We look forward to the results from the ongoing comparative vaccine immunoprophylaxis trial in progress in Avadi, Tamil Nadu, India, where *Mycobacterium w*, ICRC (contributed by Dr. C. V. Bapat), and killed *M. leprae* plus BCG are under a coded comparative trial.

We hope that the data presented above clarifies any doubt about the identity of *Mycobacterium w.*

—Ashok Mukherjee, M.D.
Syed A. Zaheer, M.B.B.S., D.V.D., Ph.D.
Amar K. Sharma, M.D.
Radhey S. Misra, M.D., D.D.
H. K. Kar, M. D.
Rama Mukherjee, Ph.D.
Gurusaran P. Talwar, D.Sc., F.I.C.A., F.A.Sc., F.N.A.

*Institute of Pathology-ICMR*
*Departments of Dermatology*
*Safdarjang and R.M.L. Hospitals and the National Institute of Immunology*
*New Delhi, India*

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# Leprosy and HIV Infection

To the Editor:

Leprosy and HIV infection are diseases of an endemic nature in Brazil and are a source of great concern for public health agencies. Brazil is second in the world, after India, in absolute numbers of leprosy cases. According to the Brazilian Health Ministry, the overall prevalence is 4–4.5 cases per 1000 inhabitants and 2 per 1000 in the Ribeirão Preto region. With respect to AIDS, Brazil is among the five countries in the world with the largest absolute number of recorded cases. According to the World Health Organization, the incidence of AIDS recorded in 1990 was 22.9 per 100,000 inhabitants. According to the Health Department of the state of São Paulo, the incidence of AIDS in the city of Ribeirão Preto is 100.7 per 100,000 inhabitants, and among injectable drug users the HIV prevalence is still 46.5 per 100,000 inhabitants.

The association of HIV infection with infection by mycobacteria such as *Mycobacterium avium* and *M. intracellulare* has been reported ([1]). Over the last few years, there have been reports of HIV infection associated with *M. leprae* infection. In the present report, we describe the first case of such

association treated at University Hospital, Faculty of Medicine Ribeirão Preto, University of São Paulo.

The patient was a 26-year-old, unmarried male mulatto, a carpenter by profession, living in Ribeirão Preto city without risk factors for leprosy such as family contacts or residence in a hyperendemic part of the region. Seven years ago he presented with skin lesions of the erythema nodosum type, as well as erythematous-edematous and nummular lesions with purple centers on the upper and lower limbs, together with fever, generalized neuritis, orchitis and bilateral inguinal adenomegaly. The diagnosis was lepromatous leprosy with type 2 reaction confirmed by skin biopsy, positive earlobe and skin lesion bacilloscopy, and a negative intradermal Mitsuda reaction. C-Reactive protein was positive. He was treated with a combination of rifampin (600 mg/day for 2 months) and dapsone (100 mg/day). Thalidomide (200 mg/day) was prescribed for 2 weeks to control the reaction. Over the subsequent months, the patient presented a number of reactional episodes of erythema nodosum leprosum (ENL) which were controlled with thalidomide. After 2 years and 9 months of leprosy treatment, he was admitted to the hospital with signs and symptoms similar to his original ones, plus generalized lymph node enlargement and discrete hepatomegaly. The hypothesis of a reactional episode of ENL was first advanced and a C-reactive protein test was positive. Thalidomide (300 mg/day) was prescribed and his signs and symptoms regressed after 10 days, although the lymph node enlargement, hepatomegaly, and fever persisted. After an exhaustive investigation, no infectious disease was detected.

At that point, the patient revealed that he had been using injectable drugs up to 2 years before. An anti-HIV test by enzyme-linked immunosorbent assay (ELISA) was negative. Ten months later, he returned with ENL reactions as described above and, again, no infectious disease was detected. Quantitation of C-reactive protein and earlobe and skin lesion bacilloscopy were negative. Within 2 days, the patient presented behavioral disorders, meningism, unconsciousness, and convulsive seizures. A spinal tap was performed and cerebrospinal fluid analysis revealed hypercellularity with a predominance of lymphocytes and increased proteins, with no other changes. The anti-HIV antibody test (ELISA) was positive (> 2000). At the end of the second day after admission the patient died. An autopsy was not performed.

Three years after the leprosy diagnosis, his anti-HIV antibody serologic test (ELISA) was negative although generalized lymph node enlargement of undefined etiology persisted. During treatment for his leprosy the patient presented a few episodes of type 2 reaction (ENL) which responded to thalidomide. During the last admission, the C-reactive protein test performed at the time of his serum conversion (anti-HIV antibody >2000) was negative. Since high levels of C-reactive protein are commonly observed in the type 2 reaction in leprosy ([2]), the generalized lymph node enlargement probably was not related to a reactional episode but, rather, to HIV infection. The stage of the viral infection could not be classified because the patient died within a few days.

The association of leprosy with HIV infection may not be random and perhaps may be more common than thought since the period of incubation of leprosy is very long and the patient may die of HIV infection before presenting overt clinical manifestations of leprosy. Furthermore, lepromatous leprosy may make the patient more susceptible to HIV, since leprosy also presents immunodepressant factors ([3]). Thus, we believe that studies of the detection of HIV infection should be continued in areas where leprosy is endemic, because of both the risk of dissemination of these mycobacteria and for a better understanding of the course of these patients.

—Ana Marcia de Almeida, M.D.
Ana Maria Ferreira Roselino, M.D., Ph.D.
Norma Tiraboschi Foss, M.D., Ph.D.
*Department of Internal Medicine (Dermatology)*
*Faculty of Medicine of Ribeirão Preto*
*University of São Paulo*
*Ribeirão Preto, S.P., Brazil*

Reprint request to Prof. A. M. de Almeida, Departamento de Clinica Medica, Faculdade de Medicina de Ribeirão Preto, 14049-900 Ribeirão Preto, SP, Brasil.

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# A Latex Agglutination Test for Rapid Serodiagnosis of Leprosy in the Field

To the Editor:

At present leprosy is diagnosed and classified according to clinical, histopathological, and bacteriological criteria ([1]). Nevertheless, in many developing countries where leprosy is endemic resources for these purposes are often very limited. Therefore, it is necessary to have simple, cost-effective, and rapid methodologies which can be complementary to, or even substitute for, conventional diagnostic procedures. The *Mycobacterium leprae*-specific phenolic glycolipid (PGL-I) in the semisynthetic disaccharide octyl bovine serum albumin (ND-O-BSA) form ([5]) has been widely used in indirect enzyme-linked immunosorbent assay (ELISA) techniques to detect anti-PGL-I antibodies in a number of diagnostic and epidemiologic studies (e.g., [2,7,11]). Even though the ELISA has many advantages, being fast, reproducible and quantitative, it does need the framework of an established laboratory and trained personnel.

The objective of this work was to develop a simple latex particle agglutination assay (LPAA) that can be performed under subtropical and tropical field conditions by untrained personnel which would give an easy, visual read-out within 5 minutes and which is cost-effective.

Latex particles (Estapor, KI-080, azure; Rhone-Poulenc, Aubervilliers, France) were covalently coupled with 400 μg/ml ND-O-BSA, obtained through WHO IMMLEP, according to the manufacturer's instructions (Rhone-Poulenc). Finally, precipitated antigen coupled latex particles were resuspended in 0.5 ml of the binding buffer (50 mM $Na_2PO_4$, 17 mM NaCl, 0.025% v/v Nonidet P-40, 0.1% w/v gelatin, 2% w/v Kollidon VA64, pH 6.6). A 5-μl suspension was spotted onto Perlalux Duplex rigid plastic films (Papierfabrik Perlen, Perlen, Switzerland) as a dot. The dots were dried at 40°C for 1 hr and stored at room temperature until used. Stability tests for binding to the plastic film, solubility, agglutination, and sensitivity were performed after 1 week, 2 weeks, 1 month, 2, 3, 6, 9, and 12 months using a standard pool of leprosy sera with a shelf-life of at least 12 months when stored at 15°C–35°C and 40%–80% humidity (data not shown). Hereby the criteria for use under subtropic and tropic conditions without refrigerating were obtained.

For the LPAA, 10 μl undiluted serum or twofold dilutions made in phosphate buffered saline, pH 7.2, of negative control serum, positive control serum and the unknown serum sample were added to the dried latex dots. After 60 sec, the dots were resuspended with a spatula and mixed gently. Four min later the results were read by visual inspection. The antibody titer was expressed as the highest dilution giving agglutination. A sample was considered positive when the titer value was 2 or more, determined on the basis of three times the standard deviation of the Ethiopian control group. The ELISA was used to test the reliability of the LPAA. The assay method was similar in principle to the one described by Cho, *et al.* ([5]). However, 2.5% w/v casein and 0.05% v/v Tween 20 were used as the blocking agent instead of 5% w/v BSA. Serum samples were tested as twofold dilu-