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Evaluation of Monitoring Antibodies to PGL-I in Armadillos Experimentally Infected with *M. leprae*[1,2]

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The nine-banded armadillo (*Dasypus novemcinctus*) is well developed as a host for the *in vivo* propagation of *Mycobacterium leprae,* and studies have begun that use this animal as a model to study the pathogenesis of leprosy ([3, 4]). The effective use of animals in research requires monitoring of the host over the course of infection. Previous methods to monitor experimental infections of *M. leprae* in armadillos have commonly relied on histologic techniques ([2]). Serologic techniques offer hope for greater efficiency in management.

Other attempts to monitor *M. leprae* antibodies in armadillos have utilized staphylococcal-A radioimmunoassay methodologies with iodinated fractions of the sonicated whole bacillus ([1]). Using undefined antigen mixtures, these assays lacked specificity and only detected antibody simultaneously with the appearance of disseminated infection ([5]). We recently developed an enzyme-linked immunosorbent assay (ELISA) that detects armadillo IgM antibodies to the chemically defined and apparently species-specific phenolic glycolipid-I (PGL-I) antigen of *M. leprae* ([6]). We report here an evaluation of this ELISA for application in the management of armadillos experimentally infected with *M. leprae.*

MATERIALS AND METHODS

Armadillos. A total of 437 plasma samples taken from 207 armadillos that had been captured from the wild and housed at the Gillis W. Long Hansen's Disease Center for the production of *M. leprae* were used in this study. The armadillos were at varying stages of experimental infection resulting from an average intravenous inoculum of 5.0×10^8 viable *M. leprae.* The status of individual animals ranged from not yet inoculated to sacrificed. The clinical history of each armadillo was documented, and histologic samples of ear tissues were examined by standard methods to monitor for dissemination of disease ([4]).

Serology. Native purified PGL-I was prepared by Dr. Patrick Brennan (Colorado State University) and obtained through contract with the National Institute of Allergy and Infectious Diseases (Dr. Darrel Gwinn, project officer). An ELISA was performed by the method previously reported ([6]).

RESULTS

Correlation of ELISA with yield of *M. leprae.* Armadillo harvests yielding less than 1.0×10^7 *M. leprae* per g of liver tissue are generally considered unsatisfactory. To determine if IgM antibodies to PGL-I could be used as indicators of successful infections, sera from 35 armadillos sacrificed after experimental infection with *M. leprae* were tested by the ELISA. For comparison purposes statistically, all unsatisfactory harvests were considered to yield 1.0×10^7 *M. leprae* per g of liver tissue, but actual yields may have been less (Fig. 1). Regressional analysis comparing the liver yield of *M. leprae* with ELISA absorbance at sacrifice showed a significant tendency ($p < 0.001$, $r = 0.7261$, $\log Y = 5.9 + 0.0021X$) for increased absorbance with higher yields. Correlation was not significant when only suc-

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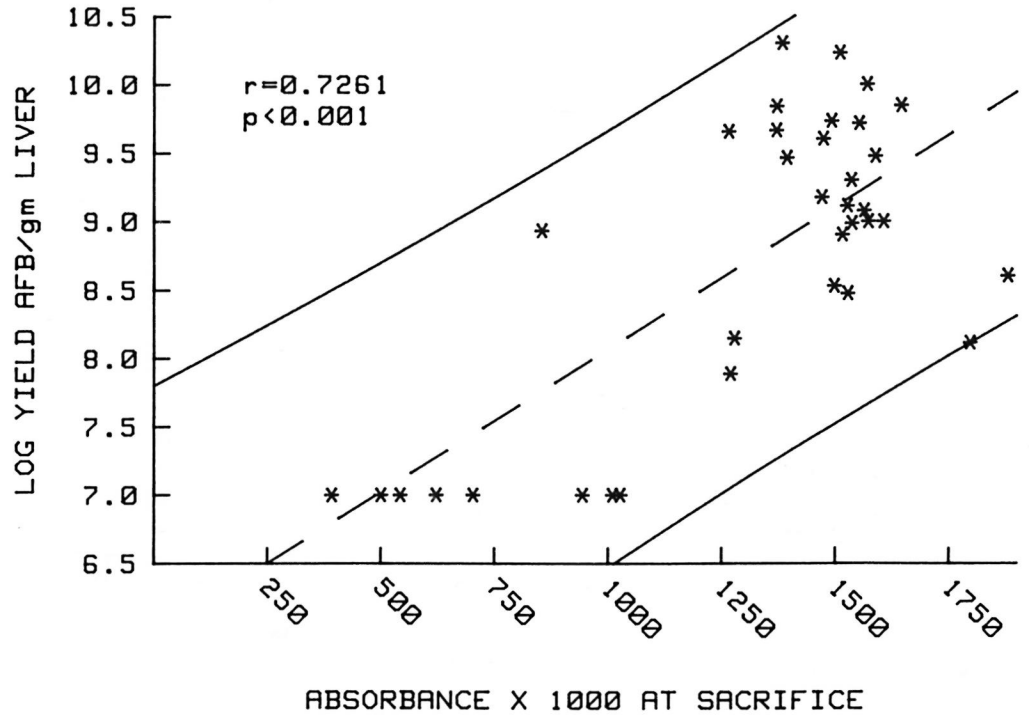

FIG. 1. Comparison of PGL-I ELISA-IgM absorbances of sacrifice sera with liver yield of *M. leprae*. Armadillos yielding less than 1×10^7 *M. leprae* per g of liver tissue are considered to yield 1×10^7. (-----) = regression line; (——) = 95% confidence interval.

cessful sacrifices yielding 10^8 to 10^{10} *M. leprae* per g of liver tissue were considered ($r = -0.3166$, $\log Y = 11.39 - 0.0014X$). The majority (25/26) of armadillos with harvests greater than 1.0×10^7 *M. leprae* per g of liver tissue had absorbances above 1.2. None (0/9) of the armadillos with lower yields had absorbances above 1.08. Based on absorbance criteria (≥ 1.2), the ELISA correctly predicted a satisfactory harvest 97% (34/35) of the time.

Kinetics of antibody response. To determine the time course for development of IgM antibody to PGL-I, serum samples from 195 armadillos at varying stages of experimental infection were tested. Those armadillos presenting from the wild with positive ELISA were presumed to be wild-type infected and not included ([7]). Sera from armadillos with unsatisfactory yields of *M. leprae* at sacrifice also were not included. The actual wild experience, as well as the eventual possibility of resistance could not be determined for all armadillos, and some wild-infected and resistant armadillos were no doubt included in this group. Mean ELISA absorbances were calculated for each group of sera taken in 82-day intervals (Fig. 2). Using the definitions of positive and negative previously derived for the ELISA ([6]), IgM antibody to PGL-I became detectable between 150 to 230 days, on the average 186 days, after experimental infection. These antibodies remained detectable over the course of the infection. The antibody response among armadillos was not homogeneous, and mathematical modeling suggested that 16% of the animals probably had detectable antibody by day 74, and that 95% of all susceptible armadillos should be positive by day 455. In comparison, an average (arithmetic mean) of 519 days postinfection was required for armadillos to exhibit a disseminated acid-fast bacterial infection detectable by histologic examination of the ear tissues. The 95% confidence interval of this average histologic-positive day exceeded the boundaries of the plot. Additionally, ELISA and histologic results from 70 armadillos were compared to determine their efficiency

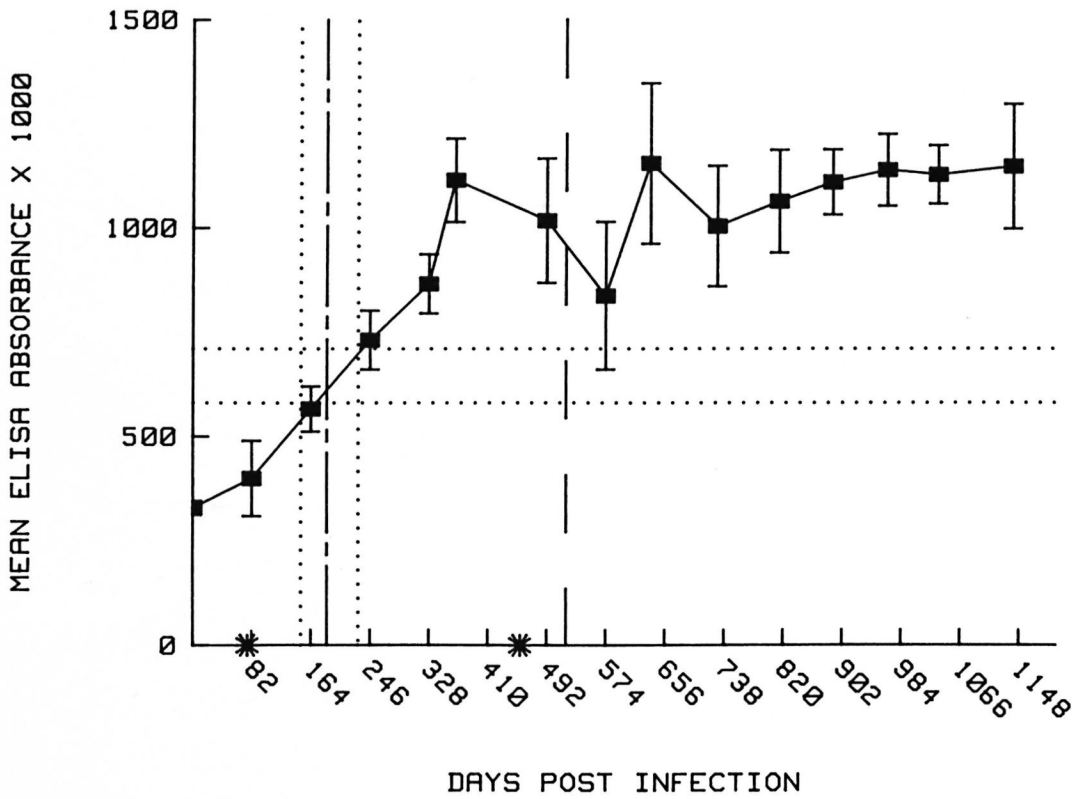

FIG. 2. Mean ELISA absorbances for IgM antibodies to PGL-I obtained from 404 armadillo plasma samples are compared to time after experimental infection in 82-day intervals. Absorbance means are plotted at the end of each interval and bracketed by their standard error (S.E.M.). Horizontal dotted lines indicate the equivocal zone, and absorbances above or below are considered positive or negative, respectively. Vertical stipple line (−·−·−) marks 50% serologic-positive day, and vertical dotted lines mark the 95% confidence interval on the 50% day. Asterisks (**) bind the 16% and 95% serologic-positive days. Vertical broken line (— — —) marks arithmetic mean day for histologic methods to detect AFB in ear tissues.

for early screening. A positive ELISA preceded by at least 82 days a positive histologic examination 93% (65/70) of the time; ELISA and histology tied 4% (3/70) of the time; and histology was positive before the ELISA 3% (2/70) of the time.

DISCUSSION

These data indicate that an ELISA for IgM antibodies to PGL-I is an effective method for monitoring armadillos experimentally infected with *M. leprae*. Serologic monitoring is more reliable and may be used effectively earlier than the histologic methods previously applied. Pre-screening the armadillos would help to assure uniformity of test animals and to preserve the integrity of culture strains by identifying armadillos that may have encountered *M. leprae* in the wild. An ELISA may be useful as a quantitative measure of bacterial proliferation in experimentally infected armadillos and deserves further investigation. Although poor correlation was observed between ELISA absorbances and yields of 10^8 to 10^{10} acid-fast bacilli per g of liver tissue, such limitation does not obviate the present qualitative utility of the assay for differentiating satisfactory and unsatisfactory yields. Protractive monitoring of infected animals will help to determine successful harvest times and to identify those armadillos probably resistant to *M. leprae*.

The extent to which armadillos may be used to model serologic events in human leprosy is unknown. The kinetics of PGL-I antibody responses have not been reported for other host systems. The apparent lag

before IgM antibodies to PGL-I become detectable in armadillos may be the result of the poor immunogenicity of the glycolipid antigen or the route or size of the inoculum. Further studies with this antigen may refine the ELISA as an aid for *in vivo* propagation, and may benefit the development of armadillos as animal models in leprosy research.

SUMMARY

An enzyme-linked immunosorbent assay (ELISA) for IgM antibodies to the phenolic glycolipid-I antigen of *Mycobacterium leprae* was evaluated for efficacy in monitoring armadillos experimentally infected with the bacillus. IgM antibodies were detected in armadillos from 186 days after experimental infection until the animals were sacrificed. The ELISA demonstrated the establishment of infection earlier and more reliably than the histologic methods previously applied. Satisfactory yields of *M. leprae* from individual armadillos could be predicted 97% of the time, and the technique may be useful in identifying appropriate harvest times or resistance among armadillos. The ELISA seems to be a valuable adjunct for managing experimental infections of *M. leprae* in armadillos.

RESUMEN

Se probó la eficiencia de un enzimo-ensayo (ELISA) para anticuerpos IgM contra el glicolípido fenólico I del *Mycobacterium leprae* para seguir la evolución de la enfermedad leprosa experimental en armadillos. Los anticuerpos IgM se encontraron en los armadillos desde el día 186 post-inoculación y permanecieron hasta que los animales fueron sacrificados. Con el enzimo-ensayo la infección se pudo demostrar más temprano y más confiablemente que con los métodos histológicos previamente utilizados. Con esta técnica se pudieron predecir rendimientos satisfactorios de *M. leprae* en armadillos individuales en el 97% de los casos, se pudieron identificar los tiempos más adecuados para la cosecha, y el estado de resistencia entre los armadillos. El método de ELISA parece ser una herramienta útil en el manejo de la infección leprosa experimental del armadillo.

RÉSUMÉ

On a évalué l'efficacité d'une épreuve ELISA dirigée contre les anticorps IgM à l'antigène phéno-glycolipidique-I (PGL-I) de *Mycobacterium leprae* pour suivre des tatous infectés expérimentalement par le bacille. Des anticorps IgM ont été décelés chez des tatous à partir du 186ème jour après l'infection expérimentale, et se sont maintenus jusqu'au moment où les animaux étaient sacrifiés. Il est apparu que l'épreuve ELISA permettait de repérer plus tôt, et de façon plus fiable, l'établissement de l'infection, que ce n'est le cas avec les méthodes histologiques utilisées jusqu'à présent. On peut espérer récolter une quantité satisfaisante de *M. leprae* chez les tatous dans 97% des cas. La technique peut être utile pour identifier le moment le plus favorable pour sacrifier les animaux et en récolter une quantité maximale de bacilles. Elle peut également être utile pour déceler une résistance chez ces tatous. L'épreuve ELISA paraît être dès lors un appoint précieux pour suivre les infections expérimentales par *M. leprae* chez les tatous.

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