Comparative Characteristics of Antigenic Profile of *M. leprae* and *M. lufu*

TO THE EDITOR:

Mycobacterium leprae, being an obligate intracellular parasite, fail to be cultivated *in vitro.* Isolation of the leprosy bacilli from *M. leprae*-infected tissues of laboratory animals is a rather intricate and multi-step process affecting the physical and chemical integrity of mycobacterial cells. Biological reagents from *M. leprae* purified from host cells in various world laboratories are not standardized, and they differ by their antigenic composition. Therefore, there is a need for cultivable mycobacteria with biological properties that might permit using them for diagnostic and other purposes instead of *M. leprae.*

M. lufu isolated from the soil in Zaire are noted for their dapsone sensitivity ([7]) which permitted using them for primary screening of antileprosy drugs. At the Leprosy Research Institute in Astrakhan, Russia, evidence for the protective properties of *M. lufu* as related to experimental infection with *M. leprae* were obtained. It was also proved that *M. lufu* were able to detect delayed-type hypersensitivity (DTH) reactions in *M. leprae*-sensitized animals ([3, 4]). We propose a test system with *M. lufu* as an antigen for the serological diagnosis of leprosy ([10]).

At the Leprosy Research Institute, *M. leprae* and *M. lufu* were comparatively studied for their protein composition, and the comparative characteristics of humoral responses of leprosy patients toward different antigenic determinants of both mycobacteria were stated.

M. leprae were isolated from the foot pads of mice with experimental leprosy infection according to Draper ([1]). *M. lufu* were cultivated on Lowenstein-Jensen medium. *M. leprae* and *M. lufu* were suspended in 0.85% solution of NaCl and then sonicated for 7 min at 18 kHz at repeated intervals in an MSE-100 sonifier. The sonified bacilli were centrifuged at 10,000 $g \times$ 10 min, and the supernatant was used as antigen. Protein in the sonicates was estimated according to Lowry, *et al.* ([5]) and equaled 1.5–2.0 mg/ml. Preparations were kept at –20°C until used.

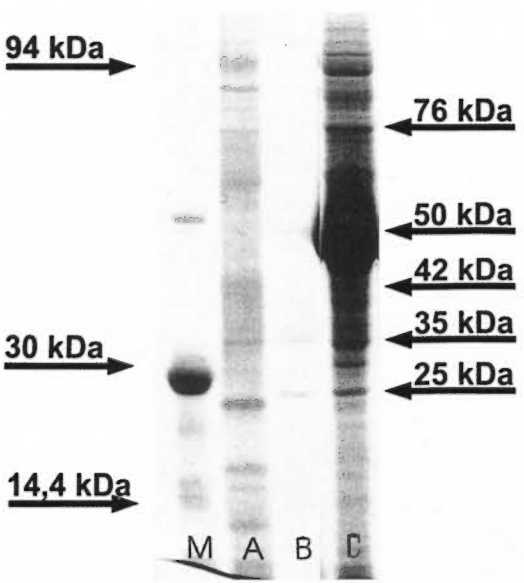

FIG. 1. Electrophoresis of mycobacterial sonicates. A = *M. leprae* isolated from nine-banded armadillos; B = *M. leprae* passed in mice; C = *M. lufu;* M = markers.

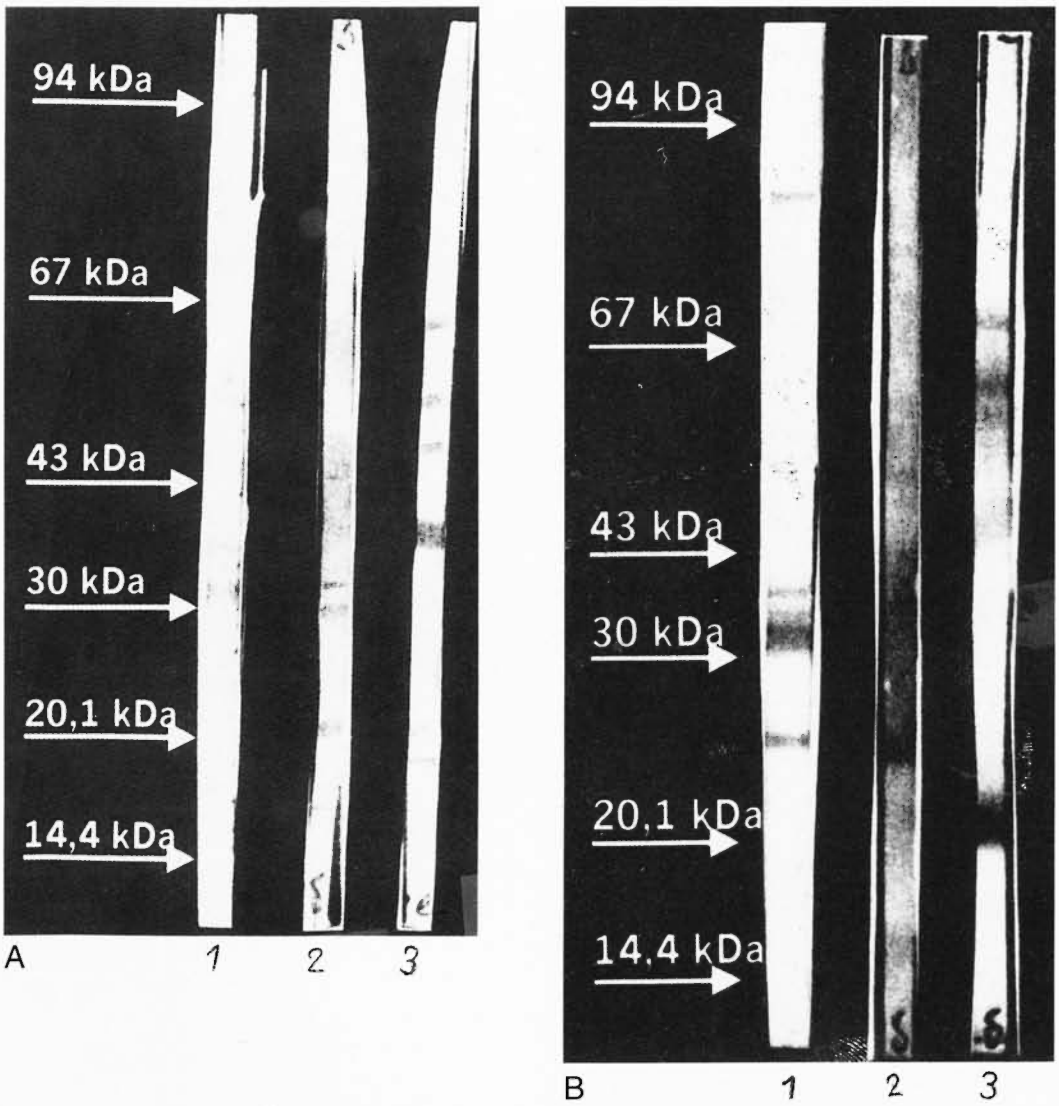

FIG. 2. Western blot of sera from leprosy patients using *M. leprae* passed in mice (**A**) and *M. lufu* (**B**) as antigens. 1 = MB patients in active stage of their disease; 2 = MB patients in regression; 3 = PB patients.

Blood sera samples from 46 multibacillary (MB) leprosy patients (18 cases with active leprosy and 28 regressed cases), 17 paucibacillary (PB) patients and 30 healthy donors were studied. Sera were grouped by their antibody spectra using cluster-analysis (Statistica, Release 5.0; StatSoft Inc., 1995). Antigens of *M. leprae* and *M. lufu* were fractionated in PAGE with sodium dodecyl sulfate (SDS-PAGE). The separated antigens were stained with Coomassie blue R-250 and scanned in a GS-700 densitometer (Bio-Rad, Hercules, California, U.S.A.). The spectrum of antibody responses of leprosy patients toward *M. leprae* and *M. lufu* antigens was characterized using Western blotting. The separated proteins were electrophoretically transferred to nitrocellulose paper (NCP) in a Trans-blot Semi-dry Transfer Cell Apparatus (Bio-Rad). The results obtained were calculated by the Molecular Analyst Program (Bio-Rad).

Electrophoresis of the sonicates of *M. leprae* and *M. lufu* showed their similarity by protein spectrum (Fig. 1). Proteins with molecular weights of 6, 12, 25, 35, 42, 50, 57, 65, 76 and 78 kDa were common, and this finding was proved by cluster analysis.

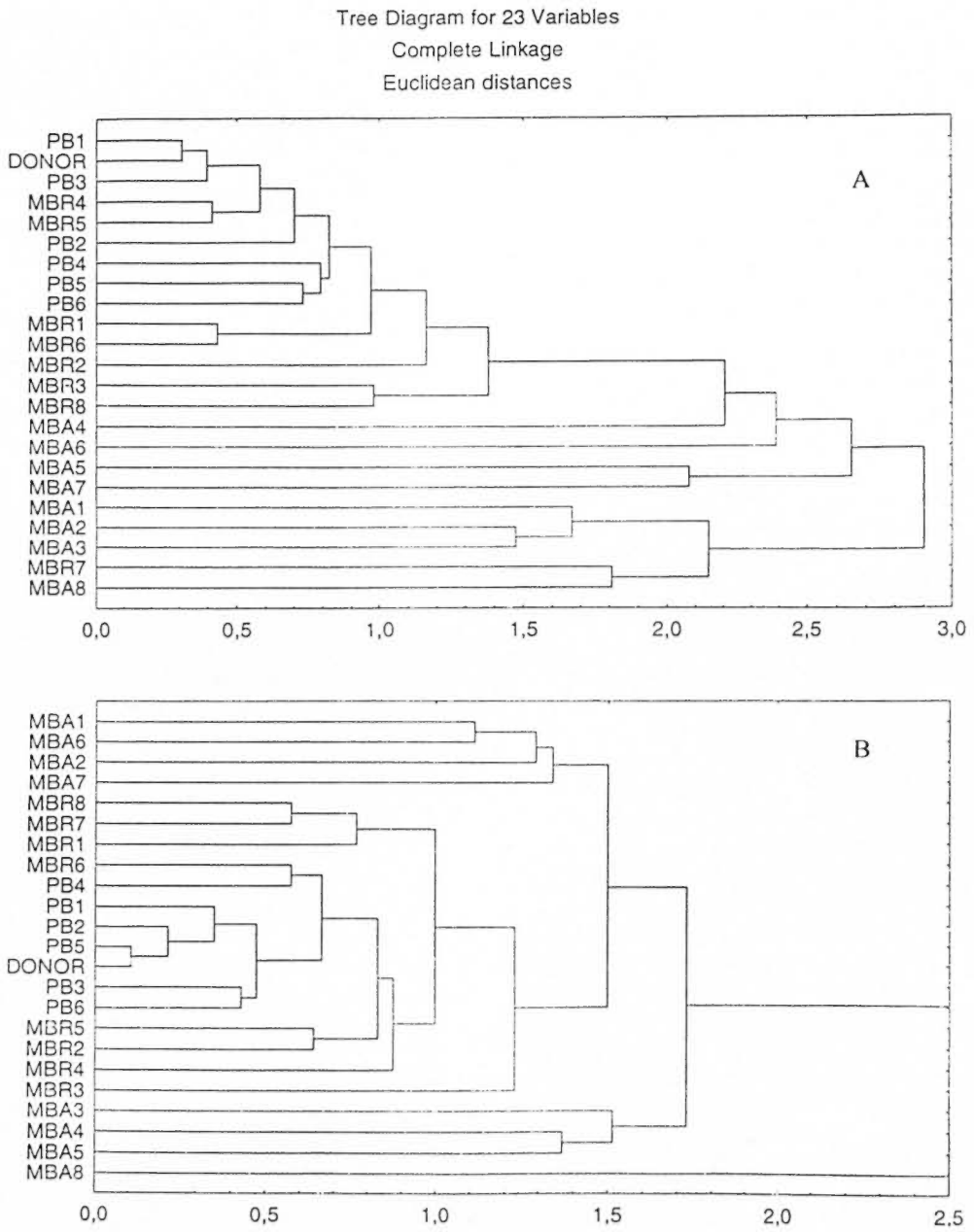

FIG. 3. Cluster analysis of individual blood sera from leprosy patients with *M. leprae* passed in mice (**A**) and *M. lufu* (**B**) antigens. MBA (1–8) = MB patients in active stage of their disease; MBR (1–8) = MB patients in regression; PB (1–6) = PB patients.

The antigenic spectrum of *M. leprae* at the range of 10–12, 25–28, 35–36 and 65 kDa corresponded to the electrophoretic picture of *M. leprae* isolates from nine-banded armadillos as reported by other authors ([2, 8, 9]).

Western blot assay of antibody responses in leprosy patients and healthy leprosy contacts toward different antigenic determinants of *M. leprae* and *M. lufu* showed some peculiarities of many-stripped picture of antibody response toward *M. leprae* antigens in leprosy pa-

tients depending on the form and severity of their disease (Fig. 2); 57% of MB leprosy patients in the active stage showed antibodies toward 64–66 and 80 kDa antigens and among 30% of sera 32–33, 41–42 and 50–60 kDa proteins prevailed. Among MB patients in regression, the antibody spectrum was significantly narrower; 60% of leprosy cases responded toward 17, 20/21, 30–33, 39–41, 62–63 kDa. Sera from PB leprosy patients also strongly responded toward *M. leprae* antigens: 71% of the samples reacted with 17 and 25 kDa; 57% with 24 and 51 kDa, and 43% with 21–22 and 60 kDa proteins.

As for antibody response toward *M. lufu,* patients with active MB leprosy had antibodies against 32, 35, 50 and 70 kDa proteins. In PB patients the antibody response was observed against 17, 20/21, 47–52 and 55–57 kDa proteins. Under the influence of antileprosy therapy, the spectrum of antibody response toward *M. lufu* also changed; 60% of cases responded with antibodies against 20/21, 29–30 kDa antigens and the remaining 40% had antibodies against 17, 31–32, 43–47 and 63–66 kDa. Using *M. leprae* and *M. lufu* as antigens, the similarity of antibody response toward 32 and 50 kDa proteins was found in patients with MB leprosy, and toward 48–52 kDa proteins in PB leprosy. In regressed leprosy, antibodies reacted with 30–32 and 63 kDa. Our results support data of other authors concerning antibody responses of leprosy patients to *M. leprae* antigens at the range of 30–40, 60 kDa ([6]) and 24, 29–45, 55–56 kDa ([8]). Cluster analysis (Fig. 3) showed a clear differentiation of serum samples by forms and stage of leprosy. Sera from PB and MB leprosy in regression were united into proximate groups, clusters of patients with active MB leprosy were the most remote and that corresponded to their clinical and bacterioscopic status.

Thus, a similarity of *M. leprae* and *M. lufu* by their protein profile was found. A comparative analysis of Western blotting data in leprosy patients also showed close similarity of antibody responses toward antigenic preparations of the mycobacterial strains studied. The data obtained will permit using *M. lufu* as an alternative source of antigens for diagnostic and other purposes in leprosy.

—Olga S. Salamatina
Margarita N. Dyachina
Anatoly A. Juscenko
Michail P. Parshin

Leprosy Research Institute
Ostrowsky passage 3
Astrakhan 414057, Russia

—Larisa N. Chernoyusova

Central Research Institute for the Study of Tuberculosis
Russian Academy of Medical Sciences
Moscow, Russia

Reprint requests to Dr. O. S. Salamatina.

Acknowledgment. We would like to thank the WHO Bank for the cell-free soluble (CFS) *M. leprae* (from armadillos) antigen batch CD 146.

REFERENCES

1. Draper, P. Cell walls of *Mycobacterium leprae.* Int. J. Lepr. **44** (1976) 95–98.
2. Ibrahim, M. A., Lamb, F. and Colston, M. J. Analysis of variation in batches of armadillo-derived *M. leprae* by immunoblotting. Int. J. Lepr. **58** (1990) 73–77.
3. Kalyanina, O. V., Irtuganova, O. A. and Urlyapova, N. G. [Screening of drugs for protective antileprosy activity.] Materials of Jubilee Scientific Conference of Astrakhan State Medical Institute, Astrakhan, 1993, pp. 210–214.
4. Kalyanina, O. V., Yushin, M. Yu., Dyachina, M. N., Korneyev, A. A., Chernyousova, L. N., Kalinina, O. A. and Salamatina, O. S. [Biochemical and immunochemical characteristics of cultures of laboratory strains M.01, M.011 and *M. lufu.*] In: Immunodiagnosis and Immune Rehabilitation in Leprosy, Tuberculosis and Other Chronic Diseases; Transaction of Symposium, Astrakhan, 1998, pp. 68–69.
5. Lowry, D. H., Rosebrough, N. J., Farr, A. L. and Randall, E. J. Protein measurement with Folin-phenol reagent. J. Biol. Chem. **193** (1951) 265–275.
6. Patil, S. A., Shivraj, L., Girdhar, B. K. and Sengupta, U. Avidin-biotin immunoblotting studies on reactivity of leprosy sera with *Mycobacterium leprae* antigen. Int. J. Lepr. **60** (1992) 280–283.
7. Portaels, F. Study of unclassified dapsone sensitive mycobacteria isolated from the environment in Zaire. Ann. Soc. Belge Med. Trop. **60** (1980) 381–386.
8. Rada, E. M. S., Santaella, C., Aranzazu, N. and Convit, J. Preliminary study of cellular immunity to *M. leprae* protein in contacts and leprosy patients. Int. J. Lepr. **60** (1992) 189–194.
9. Vadiee, A. R., Shannon, E. J., Gillis, T. P. and

HASTINGS, R. C. Partial characterization of antigens from *M. leprae* evoking IgG and IgM antibodies in armadillos. Int. J. Lepr. **56** (1988) 274–282.

10. YUSHIN, M. YU., DYACHINA, M. N., DEGTYAREV, O. V. and KALYANINA, O. V. [Leprosy serodiagnosis with using of *M. lufu* antigen.] In: Immunodiagnosis and Immune Rehabilitation in Leprosy, Tuberculosis and Other Chronic Diseases; Transactions of Symposium, Astrakhan, 1998, pp. 35–39.