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# 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. The mandate of this JOURNAL is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid, but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the JOURNAL and thus interfere with its prime purpose.*

## Dapsone-Induced Methemoglobinemia in Leprosy Patients

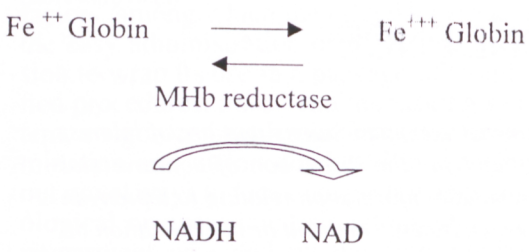
TO THE EDITOR:

Diaminodiphenylsulfone (dapsone) is one of the most widely used drugs being given to millions of leprosy patients for the last six decades. Considering the enormous number of persons receiving dapsone, severe toxic reactions are rare ([4]). Methemoglobinemia as a toxic effect with dapsone in high doses has been well documented ([3]). However, clinically significant methemoglobinemia as a hematological side effect of dapsone is rare at therapeutic doses ([4]).

Methemoglobin is an oxidized product of hemoglobin in which heme iron becomes $Fe^{3+}$ and is incapable of binding oxygen. Normally, a low level of methemoglobin is maintained in the blood due to activity of the cytochrome b5 reductase (methemoglobin reductase) enzyme ([5]). Malaria prophylaxis has been reported as provoking methemoglobinemia in unsuspected heterozygotic individuals deficient in NADH-cytochrome $b_5$ reductase enzyme ([1]). The present study was designed to investigate the role of cytochrome $b_5$ reductase activity in dapsone-induced methemoglobin production in leprosy patients.

Twenty, untreated, newly diagnosed patients with leprosy (8 BL/LL, 12 BT/TT) and 10 healthy age- and sex-matched controls attending Nehru Hospital, Postgraduate Institute of Medical Education and Research, Chandigarh, India, were enrolled in the study. Blood samples were collected from all leprosy subjects prior to and after treatment with the 1982 World Health Organization (WHO) multidrug therapy (WHO/MDT). All samples were tested for oxyhemoglobin and methemoglobin levels using standard (biochemical) methods. NADH-methemoglobin reductase enzyme activity was estimated by a slightly modified technique of Scott, *et al.* ([9]), using erythrocytes of blood conserved in ACD, removing excess nitrite, 10 ml of double distilled water was added to 0.05 ml of packed cells. Finally, absorbance was determined at 600 nm against distilled water. Then to 3 ml of hemolysate, 0.2 of 0.012 M 2, 6-dichlorobenzenone indephenol (DCIP) in 1 M Tris-HCl buffer (pH = 7.6) containing 0.011 M disodium EDTA was added. The enzyme activity was studied using 0.008 M NADH, and the enzyme activity expressed in change in absorbance per minute at 600 nm ($A \times 600/min \times 10^4$). All patients were also screened for glucose 6-phosphate dehydrogenase (G-6-PD) deficiency.

There were 12 male and 8 female leprosy patients with ages ranging from 12 to 47 years. Mean hemoglobin levels were found to be significantly decreased ($p < 0.05$) in leprosy patients after therapy (WHO/MDT) compared to before therapy. After treatment, a slight but significant increase ($p < 0.01$) in methemoglobin levels was ob-




THE FIGURE. The interconversion of hemoglobin and methemoglobin.

served in leprosy patients as compared to control subjects. There was a statistically significant decrease in the activity of the methemoglobin reductase enzyme after treatment in patients compared to controls ($p < 0.01$). Similar observations have also been made in another two studies of leprosy patients in the past ([6, 8]). None of our patients had significantly raised methemoglobin levels to manifest clinically. All patients screened for G-6-PD deficiency were found to be normal. Manfredi, *et al.* ([7]) also concluded that hemolytic anemia and methemoglobinemia in patients taking dapsone is not due to functional impairment of the G-6-PD enzyme.

Acquired methemoglobinemia results from exposure to certain drugs and chemicals, such as nitrite, chlorate, and sulfonamide compounds capable of oxidizing hemoglobin directly or indirectly. The exact mechanism by which dapsone induces methemoglobinemia *in vivo* is not known, but the drug metabolite 4-amino-4-hydroxyaminodiphenylsulfone is said to be responsible for oxidation ([9]).

The NADH-dependent reductase system associated with cytochrome $b_5$ represents one of the major electron transport systems in the body which covert methemoglobin to hemoglobin (The Figure). So, we propose that a decrease in activity of the NADH-methemoglobin reductase enzyme, as observed in our study, might be one of the major factors responsible for dapsone-induced methemoglobinemia in leprosy patients. The growing numbers of immunosuppressed patients due to the spread of HIV infection may result in increased dapsone use for *Pneumocystis carinii* pneumonia (PCP) prophylaxis ([10]). Although methemoglobinemia occurring at therapeutic doses of dapsone is generally asymptomatic, clinicians should be aware of this adverse effect, and patients presenting with respiratory distress of unknown etiology should be evaluated for methemoglobinemia.

—Inderjeet Kaur, M.D., M.N.A.M.S.

*Additional Professor*
*Department of Dermatology, Venereology and Leprology*

—Manjula Mehta, Ph.D.
Natini Agnihotri, Ph.D.

*Department of Experimental Medicine*

—Sunil Dogra, M.D., D.N.B.

*Department of Dermatology, and Leprology*

—N. K. Ganguly, M.D.

*Department of Experimental Medicine*
*Postgraduate Institute of Medical Education and Research*
*Chandigarh 160 012, India*

Reprint requests to Dr. Kaur at the above address or FAX 91-0172-744-401; e-mail: kaur_inderjeet@yahoo.com

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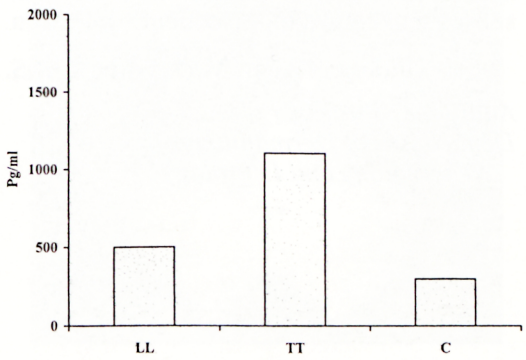
# Tumor Necrosis Factor (TNF) Production in Leprosy Patients

To the Editor:

The spectrum of host response to *Mycobacterium leprae* provides a model for investigating the role of cytokines in the pathogenesis of leprosy. Of particular interest is tumor necrosis factor (TNF), a cytokine which may have both antimycobacterial and immunopathologic effects in leprosy ([2, 7]). At one pole of the leprosy spectrum, patients with tuberculoid leprosy have few skin lesions, in which bacilli can rarely be identified with strong cell-mediated immunity (CMI); at the opposite pole, patients with lepromatous leprosy have diffuse infiltration of skin and nerves with bacilli-laden macrophages and depressed CMI to *M. leprae*. To evaluate the potential role of TNF in leprosy, we measured TNF production in response to *M. leprae* in the patients with polar forms of leprosy.

Thirty-eight new patients attending the leprosy clinic at the Postgraduate Institute of Medical Education and Research, Chandigarh, India were included in the study. Patients were classified on the basis of the clinicopathologic criteria of Ridley and Jopling ([3]). Patients had not taken treatment in the past, and they were not in any reactional state. Twenty age- and sex-matched normal healthy controls were also studied simultaneously. Heparinized venous blood (7 ml) was obtained from each patient and from the controls. Plasma was separated and stored at –20°C until used, and the TNF assay was done by the method of Silva and Foss ([8]). The Student's *t* test was used to compare the control and experimental groups; values of $p > 0.05$ were considered as nonsignificant. Of the 38 patients, 25 were in the tuberculoid pole and 13 were lepromatous leprosy. TNF release was significantly higher ($p < 0.001$) in patients with tuberculoid leprosy than in those with lepromatous leprosy and healthy controls (The Figure).

TNF functions as a macrophage/monocyte-derived immunoregulatory cytokine, with important biological effects ([6]). To investigate the potential role of TNF in mediating the clinical manifestations of leprosy, we measured the production of TNF in patients from the two poles of disease. High TNF levels were demonstrated in the plasma of tuberculoid patients; however plasma levels of TNF were low in lepromatous patients and in healthy subjects. The decreased TNF production by peripheral blood mononuclear cells (PBMC) from LL patients has been attributed to an intrinsic cellular defect or deficient production of other cytokines ([1]). Decreased production of TNF in these patients may contribute significantly to the evolution of the infection.




The Figure. TNF production in leprosy patients and controls. LL = lepromatous leprosy; TT = tuberculoid leprosy; C = controls.