Immunosuppression and Cellular Immunity Reactions in Leprosy Patients Treated with a Mixture of *Mycobacterium leprae* and BCG[1]

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Leprosy patients present different clinical, histological, and immunological forms of disease throughout a wide spectrum. The benign form, tuberculoid leprosy, is characterized by an active response measured *in vitro* by a T-cell proliferation assay. In the multibacillary malignant form, lepromatous leprosy, T cells do not proliferate in the presence of specific and crossreacting antigens of *Mycobacterium leprae* (6).

The basic immunologic defect(s) in lepromatous leprosy producing reduced or absent cellular immunity to *M. leprae* is as yet not fully understood (7). Assays for suppression of the immune response by suppressor T cells or monocytes in lepromatous leprosy patients have been presented (9). Exposure of T cells and monocytes from patients with lepromatous, but not tuberculoid, leprosy to Dharmendra lepromin preparations or to *M. leprae* phenolic glycolipid (8) suppressed the *in vitro* mitogenic response of their lymphocytes to concanavalin A. However, other assays to detect disease-related suppression in lepromatous leprosy have provided conflicting results (1, 13, 16).

The purpose of this study was to evaluate the effect of treating lepromatous patients with a mixture of *M. leprae* and BCG on the reactivity of their lymphocytes in the suppressor-cell assay described by Mehra, *et al.* (9). In addition, proliferative responses to diverse preparations of *M. leprae* and *in vivo* responses of untreated and treated patients have been compared in this study.

[1] Received for publication on 5 March 1987; accepted for publication in revised form on 12 June 1987.

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MATERIALS AND METHODS

Isolation of mononuclear cells. Mononuclear cells were obtained from 20 ml of heparinized blood from lepromatous patients and normal volunteers by centrifugation on Ficoll-Hypaque gradients (2). After three washes, the cells were resuspended in RPMI 1640 (GIBCO Laboratories, Grand Island, New York, U.S.A.) with 10% of a pool of human AB serum, 100 U penicillin, 100 μg streptomycin, and 2 μmol glutamine/ml. The cells were cultured at 2×10^5/0.2 ml medium in microtiter plates. The lepromatous patients were studied before initiation of immunotherapy and after having received two to seven vaccinations with a mixture of *M. leprae* and BCG (4) in proliferation and skin test assays; suppression assays were performed before immunotherapy and after 2 years (eight to ten vaccinations).

Suppression assay. For the development of this study, we used the same experimental conditions employed by Mehra, *et al.* (9). The antigen used in immunosuppression was Dharmendra lepromin (prepared by Dr. M. Abe, National Institute for Leprosy Research, Tokyo, Japan). Concanavalin A (ConA; Sigma Chemical Co., St. Louis, Missouri, U.S.A.) 2 μg/2×10^5 cell and 10 μl of Dharmendra lepromin were added. The cultures were incubated in 95% air-5% CO_2 for 3 days. They were treated with 1 μCi ^{3}H-thymidine (specific activity 1 Ci/mole) 18 hr before harvesting, and the cells were processed for liquid scintillation.

The percentage of suppression was calculated as follows:

$$\%S = 100 - \frac{\text{counts per minute (CPM) ConA + lepromin}}{\text{cpm ConA}} \times 100$$

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