Aggregation Phenomenon of Cultivated Blood Macrophages Isolated from Leprosy Patients

TO THE EDITOR:

We report on the aggregation phenomenon of cultivated blood macrophages derived from leprosy patients. Macrophages that were isolated formed aggregates over a coverslip (Fig. 1) in almost all of the patients with progressive stages of leprosy, such as new patients, many patients with erythema nodosum leprosum (ENL), and those having a relapse and leprous neuralgia. On the other hand, macrophages isolated from patients with leprosy in the quiescent stages were almost always dispersed (Fig. 2). Thirteen of the 16 patients whose cells formed aggregates were in a progressive stage of the disease and 17 of the 20 patients whose cells were dispersed had quiescent leprosy. As described later, the blood macrophages were isolated from leukocytes in supernatant plasma following red cell precipitation. However, as is well known, no plasma can be isolated from healthy individuals through this method. Thus, we could not use healthy subjects as controls. The differences between the two patient groups were statistically significant ($p < 0.01$, χ^2).

Subjects used in this study were randomly selected from among patients treated at the National Leprosarium Tama-Zensyo-En, without regard to age, sex, type or stage of leprosy. Fifteen ml of heparinized blood was collected from each patient using a 30-ml plastic syringe. After collection, the blood stood for 1 hr at room temperature; the red cells were precipitated; and approximately 7 ml plasma was harvested as a supernatant in which almost all of the monocytes were floating. The plasma was mixed with penicillin 100 U/ml, and approximately 1 ml of the plasma was poured into a plastic Leighton tube (Costar Co., Cambridge, Massachusetts, U.S.A.) with a Lux Thermanox coverslip (Miles Laboratories, Inc., Naperville, Illinois, U.S.A.), and cultivated in a CO_2 incubator at 37°C. Each coverslip was removed 3 days after cultivation, fixed with methanol, and stained with Giemsa's solution.

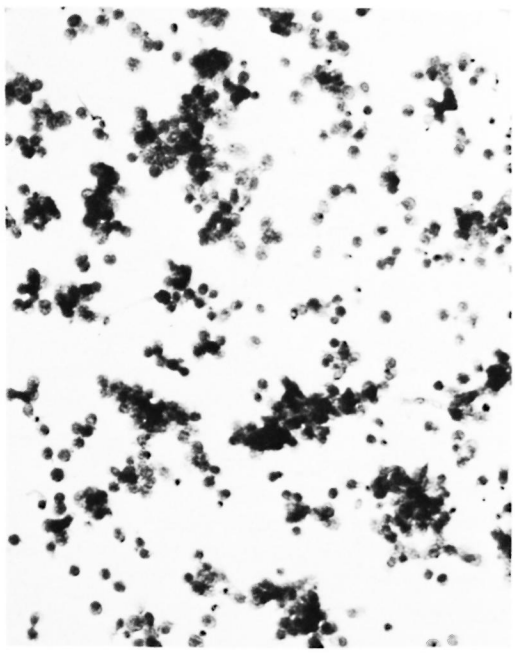

FIG. 1. Cell aggregate form of macrophages isolated from a patient in a progressive stage of leprosy (×100).

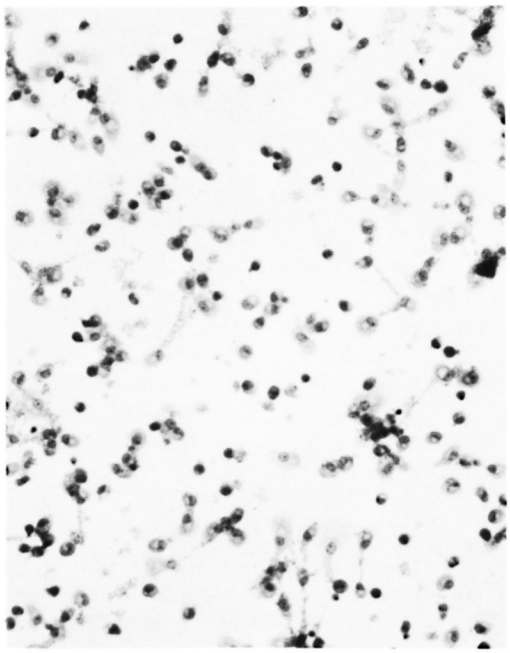

FIG. 2. Cell dispersion form of macrophages isolated from a patient in a quiescent stage of leprosy (×100).

There are a number of reports thought to be related to our study ([1-6]). However, we see no direct relation between these findings and ours, nor are there reports suggesting that macrophages in leprous plasma react with something during cultivation. The nature of this cell aggregation is such that we think the macrophages could be reacting with antigens in the plasma of leprosy patients, and forming a granuloma *in vitro*. These results indicate that cell aggregation can be used as an indicator of the progressive stages of leprosy.

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Cultivation of *Mycobacterium leprae*: A New Approach

To the Editor:

We wish to report our recent findings on our *Mycobacterium leprae* culture isolates-ICRC strains which may throw new light on the problem of cultivation of *M. leprae in vitro* and on the origin of leprosy-derived mycobacteria (LDM). In the course of our investigation on antigenic differences among *M. leprae* culture isolates and on the development of an improved vaccine, we discovered that the ICRC bacilli are not pure cultures, but consist of two mycobacteria with distinctive properties.

Since 1958, in our attempts to grow *M. leprae in vitro,* we have isolated several strains of mycobacteria designated as ICRC bacilli ([7, 8]). These cultures have been grown exclusively on liquid media, e.g., tissue culture conditioned medium and enriched Dubos' medium ([8, 21]) (The Table). On the other hand, the majority of LDM are grown on solid media, and are found to belong to the *M. avium-intracellulare-scrofulaceum* (MAIS) complex. Since some of the ICRC strains isolated during 1958–1961 had shown lepromin-like reactivity, the only marker of *M. leprae* identification available at the time, we continued the work on new isolates with detailed studies on some of the strains (The Table). They were found to share some *M. leprae*-specific characteristics, e.g., lepromin-like activity ([6]), growth in the mouse foot pad ([5]), DOPA-oxidase activity ([22]), and antigenic behavior like that of *M. leprae* in mice ([1, 20]). However, they express a biochemical profile like that of *M. avium-intracellulare* ([8, 21] and Kato, L., personal communication, 1979). A candidate antileprosy vaccine prepared from one of the strains was found to induce lepromin