discussed in the first publication dealing with experimental leprosy in the armadillo<sup>(9)</sup>, one of the reasons for selecting armadillos as a possible leprosy model was the expectation that in analogy to the human situation great differences in susceptibility to leprosy might exist.

In a subsequent publication<sup>(3)</sup>, it was explained that several fundamental areas of leprosy research such as the hypothetical modes of transmission and the mechanism of resistance can be explored only with non-leprosy individuals known to be maximally susceptible and in the latter case in addition maximally resistant, to infection with *M. leprae*. For this reason one of the major objectives of our work with nine-banded armadillos involves attempts to develop a valid test for determining the degree of susceptibility to leprosy of any particular armadillo<sup>(4,5)</sup>. In our experience, susceptibility tests with heat-killed *M. leprae* based on cell responses and fate of these bacilli in the test site often seem difficult to interpret<sup>(4)</sup>. To circumvent the difficulties posed by using whole leprosy bacilli in the test, we are developing a susceptibility skin test based on the capacity of armadillos vaccinated with heat-killed leprosy bacilli to respond with local delayed-type hypersensitivity reactions to *M. leprae* protein and increase T lymphocyte blast transformation in the presence of *M. leprae* antigens<sup>(4)</sup>. On theoretical grounds, we assume that armadillos unable to muster any parameter of increased cell-mediated immunity (CMI) following vaccination probably have a defect which makes them hypersusceptible to infection with *M. leprae*. Such armadillos will become the subject of our objective to study the nature of susceptibility on the cell level in

uninfected armadillos. We have observed that most armadillos following intramuscular vaccination with *M. leprae* A (armadillo tissue-derived) respond later with local delayed-type hypersensitivity skin reactions to 10 to 170  $\mu$ g of trichloroacetic acid precipitated protein prepared from ultrasonicates of purified *M. leprae* A. They also exhibit significantly increased lymphocyte blast transformation in the presence of heat-killed *M. leprae* A and *M. leprae* A protein, and additionally respond with strong Koch reactions at the site of intracutaneous inoculation of lepromin A<sup>(4)</sup>.

The present study was made to show if vaccination with heat-killed *M. leprae* A also increases CMI to challenge with living *M. leprae* A.

### MATERIALS AND METHODS

Six normal adult armadillos of either sex were vaccinated by intramuscular inoculation of  $3.0 \times 10^8$  heat-killed armadillo tissue-derived leprosy bacilli suspended in Freund's incomplete adjuvant. Forty-six days later the armadillos received an intracutaneous inoculation of  $4.0 \times 10^7$  heat-killed *M. leprae* A in 0.2 ml normal saline. Nodules up to  $15 \times 15$  mm in size developed in all six armadillos at these inoculation sites within 48 hours after which they declined in size. These lepromin test sites were biopsied after 28 days. Sections showed that the tissue at the test site consisted of macrophages with giant cells. Part of the tissue was necrotic and where bacilli were found they appeared disintegrated. We interpreted these responses as Koch reactions, an expression of delayed-type hypersensitivity. Eight months after the initial vaccination the armadillos were skin tested in the genital region with 29  $\mu$ g *M. leprae* A protein and 250 US tuberculin units (TU). The vaccinated armadillos reacted to the *M. leprae* protein with erythematous reactions in 24 to 72 hours. They did not react to tuberculin. Four-

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teen unvaccinated armadillos did not react to *M. leprae* protein up to 170 µg.

The lymphocytes of the vaccinated armadillos, with one exception, underwent significantly increased blast transformation in the presence of intact *M. leprae* A as measured by uptake of radioactive thymidine by the cultured lymphocytes. Transformation indices above 1.45 were found significantly higher than those in unvaccinated armadillos. To test the effect of the specific vaccine on the resistance of armadillos against *M. leprae* A, six of the vaccinated together with nine nonvaccinated armadillos were infected intracutaneously with an inoculum of 10<sup>6</sup> *M. leprae* A.

### RESULTS

One of the vaccinated armadillos had signs of disseminated leprosy after 1,005 days. Another was autopsied after 690 days and had no leprosy. The remaining three vaccinated armadillos are without signs of leprosy after 2,298 days by examination of the last blood and ear clips. The sixth vaccinated armadillo died after 94 days of unrelated causes. The bacilli in the infection site were disintegrated. Eight of the nine unvaccinated armadillos had signs of disseminated leprosy from 349 to 1,148 days after infection. The remaining unvaccinated armadillo has no signs of dissemination after 2,298

days. The immunologic responses and the results of challenge of the vaccinated armadillos with living *M. leprae* A are summarized in Table 1.

### DISCUSSION

Our assumption that in analogy to man armadillos might display marked differences in susceptibility to leprosy is supported by several observations (<sup>6,8</sup>). For example, in a group of 15 armadillos infected intracutaneously at the same time with 10<sup>7</sup> *M. leprae* from the same suspension, only three survived for more than five years without any sign of leprosy. These three armadillos had no microscopic or mouse foot pad multiplication evidence of the presence of leprosy bacilli in the infection site after 400 days. On the other hand, longer survival of *M. leprae* at the infection sites seems to signal dissemination of the infection later on (?). Furthermore, histopathologic evaluation of liver lesions of leprosy armadillos infected with the same suspension of *M. leprae* seem to fall into one of two categories. In one group the lesions are less extensive and can be compared to indeterminate leprosy in man. In the other group the involvement was much more severe and resembled lepromatous leprosy in man. The nature of these lesions did not correlate with the duration of the infection and, in the opinion of the au-

TABLE 1. Cell-mediated immunity responses of vaccinated armadillos.

Armadillo no.	Koch reactions, induration in mm			Skin test responses to 29 µg <i>M. leprae</i> -protein		Blast transformation radiothymidine uptake	Fate of armadillo
	24 hr	48 hr	72 hr	Erythema in mm		Trial/Control T/C	
191	10 × 13	8 × 8	8 × 8	4 × 4 to 170 µg		1.08	No leprosy after 2,298 days.
193	11 × 13	5 × 8	5 × 8	4 × 4	0	2.36	Leprosy at 690 days.
194	12 × 15	15 × 15	12 × 15	5 × 5	3 × 3	13.69	Died after 94 days from unrelated causes. <sup>a</sup>
195	10 × 14	5 × 5	7 × 7	7 × 8	3 × 3	8.61	No leprosy after 2,298 days.
199	13 × 14	10 × 10	7 × 10	6 × 6	2 × 2	31.48	No leprosy after 2,298 days.
201	11 × 12	8 × 10	4 × 4	5 × 5	3 × 3	8.49	No leprosy after 2,298 days.

<sup>a</sup> At the time of death, the infection site showed peripheral accumulations of histiocytes without acid-fast bacteria, central necrosis with disintegrated acid-fast bacilli.

thors, reflects differences in susceptibility (<sup>2</sup>).

In a recent publication Hastings (<sup>1</sup>), dealing with the fundamental nature of the lepromatous defect, stressed that there might be a genetic reason for the observation that only a few individuals develop disseminated leprosy from infectious doses to which most persons are completely or partially resistant. Our assumption that vaccinated armadillos which are unable to respond with correlates of CMI should be unusually susceptible leans heavily on a genetic basis for susceptibility to leprosy.

The results of the present experiments (which probably are significant; Chi square = 4 with Pearson's correction for small samples) show that armadillos in whom vaccination engenders capability to respond with correlates of CMI are also resistant to infectious challenge. The infectious dose of  $10^6$  *M. leprae* A must be considered a massive one and, therefore, the acquired immunity seems solid. This conclusion is strengthened by the additional observation that eight of ten unvaccinated armadillos infected with  $10^5$  *M. leprae* A from a different suspension, likewise prepared from a one time armadillo to armadillo passaged *M. leprae* A, had disseminated leprosy within 1,095 days.

The observation that heat-killed *M. leprae*, unlike BCG and most other pathogens, does not lose immunogenicity when it is killed confirms Shepard's observation on mice (<sup>10</sup>). His conclusions were based on such parameters of CMI as lymph node and foot pad enlargement in addition to results with infectious challenge. In respect to the latter, our observations add considerably to its value because leprosy in the armadillo is more comparable to human lepromatous leprosy.

What are the implications of our findings for the successful vaccination of leprosy susceptible human beings? Sight should not be lost of the fact that the successfully vaccinated armadillos were immune-responsive as shown by their Koch responses, delayed-type hypersensitivity reactions to *M. leprae* A protein and increase in lymphocyte blast transformation. This is, however, not analogous to highly susceptible human beings who might be more comparable to some immunologically unresponsive armadillos we expect to exist, and we believe will be found

not immunizable. We suspect that, despite our present findings, there is little hope for immunizing highly susceptible human beings with a *M. leprae* vaccine.

### SUMMARY

Six nine-banded armadillos were vaccinated intramuscularly with  $3.0 \times 10^8$  armadillo-passaged leprosy bacilli in Freund's incomplete adjuvant. After 46 days the armadillos responded with strong Koch reactions to intracutaneous challenge with  $4.0 \times 10^7$  *M. leprae* A. Eight months after the initial vaccination, they responded with delayed-type hypersensitivity reactions to intracutaneous challenge with *M. leprae* A protein.

With one exception, the vaccinated armadillos had significantly increased lymphocyte blast transformations in the presence of *M. leprae* A antigens. After simultaneous challenge of the vaccinated, as well as nine nonvaccinated armadillos by intracutaneous inoculation of  $10^6$  *M. leprae* A, eight of the nonvaccinated animals developed disseminated leprosy in from 349 to 1,148 days. The one exception is alive and has no evidence of leprosy after 2,298 days.

Of the six vaccinated armadillos, four are alive after 2,298 days without signs of leprosy. One of the other two vaccinated armadillos died after 94 days from unrelated causes. The findings at the infection site permit the conclusion that it would have escaped infection. The remaining armadillo in the vaccinated group was sacrificed at 690 days and had leprosy. It is concluded that armadillos responsive to vaccination with heat-killed *M. leprae* with parameter of CMI are also resistant to infectious challenge. The implications of these findings for vaccination of susceptible human beings are discussed.

### RESUMEN

Seis armadillos de 9 bandas se vacunaron intramuscularmente con  $3 \times 10^8$  bacilos de la lepra (mantenidos en armadillo) emulsificados en adyuvante incompleto de Freund. Después de 46 días, los armadillos respondieron con intensas reacciones de Koch al reto intradérmico con  $4.0 \times 10^7$  *M. leprae* A. Ocho meses después de la vacunación inicial, los armadillos respondieron con reacciones de hipersensibilidad tipo tardío al reto intracutáneo con proteína de *M. leprae* A.

Con una sola excepción, los armadillos va-

cunados tuvieron una incrementada transformación blastoide de sus linfocitos en presencia de antígenos del *M. leprae* A.

Después del reto simultáneo con  $10^6$  *M. leprae* A, por la vía intracutánea, de los armadillos vacunados y de 9 armadillos no vacunados, 8 de los animales no vacunados desarrollaron lepra entre 349 a 1,148 días. La única excepción es un armadillo que permanece vivo y sin lepra después de 2,298 días.

De los 6 animales vacunados, 4 están vivos y sin signos de lepra después de 2,298 días. Uno de los otros 2 armadillos vacunados murió después de 94 días por causas no relacionadas. Los hallazgos en el sitio de infección permitieron concluir que este animal debió haber escapado de la infección. El armadillo restante en el grupo vacunado fue sacrificado a los 690 días y tuvo lepra. Se concluye que los armadillos que respondieron a la vacunación con el *M. leprae* muerto por calor (usando parámetros de la inmunidad celular), también fueron resistentes al desafío infeccioso. Se discuten las implicaciones de estos hallazgos en la vacunación de los humanos susceptibles.

## RÉSUMÉ

On a vacciné par voie intramusculaire 6 armadillos à neuf bandes, avec  $3.0 \times 10^8$  bacilles de la lèpre passés sur armadillos, et suspendus dans l'adjuvant incomplet de Freund. Après 46 jours, les armadillos ont montré une des réactions de Koch fortement positives lorsqu'on les inoculait par voie intra-cutanée avec  $4.0 \times 10^7$  *M. leprae* A. Huit mois après la vaccination initiale, ces armadillos montraient des réactions d'hypersensibilité du type retardé lorsqu'on les inoculait par voie intra-cutanée avec la protéine de *M. leprae* A.

A une exception près, les armadillos vaccinés ont présenté une augmentation significative de la transformation plastique des lymphocytes en présence d'antigènes de *M. leprae* A. Après challenge simultané des armadillos vaccinés, ainsi que des 9 armadillos non vaccinés, par inoculation intra-cutanée de  $10^6$  *M. leprae* A, 8 des animaux non vaccinés ont développé une lèpre disséminée, dans un intervalle de 349 à 1,148 jours. La seule exception est toujours vivante, et ne présente aucun signe de lèpre après 2,298 jours.

Parmi les 6 armadillos vaccinés, 4 sont encore en vie après 2,298 jours, sans manifester aucun signe de lèpre. Un des 2 autres armadillos vaccinés est mort 94 jours après la vaccination pour des causes étrangères à celle-ci. Les observations faites à l'endroit de l'infection permettent d'affirmer que cet armadillo n'aurait pas développé l'infection. Le dernier armadillo du groupe

vacciné a été sacrifié après 690 jours; il était atteint de lèpre. On en conclut que les armadillos qui répondent à la vaccination par *M. leprae* tué par la chaleur, et qui présentent des signes d'immunité cellulaire, sont également résistants à un challenge infectieux. Les conséquences de ces observations en ce qui concerne la vaccination des humains susceptibles sont discutées.

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