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Low Rates of Detection of Mycobacterial Secretory Antigen 85 in Sera of Untreated Leprosy Patients

To the Editor:

Leprosy continues to be one of the major infectious diseases, affecting about 2.4 million people worldwide (estimate for December 1993) ([7]), despite concerted efforts to control this disease. The World Health Assembly call for the elimination of leprosy by 2000 A.D. and the World Health Organization (WHO) recommendation of the use of fixed duration multidrug therapy (WHO/MDT) has made the monitoring of therapy a matter of importance. In this regard, mycobacterial proteins, such as the antigen 85 (Ag85) complex, actively secreted by growing microbacteria ([8, 13]), were used for the assessment of MDT in leprosy.

Sera of leprosy patients were used for the concurrent detection of Ag85 (secretory protein) and Ag82 (cytoplasmic 65-kDa heat-shock protein) by ELISAs. A ratio of the secretory to the cytoplasmic antigen (SCR) indicated secretory efficacy and, hence, viability of *Mycobacterium leprae*. Only 6 of 21 (28.5%) untreated multibacillary (MB) and 11 of 34 (32.3%) untreated paucibacillary (PB) patients showed significant positivity for Ag85, although among the positive cases the SCR was high (> 1) and Ag85 levels between 0.1–10 ng/ml were detected, indicating bacterial viability. Immune complexes, which have been demonstrated in MB patients' sera ([6, 12]) and which could lower the level of free antigen detection, were suspected to be the cause of such low sensitivity. However, immune complex dissociation attempted with MB sera failed to improve sensitivity. Furthermore, no correlation was apparent between the bacterial index (BI) in skin smears and the positivity in ELISA.

The low sensitivity in ELISA could not be attributed to the lack of antigen secretion by intracellular bacilli since the presence of Ag85 in the skin and nerve tissue of untreated leprosy patients was clearly demonstrable by immunocytochemistry. Skin sec-

tions showed intense staining within foamy macrophages, sweat and sebaceous glands and endothelial cells with the antigen packaged in large macrophage granulomas, leaving the surrounding connective tissue free of stain. This lack of staining in connective tissue lends itself to several interpretations: a) lack of diffusion of antigen from macrophages, b) breakdown of antigen in connective tissue, c) binding of Ag85 to components of the connective tissue (e.g., fibronectin) ([1]), thus altering the configuration of its antibody-binding epitopes.

Nerve biopsies from untreated MB cases also showed diffused straining within macrophages in the neural granuloma but not in the extracellular matrix, Schwann cells or endothelial cells. Although the overall pattern remains unchanged, the tissues of untreated PB patients showed comparatively less Ag85 than did MB cases. The virtual absence of secretory antigen in Schwann cells was concordant with the lack of antigen detection in *M. leprae*-infected, murine Schwann cell cultures despite an average 15–20-fold multiplication of intracellular bacilli observed in these cultures ([2]).

On the other hand, if growth of *M. Leprae* takes place within macrophages in tissues, release of mycobacterial secretory antigens in the extracellular milieu is expected since *M. tuberculosis* H37Rv-infected macrophages secrete significant quantities of Ag85 in culture supernatants during the growth phase ([5]).

In the case of *M. leprae*-infected macrophages in tissue culture, Ag85 remains undetectable in culture supernatants despite overloading of the host cells and the use of protease inhibitors, such as aprotonin, to minimize antigen degradation by macrophage proteases. In such short-term cultures active growth of *M. leprae* is not possible although intracellular maintenance of bacilli and slow growth within macrophages has been relatively well established ([9, 11]).

Both armadillos, during experimental leprosy infections, and leprosy patients show the presence of anti-Ag85 antibodies ([4, 10, 14]), signifying its functional presence. It is possible, however, that sensitization is due to the cell-bound forms of the antigen rather than the secreted form. However, antibody detection for the monitoring of therapy has its inherent ambiguities ([3, 4, 10]) and monitoring of therapy would be infinitely more successful if antigens reflecting viable bacteria could be demonstrated. A demonstration of the process of modification of established secretory antigens or production of novel antigens by leprosy bacilli may give a much needed fillip to this objective.

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Prevalence of Antibodies to Hepatitis C Among Recently Treated Leprosy Patients in Senegal Parallels Those in Normal Populations

TO THE EDITOR:

Recent reports focused on a higher prevalence of hepatitis C (HCV) antibodies (Abs) in leprosy patients than in matched uninfected individuals ([3, 5]). Having followed a 175-patient cohort under field conditions, we do not confirm a higher carriage of hepatitis C among leprosy patients in Sénégal, and we suggest that the high levels previously observed might rather be associated with epidemiological conditions within the populations studied.

Infection with *Mycobacterium leprae* is accompanied by a profound alteration of cellular immunity leading to the different aspects of the leprosy spectrum. Little is known about the exact influence of the lack of T-cell immunity observed in leprosy and the susceptibility to viral infections. In this line, we investigated the seroprevalence to HCV using sequentially 2 third-generation ELISAs (Ortho HCV 3.0®; Chiron Corp., Emeryville, California, U.S.A.; Murex anti-HCV version III, Templehill, England) and an immunoblot assay (Ortho RIBA 3.0.®; Chiron). Genotyping of positive HCV sera was carried out using an ELISA revealing circulating antibodies to recombinant NS4 proteins (Murex HC02) ([1]). Two other markers of viral infection were also examined, i.e., the presence of hepatitis B virus antigen (HBs Ag) and HIV antibodies using commercially available kits; Monolisa® and Elavia mixte®, respectively (Sanofi-Pasteur, Marne la Coquette, France).

Subjects consisted of 147 newly diagnosed patents and 28 previously treated (monotherapy) patients entering a new survey for the efficacy of a fully supervised, intermittent, single-dose poly-antibiotherapy. Enrollment was done from March to December 1995; patients received full information regarding this investigation and an initial blood sample was taken at the time of the enrollment visit. During the survey, serum was kept in a dry sterile tube at 4°C (for approximately 12 hr) in the field and then frozen at –20°C until further assays.

A similar prevalence of the viral markers was found in sera from leprosy patients and a normal population. The HIV antibody prevalence reported here is in line with previous studies conducted on West African populations ([2, 6]). Secondly, the HBs antigen carriage found in this study was not higher than that found in recent determinations