to ascertain whether the inability of T lymphocytes from LL patients to proliferate in response to PHA stimulation was also reversible by preincubating those cells.

This hypothesis was not confirmed; our results showed that in only 3 of 23 cases did T lymphocytes from LL patients recover proliferation capacity. These results are not in contradiction with those obtained by Mohagheghpour, *et al.* who used specific antigen for proliferation of T cells. They only show that PHA cannot induce proliferation under the above-mentioned conditions, that perhaps the failure of T lymphocytes to respond to the mitogen stimulus is due to inadequate calcium metabolism, T-cell cycle and IL-2 synthesis ([8]) and not to the steric obstruction of the receptor to PHA with *M. leprae* antigen.

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Mycobacterial Cell Surface Proteins Revealed by Labeling with ^{125}I

To the Editor:

Surface antigens of infecting organisms play an important role in host immunity, since they are the first antigens encountered by the cells of the immune system. In our laboratory, ICRC bacilli—mycobacteria cultured from biopsies of leprosy patients (mainly *Mycobacterium avium-intracellulare*)—are used to prepare an antileprosy vaccine ([4]). A very high molecular weight, glycolipoprotein fraction of these bacilli, named PP-I, has been found to have good immunogenicity; hence, it is used for the preparation of a subunit vaccine ([1]). Since this PP-I is purified from the sonicate of ICRC bacilli, its exact location was not known. However, its chemical composition suggested that it may be a cell-wall component. We have now studied this using the technique of iodination of surface proteins in intact cells.

The ICRC bacilli were cultivated as described earlier ([3]). The organisms were harvested after 12 to 13 days of culture, washed three times with sterile phosphate buffered saline (PBS), and then labeled with ^{125}I using the iodogen method ([5]). In brief, 10^9 bacilli suspended in 50 μl of PBS were added to a tube coated with 100 μg of iodogen. Carrier-free Na ^{125}I (0.5 mCi; Amersham, U.K.) was then added and the mixture kept on ice for 10 min with intermittent shaking. The reaction was stopped by adding 5 mM potassium iodide (KI) in PBS, and the bacilli were then washed three times with PBS-KI and three times with PBS to remove nonspecifically bound iodine and KI. Radioactivity associated with the cell pellet was recorded; it was found to be in the range 0.5 to 1 $\times$ 10^7 counts per minute (cpm).

The labeled bacilli were sonicated and the soluble extract prepared was analyzed in SDS-PAGE in 10% separating gel and 4.5% stacking gel using the Laemmli buffer system ([7]). The dried gels were exposed to X-ray plates and autoradiographs developed after 24 to 48 hr of exposure. Two bands of radiolabeled proteins were observed as shown in Figure 1. These proteins did not enter the separating gel; instead, one band was observed at the interphase between stacking and separating gel, and the other at the top of the stacking gel, indicating a very high molecular weight of these proteins. Identical results were obtained under reducing as well as nonreducing conditions (Fig. 1) and even in 5%–20% gradient gels (data not shown).

Simultaneously, PP-I purified from the sonicate of unlabeled ICRC bacilli as described earlier was also analyzed in SDS-PAGE. Two high molecular weight bands as described above were also observed in the Coomassie blue stained gel (Fig. 2).

The high molecular weight of the surface labeled proteins was confirmed by gel filtration. The sonicate of labeled bacilli was fractionated on a Sephacryl S-300 gel permeation column. The major radioactive peak obtained was in the void volume of the column (Fig. 3) and contained the high molecular weight proteins mentioned above. A similar pattern was obtained with PP-I (data not shown). Exclusion from Sephacryl S-300 indicates a molecular weight greater than 1.5 $\times$ 10^6 daltons. These results indicate that proteins from the PP-I preparation are located at the surface of the bacilli.

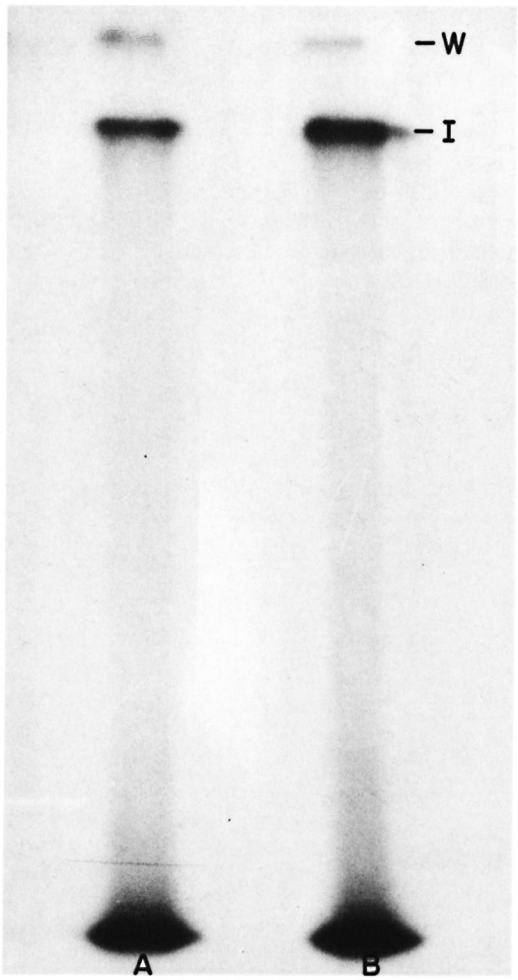

FIG. 1. Autoradiograph of SDS-PAGE gels including **A** unreduced and **B** reduced sonicate of ^{125}I-labeled ICRC bacilli. W = position of the wells; I = position of the interphase between the stacking and the separating gel.

Another objective of the work undertaken was to radiolabel *M. leprae* for studies on the processing and presentation of its antigens. Since *M. leprae* has not yet been cultivated, internal or metabolic labeling of its proteins cannot be carried out. The technique of iodinating whole bacilli, if successful, would be appropriate for such studies since it offers two advantages. It restricts the studies only to the surface proteins, which have been shown to be the major immunodominant antigens of *M. leprae* ([10]).

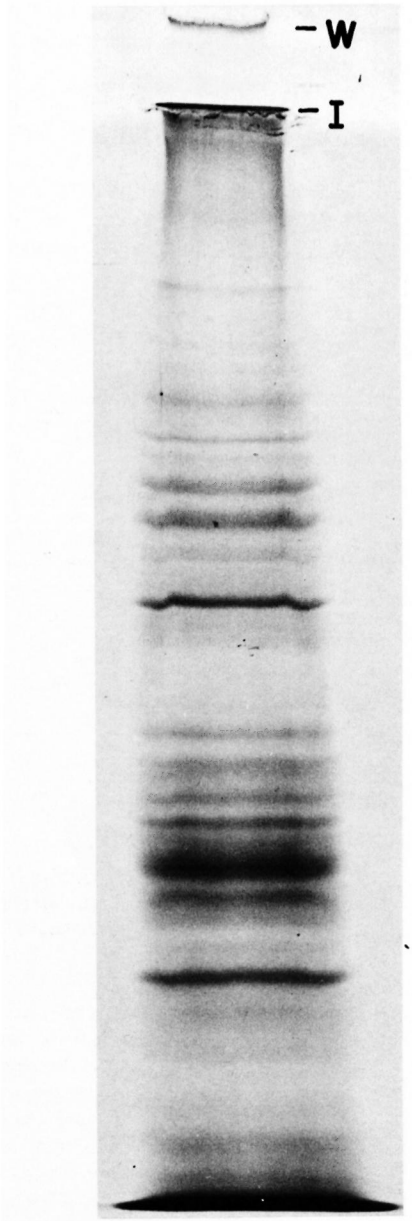

FIG. 2. Coomassie blue-stained gel showing SDS-PAGE analysis for sonicate of unlabeled ICRC bacilli. W = position of the well; I = interphase between stacking and separating gel.

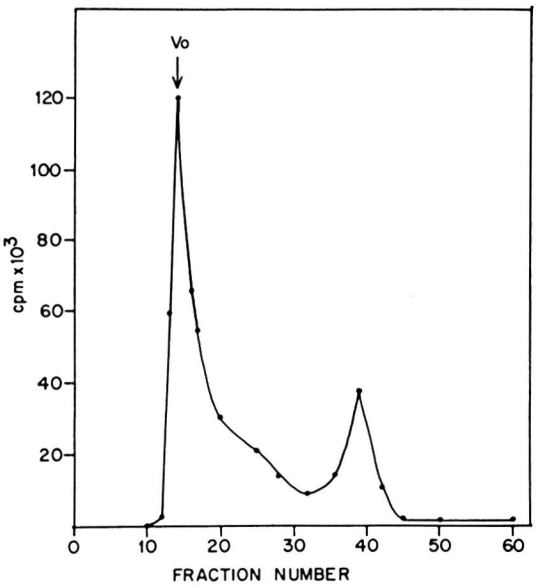

FIG. 3. Fractionation of sonicate of ^{125}I-labeled ICRC bacilli on Sephacryl S-300. Vo = void volume.

In addition, studies using these whole, labeled bacilli simulate a natural infection in which proteins are encountered by cells, not in soluble form but in intact organisms.

M. leprae obtained from biopsies of leprosy patients as well as from a lepromin preparation could be successfully labeled with ^{125}I using the method described above. SDS-PAGE analysis of the sonicate of these bacilli also indicated high molecular weight bands similar to those in the ICRC bacilli (Fig. 4 a and b). Our finding of high molecular weight proteins located at the surface of *M. leprae* agrees with the results reported from Dr. Brennan's laboratory. Similar proteins have been demonstrated by them in a peptidoglycan-protein component prepared from cell walls of *M. leprae* ([6, 9]).

Since the *M. leprae* used in these studies were obtained from human tissue and the ICRC bacilli were cultured in a medium containing human serum, we wanted to rule out the possibility that the high molecular weight radioiodinated proteins were components of the human serum or tissue. Therefore, sonicates prepared from radiolabeled ICRC bacilli and *M. leprae* were immunoprecipitated as described by Richard, *et al.* ([11]), with a few modifications. The sonicates were allowed to react with rabbit antisera against ICRC bacilli and *M. leprae* at 37°C for 2 hr. The antigen antibody complexes formed were allowed to react with Protein A coupled to Sepharose CL-4B beads (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) at 4° for 1 hr, and the beads were then washed three times with PBS. The im-

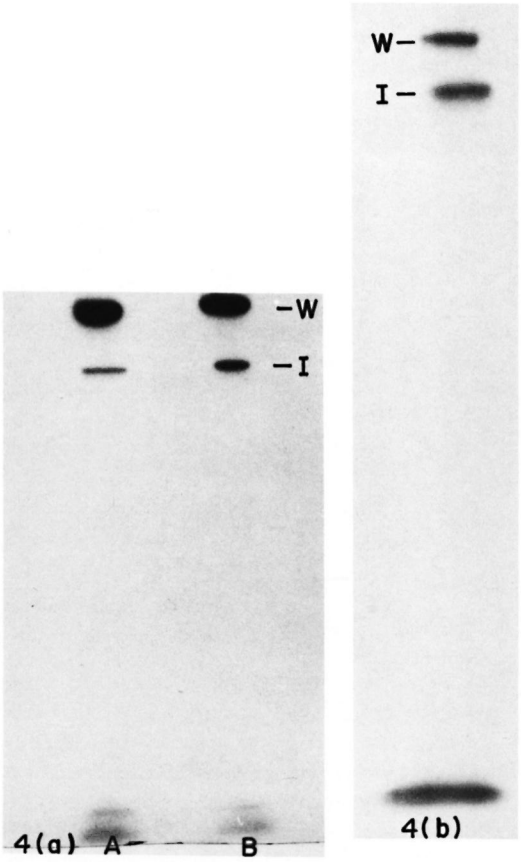

FIG. 4. Autoradiograph of SDS-PAGE gels including sonicates of ^{125}I-labeled (**a**) unreduced *M. leprae* (A) and reduced *M. leprae* (B); (**b**) reduced lepromin. W = position of wells; I = interphase between stacking and separating gel.

munoprecipitated proteins were then eluted by boiling in sample buffer and subjected to SDS-PAGE followed by autoradiography. Both the bands observed for the sonicate in earlier studies were found to be precipitated (data not shown). This indicated that the proteins in the sonicates are derived from the bacilli and ruled out that they were due to contamination with human serum or tissue components.

The technique of surface iodination of whole cells has been widely used for eukaryotic cells and has also been applied to microorganisms, such as *Pseudomonas* ([8]) and *Trypanosoma* ([13]) species. There is one report of surface iodination of *M. smegmatis* ([2]) but, to the best of our knowledge, it has not been applied to *M. leprae* and hence is reported here. This would be important in experiments aimed at studying the intracellular processing of surface proteins of *M. leprae* and other mycobacteria.

Leprosy presents a spectrum of cell-mediated immune responses against *M. leprae,* ranging from hypersensitivity in the tuberculoid form to unresponsiveness in lepromatous leprosy. Several possibilities have been suggested to explain these responses ([12]), e.g., processing of *M. leprae* antigens by macrophages may differ in different individuals leading to inappropriate antigen presentation. Surface labeled *M. leprae* offers a very good tool for exploring these possibilities using macrophages and Schwann cells.

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In Vitro Effect of Dapsone on NADH-Methemoglobin Reductase

TO THE EDITOR:

Leprosy patients under sulfone therapy exhibit both a wide variability and a raised NADH-methemoglobin reductase activity ([3]). The enzyme activity is also negatively correlated to the patients' hemoglobin level ([3]) but not to their reticulocyte rate ([2]). Considering that NADH-methemoglobin reductase (actually a NADH-cytochrome b_5 reductase) is a membrane enzyme ([6]) and that dapsone exerts significant hemolysis ([1]) due to its property to oxidize membrane lipid and protein components ([4]), one possible hypothesis to explain the rise in enzyme activity is that the action of dapsone upon the cell membrane could somehow augment the soluble fraction of NADH-methemoglobin reductase. According to this hypothesis, both the great variability and the rise in enzyme activity might be explained.

We carried out experiments on blood samples obtained from 14 volunteers. After washing the red cells with an isotonic-buffered saline solution, each sample was suspended in the same medium at approximately 50% hematocrit and divided into six portions of 1 ml each. Five portions received 2–10 μg dapsone/ml erythrocyte suspension; the other portion received none. They were incubated for 2 hr at 37°C with gentle stirring. After incubation, NADH-methemoglobin reductase activity was determined according to Scott ([5]) in both the crude hemolysate and unsealed ghosts, the latter prepared according to Steck and Kant ([7]). Scott's method ([5]) is based on a dye reduction (dichloro-indophenol, DCIP) by NADH in the presence of either crude hemolysate or cell membrane suspension after treatment of the erythrocytes with 1% $NaNO_2$ in isotonic-buffered saline followed by additional washing. Enzyme activity was expressed as (ΔA_{600}/min per mg protein) × 10^4.

We also measured the enzyme activity by applying the same method ([5]) to the supernatant of erythrocyte ghosts which were not previously treated with dapsone, before and after the addition of 5 μg dapsone/ml membrane suspension. A period of incubation of 10 min at room temperature was followed by another centrifugation for 15 min at 22,000 × *g*. The enzyme reactions in both cases proceeded for 5 min, and the results were expressed as $\Delta A_{600} \times 10^3$.

For the erythrocytes previously incubated with dapsone, we found a dose-dependent increase of NADH-methemoglobin reductase activity measured in the crude hemolysate ($r = 0.40$; $N = 36$; $p < 0.05$) and a negative correlation between this variable