A Longitudinal Study of Immunologic Reactivity in Leprosy Patients Treated with Immunotherapy[1]

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Leprosy is a chronic granulomatous disease caused by *Mycobacterium leprae*. The diverse clinical forms of the disease are closely related to the degree of cell-mediated immunity of the patient. The role played by regulatory T lymphocytes has been reported, mainly of helper phenotype CD4+ cells ([13]). Patients with tuberculoid leprosy, who develop typical granulomatous lesions formed by well-differentiated macrophages with few intracellular bacilli and many CD4+ T cells surround by a cuff of CD8+ cells, show strong cell-mediated immunity and low levels of antibodies against phenolic glycolipid-I (PGL-I). Patients with lepromatous, borderline lepromatous and Mitsuda-negative indeterminate leprosy do not develop resistance nor cell-mediated reactions associated with delayed-type hypersensitivity. Whether this type of persistent anergy reflects a primary macrophage defect resulting in inadequate antigen presentation or other mechanisms has not been clarified. The inappropriate response results in an inadequate cell-mediated immune response characterized, together with other features, by activation of suppressor cell populations ([10, 14]) and lack of an adequate stimulus of effector T cells. In some cases this defect can be restored *in vitro* by adding exogenous lymphokines ([6]) or by stimulation with components of fractionated mycobacteria ([6, 15]). In multibacillary leprosy, antibody levels can be used to monitor the elimination of the bacillary load in patients submitted to various types of treatment ([1, 3, 12]).

In the search for a vaccine against leprosy, a mixture of heat-killed *M. leprae* and viable BCG has been evaluated in the immunoprophylaxis and immunotherapy of the disease at the Institute of Biomedicine, Caracas, Venezuela. Convit, *et al.* ([4, 5]) have reported clinical and bacteriological improvement as well as the development of a positive skin-test reactivity in 60% to 70% of borderline lepromatous and lepromatous patients after 8 to 10 doses of the combined vaccine. An independent study has demonstrated histopathological changes toward more resistant forms of the disease in a high percentage of these patients ([11]).

In this study, we have evaluated the response of patients submitted to immunotherapy (IMT) with this combined vaccine together with multidrug therapy (MDT) during a 5- to 10-year period. Based on the use of multiple criteria, we have been able to analyze the diverse manifestations of the immune responses in these patients, most of whom had multibacillary disease. The parameters evaluated included determinations of the levels of antibodies against PGL-I, lymphocyte transformation assays, bacterial indexes and intradermal tests before each IMT treatment and at regular intervals for a period of at least 5 years.

MATERIALS AND METHODS

Patients. The patients were seen in the Clinical Section of the Institute of Biomedicine. Clinical, bacteriological and histopathological criteria were used to classify the form of disease according to the Ridley-Jopling scale ([16]). Twenty percent of the multibacillary patients had received sulfone therapy for more than 20 years prior to IMT and the remainder had received previous chemotherapy for an average of 6 years; all received multidrug therapy (MDT) together with IMT in this evaluation. The study in-

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té suivis pendant 5 à 10 ans avec détermination ré-étée des taux d'anticorps vis-à-vis du glycolipide phé-olique-I, des réponses de prolifération lymphocytaire PLT) à de l'extrait soluble de *M. leprae,* à des bacilles ntiers et au BCG, des réponses à des tests cutanés et es indices bactériens (IB). Après PCT plus IMT, il y vait une diminution statistiquement significative des aux d'anticorps dans le groupe des multibacillaires MB). L'IB diminuait de manière proportionnelle aux ésultats du test ELISA. La PLT augmentait vis-à-vis es antigènes de *M. leprae,* particulièrement vis-à-vis e l'extrait soluble, dans un grand pourcentage de ces atients (34% des patients LL positifs). La positivité à a lépromine augmentait chez les patients MB, de 5% ositifs initialement à 75% durant cette période. Ces ésultats montrent une réactivité à médiation cellulaire ignificative, précoce et persistante vis-à-vis de *M. lep-ae* chez de nombreux patients MB taités par PCT-MT, ce qui confirme et développe des données pu-liées antérieurement.

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REFERENCES

1. Bach, M.-A., Wallach, D., Flageul, B., Hoffenbach, A. and Cotten-T, F. Antibodies to phenolic glycolipid-I and to whole *Mycobacterium leprae* in leprosy patients; evolution during therapy. Int. J. Lepr. **54** (1986) 256–267.
2. Brennan, P. J. and Barrow, W. W. Evidence for species-specific lipid antigens in *Mycobacterium leprae.* Int. J. Lepr. **48** (1980) 382–387.
3. Chaturvedi, R. M., Chirmule, N. B., Yellapurkar, M. V., Shaikh, S. V. and Madhar, G. D. Effects of ICRC antileprosy vaccine in healthy subjects. Int. J. Lepr. **55** (1987) 657–666.
4. Convit, J., Aranzazu, N., Ulrich, M., Pinardi, M. E., Reyes, O. and Alvarado, J. Immunotherapy with a mixture of *Mycobacterium leprae* and BCG in different forms of leprosy and Mitsuda-negative contacts. Int. J. Lepr. **50** (1982) 415–424.
5. Convit, J., Ulrich, M., Aranzazu, N., Castellanos, P. L., Pinardi, M. E. and Reyes, O. The development of a vaccination model using two microorganisms and its application in leprosy and leishmaniasis. Lepr. Rev. **57** (1986) 263–273.
6. Haregewoin, A., Godal, T., Mustafa, A. S., Belehu, A. and Yemaneberham, T. T-cell conditioned media reverse T-cell unresponsiveness in lepromatous leprosy. Nature **303** (1983) 342–344.
7. Hunter, S. W. and Brennan, P. J. A novel phenolic glycolipid from *Mycobacterium leprae* possibly involved in immunogenicity and pathogenicity. J. Bacteriol. **147** (1981) 728–735.
8. Lee, S. P., Stoker, N. G., Grant, K. A., Handzel, Z. T., Hussain, R. M., McAdam, K. P. W. J. and Dockrell, H. M. Cellular immune responses of leprosy contacts to fractionated *Mycobacterium leprae* antigens. Infect. Immun. **57** (1989) 2475–2480.
9. Leiker, D. L. and McDougall, A. C. *Technical Guide for Smear Examination for Leprosy.* Amsterdam: Leprosy Documentation Service, 1983, pp. 7–29.
10. Mehra, V., Brennan, P. J., Rada, E. C., Convit, J. and Bloom, B. Lymphocyte suppression in leprosy induced by unique *M. leprae* glycolipid. Nature 308 (1984) 194–195.
11. Meyers, W. M., McDougall, A. C., Fleury, R. N., Neves, R., Reyes, O. and Binford, C. H. Histologic responses in sixty multibacillary leprosy patients inoculated with autoclaved *Mycobacterium leprae* and live BCG. Int. J. Lepr. **56** (1988) 302–309.
12. Miller, R. A., Gorder, D. and Harnisch, J. P. Antibodies to phenolic glycolipid-I during long term therapy; serial measurements in individual patients. Int. J. Lepr. **55** (1987) 633–645.
13. Modlin, R., Gersuk, G. M., Nelson, E. E., Patengale, P. K., Gunter, J. R., Chen, L., Cooper, C. L., Bloom, B. and Rea, T. H. T-lymphocyte clones from leprosy skin lesions. Lepr. Rev. **57** (1986) 143–147.
14. Rada, E., Convit, J., Ulrich, M., Gallinoto, M. E. and Aranzazu, N. Immunosuppression and cellular immunity reactions in leprosy patients treated with a mixture of *Mycobacterium leprae* and BCG. Int. J. Lepr. **55** (1987) 646–650.
15. Rada, E., 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.
16. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity; a five-group system. Int. J. Lepr. **34** (1966) 255–276.
17. Ulrich, M., Smith, P., Sampson, C., Zuñiga, M., Centeno, M., Garcia, V., Manrique, X., Salgado, A. and Convit, J. IgM antibodies to native phenolic glycolipid-I in contacts of leprosy patients in Venezuela; epidemiological observations and a prospective study of the risk of leprosy. Int. J. Lepr. **59** (1991) 405–415.
18. Waters, M. F. R., Ridley, D. S. and Lucas S. B. Positive Mitsuda lepromin reactions in long-term treated lepromatous leprosy. Lepr. Rev. **61** (1990) 347–352.