CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters.

Fate of *Mycobacterium leprae* In Macrophages of Patients with Lepromatous or Tuberculoid Leprosy

TO THE EDTOR:

Barbieri and Correa [2] and Beiguelman [3, 4] have reported that blood-derived macrophages from Mitsuda negative, unlike Mitsuda positive individuals, with or without leprosy, lack the ability to lyse or digest killed *Mycobacterium leprae* in tissue culture. This deficiency appeared to be specific to *M. leprae* since macrophages of Mitsuda negative individuals were able to lyse killed *M. lepraemurium* and *M. tuberculosis*. Mycobacterial lysis was complete in 10 to 16 days, by which time no bacilli stained with carbol-fuchsin by the Ziehl-Neelsen method. Our attempts to repeat these findings have been unsuccessful.

Blood was collected from five pairs of patients—one lepromatous and one tuberculoid on each occasion. The type of leprosy was based on clinical and histologic assessments according to the Ridley-Jopling scale [11]. All the lepromatous patients (LL-LI) were Mitsuda negative, while all the tuberculoid patients (TT-BT) were Mitsuda positive, four giving a +++ reaction, the fifth a ++ reaction.

After defibrination the white cells were separated by addition of 3 per cent dextran (MW 250,000; BDH—one volume dextran to two volumes blood). The red cells were allowed to sediment at room temperature for 30 to 60 minutes. The leukocytes were washed once and made up to a concentration of 4-6 x 10^6 cells/ml. in Eagle's medium containing a 1.37 gm./1 $NaHCO_3$ (one half of the usual concentration), 20 per cent autologous serum, 0.1 mg./ml. *L*-glutamine and 100 units/ml. penicillin. The macrophages were cultured on coverslips in Leighton tubes. One ml. of the cell suspension was added to each tube, gassed in 5 per cent CO_2/air and incubated at 37°C.

In order to remove most of the lymphocytes before *M. leprae* was added, the medium was changed and the cells washed on the second day of cultivation. On the third day, *M. leprae* prepared from Mitsuda-type lepromin analogous to the method of Barbieri and Correa, was added to the cells in medium containing 20 per cent normal human serum (T.G. or R.J.W.R.). The macrophages were infected by adding to the cultures either 2.5 x 10^5 bacilli, counted by the method of Hart and Rees [8], and incubating for three days or by adding 1 x 10^6 bacilli for two hours. The cells, after changing the medium, were then incubated for 10 days.

Changes in the macrophage population and the fate of *M. leprae* within these cells was assessed by comparing Ziehl-Neelsen stained [1] coverslips immediately following infection with coverslip preparations after incubation for 10 days. The number of bacilli taken up per cell was on average 1.8 in the lepromatous group and 1.5 in the tuberculoid group. The total number of cells in each culture did not change significantly during this period of incubation. Because it was apparent from preliminary assessments that acid-fast bacilli persisted within macrophages from all the patients throughout the culture period, more precise comparisons were made of the morphologic appearances of the bacilli within the cells before and after incubation. These assessments were made first by T.G. and then by R.J.W.R., on coded cover slips. Similar results were obtained by the two observers. Bacilli were grouped into those that stained uniformly and brightly with carbol fuchsin and those that stained irregularly or faintly. The latter were considered to represent degenerative changes due to the loss of

cytoplasmic material from the organisms ([10]). In the baseline preparations the average percentage of such degenerate bacilli was 40 in the lepromatous and 44 in the tuberculoid group. After 10 days the corresponding figures were 64.4 per cent and 66 per cent. Thus, as shown in Figure 1, there was a similar overall increase in percentage of degenerate bacilli in the two groups of patients and, moreover, the proportion of bacilli showing the most advanced forms of irregular staining was also similar in both groups of patients. Furthermore, parallel investigations with *M. lepraemurium* and *M. tuberculosis* in four out of the five pairs (Fig. 1) indicate that there are no major differences in the ability of macrophages from the two groups of patients to lyse or digest these strains of mycobacteria and that little or no lysis occurred within the period of observation.

The original observations of Barbieri, Correa and Beiguelman showed that macrophages from tuberculoid patients, or from Mitsuda positive individuals, so completely lysed or digested phagocytosed heat-killed *M. leprae* that no acid-fast bacilli remained after 10 to 16 days of incubation and, moreover, the lytic effect of these macrophages was specific only for *M. leprae* ([3, 4]). Therefore our studies failed completely to confirm their observations. We introduced more refined assessments in an attempt to detect intermediate degrees of lysis, but even these failed to confirm any such tendency. Because of the considerable variations between the individual cell-lines and the small number of patients included in our own study, small but significant differences between the two types of leprosy may still have been missed. However, they could not account for the complete failure of our study to confirm the results of Barbieri *et al.*

In attempting to explain the discrepant results obtained by Barbieri *et al.*, and ourselves, consideration of a possible mechanism for the phenomenon seemed to be appropriate. The two most likely mechanisms were that macrophages from lepromatous patients and Mitsuda negative individuals: (1) failed to digest *M. leprae* because they lack the appropriate enzyme systems (possibly lysosomal in origin and a genetically determined deficiency) and therefore comparable to the situation in children with chronic granulomatous disease ([5]) or, (2) a cell-mediated immune deficiency in such patients and individuals affecting either the antigen-processing

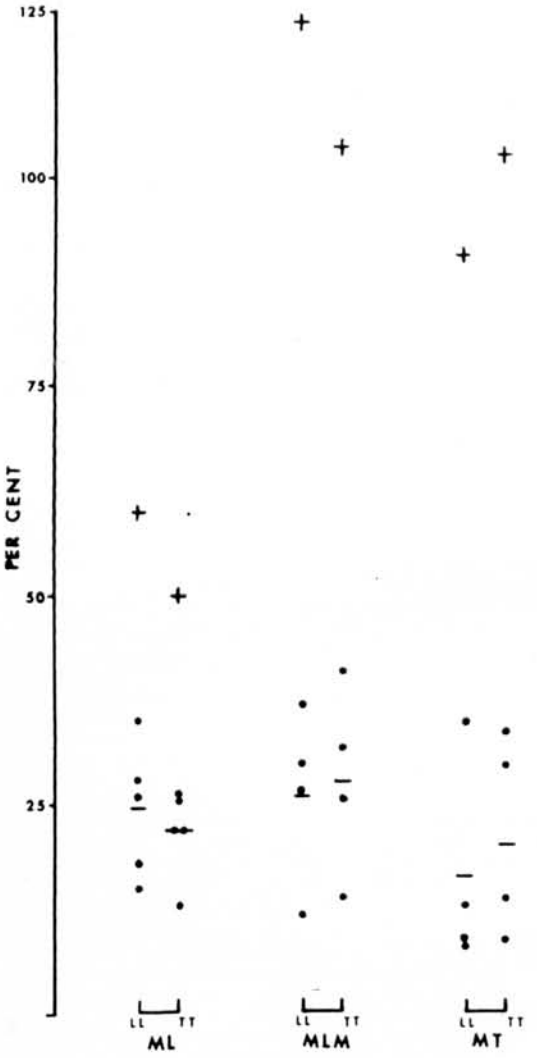

FIG. 1. Difference between percentages of degenerate bacilli before and after 10 days of cultivation in macrophages; degeneracy increases during activation. Horizontal lines = mean of each group; + = $\frac{\text{Increase}}{\text{Degeneracy at base line}} \times 100$.

LL = macrophages from lepromatous. **TT** = macrophages from tuberculoid patients. **ML** = *M. leprae*. **MLM** = *M. lepraemurium*. **MT** = *M. tuberculosis*.

mechanism of the macrophages or the immune capacity of their lymphocytes, again possibly a genetically determined deficiency. If the first mechanism was relevant, then the methods for collecting peripheral-blood monocytes to the exclusion of lymphocytes are unimportant, but clearly there could be important genetic differences between the population studied by Barbieri *et al.* in Brazil, compared with our patients who, with the exception of one from Fiji, were all Indian or Anglo-Indian. On the other hand, if there is an immunologic mechanism then the results of recent studies would indicate the importance of technical details in the preparation of monocytes to the exclusion of significant numbers of lymphocytes in order to distinguish between the role of these two cell types. For example, our own studies on peripheral-blood macrophages from lepromatous and tuberculoid patients ([7]) indicate the essential role of lymphocytes from the tuberculoid compared to the lepromatous patients in inhibiting the multiplication of mycobacteria *in vitro* within these macrophages. The role of immune lymphocytes in determining the multiplication of *M. tuberculosis* in mouse peritoneal macrophages from immunized and nonimmunized animals has also been established ([9]). These recent results underline the necessity for futher detailed studies based on methods designed for carefully excluding lymphocytes at the time of incubation of blood-derived macrophages with *M. leprae* or other mycobacteria.

The clear-cut inability of peripheral-blood macrophages from lepromatous patients or Mitsuda negative individuals to specifically digest or lyse heat-killed *M. leprae* has not been confirmed by our own studies using, apparently, identical methods. More recently, another group ([6]), using apparently similar conditions, has also failed to confirm the original observation by Barbieri *et al.* The latter studies were undertaken in San Francisco, and therefore like our own patients are likely to be from different races than those studied first in Brazil. On the basis of these racial differences and the other possible differences discussed here, further studies must be undertaken before the relevance of this phenomenon in determining the outcome of leprosy in man can be adequately assessed.

—T. Godal
—R. J. W. Rees
Laboratory for Leprosy and Mycobacterial Research
National Institute for Medical Research
Mill Hill, London, N.W.7., England

Acknowledgment. We are indebted to Dr. W. H. Jopling of the Hospital for Tropical Diseases, London, for arranging the attendance and collection of plasma from the patients on which this study was based.

REFERENCES

1. Allison, A. C. and D'Arcy Hart, P. Potentiation by silica of the growth of *Mycobacterium tuberculosis* in macrophage cultures. British J. Exper. Path. **49** (1968) 465.
2. Barbieri, T. A. and Correa, W. M. Human macrophage culture. The leprosy prognostic test (LPT). Internat. J. Leprosy **35** (1967) 377-381.
3. Beiguelman, B. Leprosy and genetics. A review of past research with remarks concerning future investigations. Bull. WHO **37** (1967) 461-476.
4. Beiguelman, B. Some remarks on the genetics of leprosy resistance. Acta Genet. Med. et Gemellol. (Roma) **17** (1968) 584-594.
5. Douglas, S. D. and Fudenberg, H. H. Genetically determined defects in host resistance to infection: Cellular immunologic aspects. Med. Clin. North America **53** (1969) 903-922.
6. Drutz, D. J. and Cline, M. J. Polymorphonuclear leukocyte and macrophage function in leprosy. Fifth Annual Leprosy Research Conference, Boston, Mass., 1970.
7. Godal, T., Rees, R. J. W. and Lamvik, J. O. Lymphocyte mediated modification of blood-derived macrophage function *in vitro:* including inhibition of growth of intracellular mycobacteria. Clin. Exper. Immunol. **8** (1971) (*In press*).

8. HART, P. D'ARCY and REES, R. J. W. Effect of macrocyclon in acute and chronic pulmonary tuberculosis infection in mice as shown by viable and total bacterial counts. British J. Exper. Path. **41** (1960) 414-421.
9. PATTERSON, R. J. and YOUMANS, G. P. Demonstration in tissue culture of lymphocyte-mediated immunity to tuberculosis. Infect. Immunol. **1** (1970) 600-603.
10. REES, R. J. W. and VALENTINE, R. C. The appearance of dead leprosy bacilli by light and electron microscopy. Internat. J. Leprosy **30** (1962) 1-9.
11. RIDLEY, D. S. and JOPLING, W. H. Classication of leprosy according to immunity. A five-group system. Internat. J. Leprosy **34** (1966) 255-273.