

Reply to Dr. Harboe's and Dr. Closs' Letter to the Editor

TO THE EDITOR:

We have no explanation why Harboe and Closs were unable to reproduce our lithium acetate extraction procedure to obtain an extract containing antigens with reactivities that we described as *M. leprae* "specific" in 1979 (²). We have reproduced this extraction procedure with comparable results more than 15 times and would welcome Harboe or Closs trying the same procedure in our laboratory. Perhaps small but relatively important differences in technique would prove responsible for their inability to detect the antigen(s) that we described.

Harboe and Closs state that our "antigen" cross-reacts with *M. avium* and BCG. These authors have demonstrated that our antigenic extract reacts with antiserum to *M. avium* and BCG without demonstrating that the antigen(s) with *M. leprae* "specific" activity react with the BCG and *M. avium* antiserum. We have never claimed antigenic purity of the lithium acetate extract, and an SDS polyacrylamide gel of this extract reveals that more than ten separate proteins as well as carbohydrate and glycolipid molecules are present. One would estimate therefore that there may be 10 to 20 separate antigens in this extract, considerably more than just the antigens 4, 5, and 7 recognized with CIE by Harboe and Closs. The basis for the claim of "specificity" of protein antigenic determinants for *M. leprae* was the use of a pool of sera from LL patients adsorbed by Abe, *et al.* (¹) with BCG, *M. vaccae*, cardiolipin, and lecithin. This adsorption made the serum pool "specific" for *M. leprae* in an IFA test and specific for *M. leprae* as compared to four other mycobacterial species using double diffusion in gel. The proof that the *M. avium* or BCG antisera recognized the same *M. leprae* antigen(s) in our extract as that recognized by Abe's adsorbed serum pool would require using Abe's adsorbed serum pool in the same double diffusion in

gel experiment. This critical experiment was not performed by Harboe and Closs. Therefore, their claim of cross-reactivity between the antigen(s) that we described and antigens of *M. avium* and BCG, while potentially correct, is not proven.

We have extended our studies by reacting Abe's adsorbed serum with lithium acetate extracts from 21 species of mycobacteria in double diffusion in gels. In September 1979 we reported that Abe's adsorbed serum recognized a shared antigenic determinant between *M. leprae* and *M. lepraemurium* (³). Subsequent studies with Abe's adsorbed serum also showed shared reactivity between *M. leprae* and *M. bovis* (BCG), *M. gordonae*, *M. nonchromogenicum*, *M. flavescens*, and *M. gastri* and no shared reactivity with 15 other mycobacterial species including significant human pathogens such as *M. tuberculosis*, *M. intracellulare*, *M. scrofulaceum*, *M. kansasii*, and *M. marinum*. These results are summarized in an abstract (⁴) which has appeared since the letter of Harboe and Closs was written.

It is our opinion that definite proof of whether or not *M. leprae* contains unique antigenic determinants will require monoclonal antibodies or extensive antigen purification. It cannot be answered by double diffusion in gel or CIE experiments.

—Thomas M. Buchanan, M.D.

—Thomas P. Gillis,

—Harlan D. Caldwell, Ph.D.

*Immunology Research Laboratory
USPHS Hospital &
University of Washington School of Medicine
Seattle, Washington 98114, U.S.A.*

REFERENCES

1. ABE, M., MINAGAWA, F., YOSHINO, Y., OZAWA, T., SAIKAWA, K. and SAITO, T. Fluorescent leprosy antibody absorption (FLA-ABS) test for de-

- fecting subclinical infection with *Mycobacterium leprae*. *Int. J. Lepr.* **48** (1980) 109–119.
2. CALDWELL, H. D., KIRCHHEIMER, W. F. and BUCHANAN, T. M. Identification of a *Mycobacterium leprae* specific protein antigen(s) and its possible application for the serodiagnosis of leprosy. *Int. J. Lepr.* **47** (1979) 477–483.
 3. GILLIS, T. P. and BUCHANAN, T. M. Fractionation of antigens of *Mycobacterium leprae*. (Abst.) *Int. J. Lepr.* **47** (1979) 674.
 4. GILLIS, T. P. and BUCHANAN, T. M. Characterization of *M. leprae* "specific" antigens. (Abst.) *Int. J. Lepr.* **48** (1980) 505.