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## Effects of Vaccination with Several Mycobacterial Proteins and Lipoproteins on *Mycobacterium leprae* Infection of the Mouse

TO THE EDITOR:

Continuing our study<sup>(5)</sup> of the protective effects of various components of *Mycobacterium leprae* and other mycobacteria against infection of the mouse foot pad with *M. leprae*, we examined the potential of several proteins and lipoproteins derived from both *M. leprae* and *M. tuberculosis* to protect mice against challenge with *M. leprae* in the hind foot pad.

One-hundred-seventy, female BALB/c +/+ mice, purchased from CLEA Japan, Inc., Meguro-ku, Japan, and housed locally at the Sasakawa Research Building under specific-pathogen-free conditions, were divided among 11 groups as shown in Table 1. The four antigen preparations, which were generated at Colorado State University, Fort Collins, Colorado, U.S.A., or Yonsei University, Seoul, Korea, were: *M. leprae* soluble antigen from which lipoarabinomannan had been removed (MLSA-LAM); a pool of recombinant *M. leprae* proteins [antigen 85A (32 kDa) and antigen 85C (30 kDa)]; a pool of recombinant *M.*

*tuberculosis* proteins [38 kDa, antigen 85A (32 kDa), antigen 85C (30 kDa), 19 kDa, 23 kDa, and 14 kDa]; and a pool of native *M. tuberculosis* lipoproteins prepared by extraction of *M. tuberculosis* with the detergent TX114<sup>(4)</sup>. These were employed with two adjuvants—Freund's incomplete adjuvant (FIA) and monophosphoryl lipid A (MPL) solubilized in triethanolamine (TeoA)<sup>(1)</sup>.

The antigens, which were emulsified in one of the adjuvants in every case but one, were injected intradermally in each flank to groups of 15 mice in a dosage of 20 µg per mouse on three occasions 3 weeks apart. The pooled *M. tuberculosis* lipoproteins were administered to one group of mice without added adjuvant in the hope that the inherent lipid element would provide its own adjuvanticity. Additional groups of 15 mice were administered one of the adjuvants, and a final group of 20 mice, not treated, served as the controls. Twenty-eight days after the third injection, the antigen-treated mice were inoculated, each with  $5 \times 10^3$  *M. leprae* into the right hind foot pad

TABLE 1. Treatment of mice.<sup>a</sup>

Group no.	No. mice	Challenge site	Treatment
1	20	BHF	Untreated controls
2	15	BHF	FIA
3	15	BHF	MPL
4	15	RHF	MLSA-LAM in FIA
5	15	RHF	MLSA-LAM in MPL
6	15	RHF	r <i>M.lep</i> proteins in FIA
7	15	RHF	r <i>M.lep</i> proteins in MPL
8	15	RHF	r <i>M.tb</i> proteins in FIA
9	15	RHF	r <i>M.tb</i> proteins in MPL
10	15	RHF	<i>M.tb</i> lipoproteins in FIA
11	15	RHF	<i>M.tb</i> lipoproteins

<sup>a</sup> Abbreviations: BHF = mice were inoculated in both hind foot pads; RHF = mice were inoculated in the right hind foot pad only; FIA = Freund's incomplete adjuvant; MPL = monophosphoryl lipid A; MLSA-LAM = *M. leprae* soluble antigen from which lipoarabinomannan and other lipids had been extracted; r*M.lep* = recombinant *M. leprae*; r*M.tb* = recombinant *M. tuberculosis*.

TABLE 2. Results of harvests of *M. leprae* from untreated control mice.

No. days after inoculation	No. <i>M. leprae</i> per foot pad ( $\times 10^5$ )	
	Individual foot pads	Median
99-112	0.089 (2) <sup>a</sup> , <0.089 (6)	<0.089
141-148	<0.089 (12)	<0.089
178	0.266, <0.089 (3)	<0.089
210-231	7.72, 4.08, 1.60, 0.71, 0.62, 0.089 (2), <0.089 (9)	<0.089

<sup>a</sup> Numbers in parentheses are number of foot pads demonstrating this result. In all other cases, only a single foot pad yielded the result shown.

(RHF), and the control and adjuvant-treated mice were similarly inoculated in both hind foot pads (BHF). Harvests of *M. leprae* from the inoculated mice were subsequently performed by Shepard's method (<sup>6,7</sup>).

Because the organisms had not multiplied in large proportions of the control mice that had been subjected to harvest earlier than 200 days after inoculation, the results (Table 2) suggest that the inoculum had contained only a small proportion of viable *M. leprae*. Assuming that the organisms would have multiplied in all of the foot pads inoculated with  $5 \times 10^4$  *M. leprae*

per foot pad, and in none of those inoculated with 500 organisms per foot pad, one may calculate by means of the Halverson-Ziegler equation (<sup>3</sup>) that the inoculum contained only about 4 viable organisms per  $5 \times 10^4$  total, i.e., fewer than 1 per 10,000.

Nevertheless, the outcome of this vaccine trial is clear-cut (Table 3). In interpreting this table, one must compare like with like. For instance, the results in mice treated with antigen in FIA must be compared with the results in mice administered FIA alone; similarly with MPL. Only the mice administered the native lipoproteins without adjuvant should be compared with the untreated control mice. The results of harvests performed between 210 and 231 days after inoculation from all groups of mice suggest that the mice were protected against challenge with *M. leprae* only by MLSA-LAM, if the antigen had been suspended in MPL. The recombinant proteins were without effect, as was the pool of native *M. tuberculosis* lipoproteins, which, if administered without adjuvant, appeared to enhance multiplication of the organisms.

The lack of effectiveness of the pools of recombinant proteins was disappointing, because recombinant products provide the best

TABLE 3. Results of harvests of *M. leprae* >200 days after inoculation from all groups.

Mouse group no. (treatment)	No. <i>M. leprae</i> per foot pad ( $\times 10^5$ )	
	Individual foot pads	Median
1 (Untreated Control)	7.72, 4.08, 1.60, 0.71, 0.62, 0.089 (2) <sup>a</sup> , <0.089 (9)	<0.089
11 ( <i>M.tb</i> lipoproteins)	4.88, 4.79, 4.53, 3.46, 3.37, 2.93, 2.57, 1.15, 0.71, 0.53, 0.44, 0.27, 0.18, <0.089	1.86 <sup>b</sup>
2 (FIA Control)	13.5, 10.7, 7.19, 2.31, 2.13, 1.86, 1.51, 1.33, 0.71, 0.62, 0.27, 0.18 (2), 0.089, <0.089 (9)	0.18
4 (MLSA-LAM in FIA)	4.26, 3.46, 3.02, 0.27 (2), 0.089 (2), <0.089 (8)	<0.089
6 ( <i>rM.lep</i> proteins in FIA)	9.76, 8.16, 6.48, 6.12, 5.95, 3.90, 3.20, 2.04, 1.86, 1.69, 1.24, 0.44, 0.18, <0.089	2.62
8 ( <i>rM.tb</i> proteins in FIA)	7.99, 5.32, 3.02, 2.66 (2), 2.31, 2.22, 2.13, 1.78, 1.33, 0.98, 0.44 (2), 0.36 (2)	2.13
10 ( <i>M.tb</i> lipoproteins in FIA)	7.72, 7.54, 5.86, 4.53, 2.66, 0.89, 0.62, 0.53, 0.36 (2), 0.27, 0.089, <0.089	0.62
3 (MPL Control)	8.52, 3.46, 2.75, 2.63, 2.31, 1.60, 1.42, 1.33, 0.89, 0.80 (4), 0.71, 0.62, 0.27, 0.18, 0.089 (3), <0.089 (4)	0.80
5 (MLSA-LAM in MPL)	1.06 (2), 0.71, 0.44, 0.36 (2), 0.27, 0.089 (2), <0.089 (5)	0.18 <sup>c</sup>
7 ( <i>rM.lep</i> proteins in MPL)	8.34, 5.06, 3.73, 3.28, 2.57, 2.04, 1.76, 1.69, 1.15, 0.80, 0.62, 0.18, <0.089 (2)	1.72
9 ( <i>rM.tb</i> proteins in MPL)	4.53, 4.08 (2), 3.28, 3.20 (2), 3.02, 1.69, 1.06, 0.98, 0.89, 0.71, 0.27, 0.18, <0.089	1.69

<sup>a</sup> Numbers in parentheses are number of foot pads demonstrating this result. In all other cases, only a single foot pad yielded the result shown.

<sup>b</sup> Significantly greater than the median value among the untreated control mice as determined by the Mann-Whitney U test ( $p = 0.0076$ ).

<sup>c</sup> Significantly smaller than the median value among the mice administered MPL only as determined by the Mann-Whitney U test ( $p = 0.0352$ ). None of the remaining median values was significantly different from that of the corresponding control group.

hope for a subunit vaccine against leprosy in an era of diminishing availability of armadillo-derived *M. leprae*. The evidence of efficacy of whole, complex fractions of *M. leprae* containing a multitude of immunogenic proteins, in addition to some carbohydrates, lipoglycans, and lipids, is in accord with other successful studies<sup>(2)</sup>, demonstrating that only a complex array of immunogens provides protection against leprosy.

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