Vaccination of Mice Against the Leprosy Bacillus with Skin-Test Antigens

TO THE EDITOR:

The specter of approximately 700,000 new leprosy cases a year, despite a prevalence of only about 805,000 ([11, 12]), haunts us. Many, perhaps the majority, of these cases are detected in the course of intensive case finding ([11]), and are single-lesion cases which are difficult to diagnose definitively. Measurement of the true incidence of leprosy requires new diagnostic tools, more sensitive than physical examination supported at times by slit-skin smear ([9]). Neither serology, even the modern, user-friendly, sensitive phenolic glycolipid-I (PGL-I)-based kits ([5]), nor gene-amplification based on a variety of primers provides the necessary sensitivity ([10]). A promising approach to the diagnosis of asymptomatic leprosy is provided by the availability of two new skin-test antigens—MLSA-LAM (soluble antigens of *Mycobacterium leprae*

THE TABLE. *Results of harvests of* M. leprae.

Treatment[a]	No. AFB per foot pad ($\times 10^5$)		Probability
	Individual feet	Median	
Control	9.14, 5.50, 4.44, 2.40, 1.24, 0.888	3.42	0.0037[b]
IFA	10.7, 10.0, 9.50[c], 8.70, 4.44	9.50	0.0014[b]
MLSA-LAM in IFA	6.04[c], 3.73, 2.31, 1.78, 1.42, 1.18, 0.888, 0.710, 0.710, 0.710	1.78	0.00046[d]
MLCwA in IFA	4.79[c], 2.40, 1.86, 0.444	4.68	0.0047[d]
MLCwA-LAM in IFA	6.30, 5.68, 5.15, 3.20, 2.38[c], 0.355, 0.178	2.56	0.00089[d]
APOH	39.8, 13.7[c], 8.08, 6.83, 6.66, 5.59, 3.99, 2.04, 1.42, 1.15, 0.799	6.74	0.45[b]
MLSA-LAM in APOH	26.4, 13.6, 6.57[c], 6.30, 5.77, 4.79, 3.37, 2.13, 2.01	6.13	0.72[d]
MLCwA in APOH	17.2[c], 6.30, 2.84, 2.57, 0.533, 0.444	6.30	0.85[d]
MLCwA-LAM in APOH	4.88, 3.46[c], 0.799	3.46	0.091[d]

[a] IFA = incomplete Freund's adjuvant; APOH = aluminum hydrogel; MLSA-LAM = *M. leprae*-soluble antigen from which lipoarabinomannan has been removed; MLCwA = *M. leprae* cell-wall antigen; MLCwA-LAM = *M. leprae* cell-wall antigen from which lipoarabinomannan has been removed.

[b] The probability, determined by the Kruskal-Wallis one-way analysis of variance, that the results from all of the relevant groups of mice were drawn from a single population.

[c] This harvest was performed from the pooled tissues of four foot pads.

[d] The probability, determined by the Mann-Whitney *U* test, that the results from the group of mice indicated were drawn from the same population as were those from the relevant control group.

Additional probabilities: Control vs. IFA vs. APOH: 0.072; IFA vs. APOH: 0.41; MLCwA in IFA vs. MLCwA-LAM in IFA: 0.92; MLCwA in APOH vs. MLCwA-LAM in APOH: 0.55.

from which the immunosuppressive lipoarabinomannan has been removed) and MLCwA (*M. leprae* cell-wall antigens) ([2]). These materials have successfully undergone Phase I safety studies in the U.S.A., and Phase II studies are currently being planned for several areas of high leprosy endemicity. Awaiting the outcome of these studies, we examined the protective potency of these products in the BALB/c mouse.

M. leprae harvested from the livers and spleens of *M. leprae*-infected armadillos were disrupted by sonication and centrifuged to yield two major fractions—the soluble supernatant (MLSA) and the insoluble pellet (MLCwA) ([2]). Much of the soluble carbohydrates and lipids in the preparations, especially lipoarabinomannan (LAM) and the phosphatidylinositol mannosides, were removed by extraction with Triton X-114 to yield the two fractions, MLSA-LAM and MLCwA-LAM ([1]). These materials were produced at Colorado State University under the conditions required for approval of their use as skin-test antigens in humans ([1]). BALB/c mice were bred under specific pathogen-free (SPF) conditions in the animal facility of the Sasakawa Research Building, Ministry of Public Health, Nonthaburi, Thailand, from a nucleus purchased from CLEA Japan, Inc., Kawasaki, Japan. The inoculum of *M. leprae* was prepared from the foot pads of BALB/c mice that had been inoculated approximately 4 months earlier with a suspension of organisms of the "Thai 53" strain that had been provided by Dr. J. L. Krahenbuhl, Baton Rouge, Louisiana, U.S.A. When the organisms were found to have multiplied to a number approaching 10^6 per foot pad, the bacterial suspension was diluted to a concentration of 5×10^3 organisms per 0.03 ml.

For the purpose of this experiment, the mice of one group were held as untreated controls, and those of other groups were administered incomplete Freund's adjuvant (IFA) or aluminum hydrogel (APOH), an adjuvant suitable for use in humans, or one of the two antigen preparations suspended in one of the adjuvants intracutaneously in the flank. After 1 month, the mice were challenged in the right hind foot pad with 5×10^3 *M. leprae*. At intervals thereafter, the organisms were harvested from the pooled hind foot pads of four control mice, stained by a standard, room temperature acid-fast stain, and counted directly ([6–8]).

The results of this experiment are presented in The Table as the actual number of acid-fast bacilli (AFB) per individual foot pad as well as the median number of AFB per foot pad for each group. These results demonstrate that all three antigens were protective when administered to mice after

emulsification in IFA; whereas none of the three antigens protected mice when administered in APOH.

Previously, we reported that immunization of mice with various cell-wall fractions of *M. leprae,* progressively depleted of lipids, carbohydrates, and soluble proteins, conferred significant protection against subsequent infection with live *M. leprae* ([3]), and subsequent studies ([4]) demonstrated that a mixture of proteins derived from the *M. leprae* cell wall conferred long-term protection, compared to the shorter-term protection provided by individual proteins. The conclusion from this experiment was that multiple *M. leprae* protein epitopes are critical for solid vaccine protection against the infection. The activity of the fractions studied in this experiment, MLSA and MLCwA, from which LAM and other apparently immunosuppressive lipids and lipoglycans have been removed, provides additional evidence of the utility of multicomponent fractions as distinct from individual proteins.

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