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 ([3]). The authors have reported 89 nmol of H_2O_2 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_2O_2 as shown by the following reactions: Superoxide is formed by the one electron reduction of oxygen: $2O_2 + NADPH \rightarrow O_2^- + NADP^+ + H^+$. Superoxide is converted to H_2O_2 by the reaction $2O_2 + 2H^+ \rightarrow O_2^- + H_2O_2$. 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_2O_2 by dismutation ([1]), it is difficult to understand from the paper ([3]) how 89 nmol H_2O_2 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_2O_2 ([2]), as shown by the reaction: $NADH + O_2 + H^+ \rightarrow H_2O_2 + NAD^+$. 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_2O_2 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 ([3]) 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 ([4]). 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.