Department of Anatomy
Charing Cross Hospital
Medical School
Fulham Palace Road
London W6 8RP, England

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Reply to Dr. Crawford's Letter to the Editor

TO THE EDITOR:

I am grateful for the opportunity to reply to Dr. Crawford's letter on this interesting subject and hasten to apologize to him for overlooking his previous observations on the presence of inflammatory cell infiltrates in biopsies from patients with edema of the extremities. His belief that a sensory polyneuritis may occur in these patients in the absence of bacilli, with serious consequences, confirms my suspicion that the subject of edematous swelling of the extremities in patients with borderline reactions has never been fully investigated and explained. If there is indeed an inflammatory infiltrate of cells in these edematous areas, *unrelated* to the obvious skin lesions of borderline leprosy, then the subject is surely worth further investigation since it may reveal something new concerning the pathogenesis of leprosy, at least in this part of the spectrum.

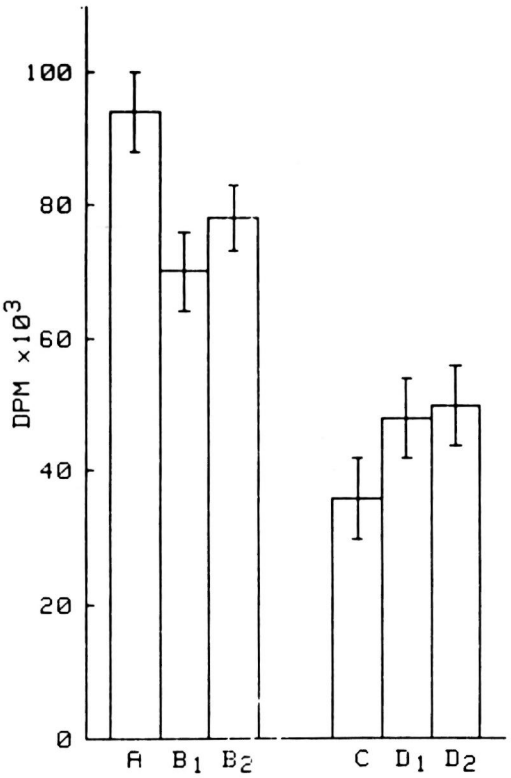
—A. Colin McDougall, M.D., F.R.C.P.
The Slade Hospital
Headington
Oxford OX3 7JH England

Adherent Suppressor Cells in Polar Lepromatous Leprosy

TO THE EDITOR:

Patients with polar lepromatous leprosy exhibit tolerance to *M. leprae* specific antigens in a variety of systems to test for delayed-type hypersensitivity and cell-mediated immunity. We wish to report results of preliminary investigations into the type of cells responsible for this immunological tolerance.

Mononuclear cells from three patients with lepromatous leprosy were isolated from whole blood by conventional methodology ([1]). The patients had been treated with dapsone (DDS) for one to two years and had not experienced complications during the period of treatment. Adherent cells were removed from the mononuclear cell suspension in a two-step procedure. In brief, the mononuclear cells were cultured on plastic petri dishes in RPMI 1640 containing 20% heat inactivated fetal calf serum but without antibiotics for 2 hr at 37°C in 5% CO_2 and 98% humidity. Nonadherent cells were removed by decantation and incubated overnight under the same conditions. After overnight incubation, nonadherent cells were removed and set up in culture. The procedure was, in our hands, highly effective and resulted in a depletion of adherent cells, which are activated mac-



A = whole cells plus concanavalin A, mean ± S.D., N = 3.

B_1 = whole cells plus concanavalin A plus Dharmendra, mean ± S.D., N = 3, $p < 0.005$ between B_1 and A.

B_2 = whole cells plus concanavalin A plus *M. leprae*, mean ± S.D., N = 3, $p < 0.010$ between B_2 and A.

C = cells depleted of adherent cells plus concanavalin A, mean ± S.D., N = 3.

D_1 = cells depleted of adherent cells plus concanavalin A plus Dharmendra, mean ± S.D., N = 3, $p < 0.005$ between D_1 and C.

D_2 = cells depleted of adherent cells plus concanavalin A plus *M. leprae*, mean ± S.D., N = 3, $p < 0.005$ between D_2 and C.

THE FIGURE. The effect of Dharmendra antigen and *M. leprae* on the lymphocyte transformation of mononuclear cells from polar lepromatous leprosy patients in response to concanavalin A. Statistical tests were performed using the Student's *t* test.

rophages, and permitted us to retain for cultures a percentage of nonadherent cells showing monocyte characteristics by staining with peroxidase and nonspecific esterase. This percentage of 5.0 ± 0.9% is compulsory for lymphocyte blast transformation tests. Three day cultures were set up with concanavalin A using either unfractionated mononuclear cells or mononuclear cell suspensions depleted of adherent cells. Cells exposed to *M. leprae* specific antigens (either Dharmendra antigen or armadillo-derived integral *M. leprae*) were handled in an identical fashion except that they were exposed to the antigens for a period of three days before the addition of concanavalin A. The antigens remained in the cultures during the final three days' incubation with concanavalin A. The cultures were pulsed with ^{3}H-thymidine and counted.

We observed (The Figure) that unfractionated mononuclear cells from these lepromatous cases are suppressed in their response to concanavalin A by either Dharmendra antigen or integral *M. leprae*; cells which have been depleted of adherent cells show enhanced ^{3}H-thymidine incorporation in the presence of Dharmendra antigen or integral *M. leprae*. Thus, the cells responsible for suppression appear to be adherent cells which are activated macrophages. These macrophages or supernatants from their culture are able to suppress blast transformation in mixed leukocyte cultures with cells from the blood of healthy individuals (data not shown). This suggests the production of a "monokine" by these macrophages, but the mechanisms of this inhibitory effect are not clear and require further investigation.

—Pierre-Richard Ridel
—Pierre Saint-André, M.D.
—Gerard Baquillon, M.D.
—Claude Ferracci, M.D.
—Pierre Boucher, M.D.

Institut Marchoux
B.P. 251
Bamako, Mali

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