Correspondence

Macrophage Microbicidal Mechanism

TO THE EDITOR:

Jolly and Mahadevan have attempted to study the role of reactive oxygen intermediates in the intracellular killing of Mycobacterium leprae in human macrophages (1). The authors have reported 89 nmol of H$_2$O, and 0.3 nmol of superoxide (O$_2^-$) in the macrophages of lepromatous leprosy patients.

During phagocytosis, macrophages produce substantial quantities of O$_2^-$ and H$_2$O, as shown by the following reactions: Superoxide is formed by the one electron reduction of oxygen: 2O$_2$ + NADPH → O$_2^-$ + NADP$^+$ + H+. Superoxide is converted to H$_2$O, by the reaction 2O$_2^-$ + 2H$^+$ → O$_2^- + H_2$O. This reaction is catalyzed by the enzyme superoxide dismutase.

Considering the fact that all of the oxygen taken up during the respiratory burst is converted to O$_2^-$, and that 80% of this O$_2^-$ is converted to H$_2$O, by dismutation (1), it is difficult to understand from the paper (3) how 89 nmol H$_2$O, could be accounted for when only 0.3 nmol O$_2^-$ was produced (Table 1).

However, there is a report which claims a direct conversion of molecular oxygen to H$_2$O, (2), as shown by the reaction: NADH + O$_2$ + H$^+$ → H$_2$O + NAD$^+$. This has been reported in guinea-pig neutrophils in vitro. It has also been argued that the rather high Km (0.4 mM) for NADH observed in vitro for this enzyme militates against significant activity during phagocytosis (2).

Under these circumstances, more confirmation is needed regarding the role of superoxide and H$_2$O, in the killing of M. leprae by human macrophages.

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A Comparison of the Ziehl-Neelsen and Kinyoun Methods in Staining Smears from Leprosy Patients

TO THE EDITOR:

The cold staining method of Kinyoun (2) for acid-fast bacilli is widely used in tuberculosis bacteriology, and is recommended by the U.S.A. Centers for Disease Control (CDC) (2) and the Institut Pasteur, France (1). To our knowledge, this method was not evaluated in leprosy diagnosis. Because the Kinyoun method was applied in our mycobacteriology laboratory, we decided to evaluate it for leprosy diagnosis since this disease is highly endemic in the state of Amazonas, Brazil (estimated incidence and prevalence were 69/100,000 and 11/1000 inhabitants in 1987).

Smears from leprosy patients were prepared in the outpatient leprosy clinic of the Centro de Dermatologia Tropical e Venereologia “Alfredo da Matta,” Manaus, Brazil. One smear from each site was stained in the outpatient clinic laboratory using the Ziehl-Neelsen method as recommended by Leiker and McDougall (2). The method of Kinyoun was applied as recommended by the CDC and Institut Pasteur (1, 2), except that the destaining solution was 1% instead of 3% hydrochloric acid in ethanol. The smears stained by the Ziehl-Neelsen method were read and classified by an experienced microscopist at the outpatient clinic.
Duplicate smears were stained at the Instituto Nacional de Pesquisas da Amazonia (INPA), and were read and classified at INPA. The readings and classifications of the smears were done blindly and independently in the participating laboratories. The results were checked only after complete data were collected.

The two-sample analysis results are shown in Figures 1 and 2. Figure 1 depicts the frequency polygon of the bacterial index (BI) and morphological index (MI) for all the smears (N = 145). The statistics indicated that there were no significant differences between the two staining procedures. Figure 2 shows the correlation analysis of the paired data on the same smears (N = 145). The correlation coefficients were 0.9 and 0.8, respectively, for the BI and MI, also indicating that there were no differences between the two methods.

From the above data, we conclude that the cold method of Kinyoun for acid-fast microscopy is satisfactory for leprosy bacteriology provided that the concentration of the acid in the destaining solution is reduced.
to 1%. We have also observed that smears stained with the Kinyoun method keep better in storage, which might be of interest in preparing teaching materials and in quality control programs. One possible disadvantage of the Kinyoun method over the Ziehl-Neelsen method is that it uses a higher concentration of fuchsin, which may slightly increase the cost of microscopy.

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