

CORRESPONDENCE

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Demonstration of Antidapsone Antibody in
Leprosy Patients

TO THE EDITOR:

It is well known that chemotherapy of leprosy with dapsone (DDS) is very effective but takes years to achieve a cure. Even for the tuberculoid type, treatment has to be continued for at least 2 years after the regression of the clinical lesion. Moreover, DDS is known to induce reactions^(4, 8) leading to deformities⁽¹⁰⁾, and the patient may also become refractory to the drug. The exact cause of the reactions induced by DDS and the reason for its slow action and the fact that some patients do not respond to DDS therapy are not fully understood. However, nonresponsiveness to DDS therapy has been amply explained by the emergence of DDS-resistant strains of *Mycobacterium leprae*⁽⁹⁾. It is known that DDS combines with many body proteins^(1, 2, 6). In that case, the drug can act as a hapten with a distinct possibility to elicit an antibody response. These antibodies could neutralize biologically active units of the drug, leaving the patient with a much lower amount of the drug than indicated by crude blood level estimates. An inadequate dose of the biologically active drug may facilitate the emergence of resistant strains of *M. leprae*. These antibodies may also form DDS/anti-DDS complexes which could contribute to the occurrence of reactions in leprosy. Before investigating these aspects, it should first be established if DDS therapy evokes an antibody response in the patients.

In all, 178 subjects were studied. They included 140 leprosy patients on DDS ther-

apy for at least 3 months, with a bacteriological status ranging from 0 to 5+. These patients included 71 cases of lepromatous or borderline lepromatous (LL/BL), 47 tuberculoid or borderline tuberculoid (TT/BT), 14 mid-borderline (BB), and 8 neuritic types of leprosy. The 30 patients who were released from chemotherapy (RFC) during the preceding 12 months or more and who had maintained a negative bacterial status after stopping chemotherapy were also included. This group was composed of 15 cases of LL/BL, 5 TT/BT, 4 BB, and 6 neuritic types of leprosy. Eight normal controls were also included.

The proteins bovine serum albumin (BSA) or casein were conjugated to DDS by the diazotization reaction⁽¹¹⁾. Antibodies were raised in two albino rabbits by intradermal inoculation of an emulsion of 1 ml of DDS-BSA (3 mg/ml) in 1 ml of Freund's incomplete adjuvant over 5 months with repeated booster injections every 3 weeks. One week after the last injection, the rabbits were bled and antibody titers were estimated. Fifty μ g of this antibody (globulin fraction) was adsorbed with BSA, labeled with ¹²⁵I (BARC, Bombay, India) employing the Iodogen⁽³⁾ technique, and was used in the serum antibody competition assay (SACT)⁽⁷⁾.

The mean binding of ¹²⁵I-anti-DDS antibody to wells coated with casein alone was taken as 0%. This count was subtracted from each of the test binding values, and the relative binding values for each serum dilution were then calculated. Binding of ¹²⁵I-anti-

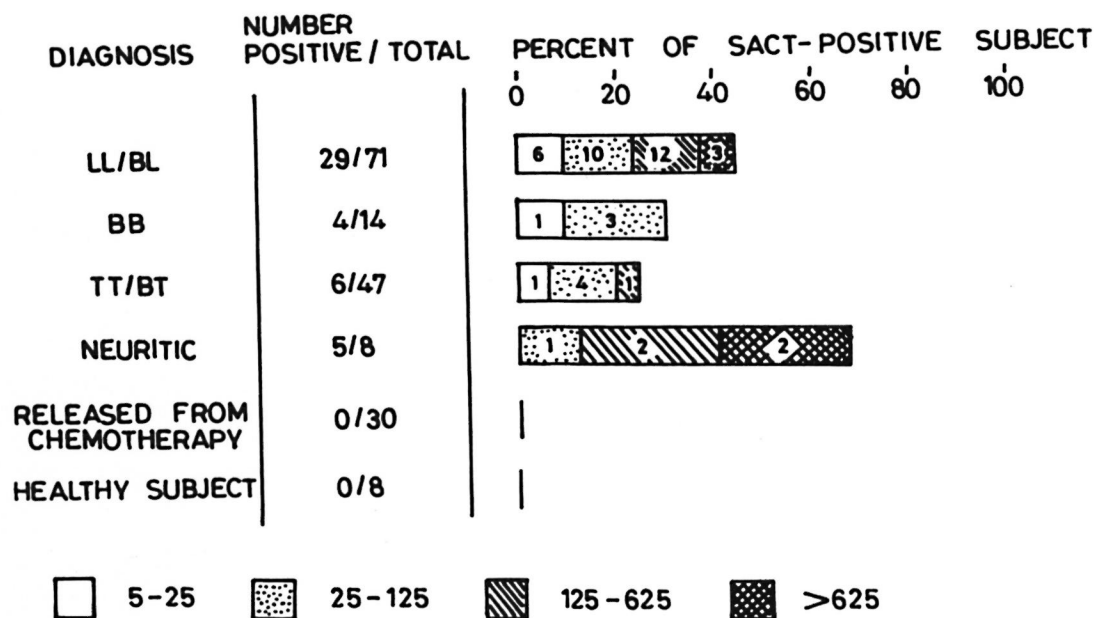


FIG. 1. Percent positivity and ID₂₅ titers of anti-dapsone (DDS) antibody in leprosy patients. Sera from a total of 178 subjects were tested in duplicate at four log-5 serial dilutions by the SACT assay. Sera which failed to give 25% inhibition of ¹²⁵I-anti-DDS antibody binding were taken as negative. Numbers of patients giving corresponding ID₂₅ titers are shown within the bars.

DDS alone to casein-DDS [80 µg/ml in phosphate buffered saline (PBS), pH 7.2] coated wells in the presence of casein-PBS was taken as 100%. The results were expressed in terms of ID₂₅ values which represent the serum dilutions causing 25% inhibition of ¹²⁵I-anti-DDS antibody binding to the antigen (casein-DDS) coated wells. The cut-off point for positivity was calculated by adding two standard deviations (S.D.) to the mean ID₂₅ value of the control sera.

The rabbit antisera showing the maximum titers (2000 and 3000 in the two rabbits, respectively) of anti-DDS antibody were pooled and used as an antibody source. These antibodies did not crossreact with other dapsone derivatives (DADDS, MADDS, diphenylsulfone, sulfadiazine, etc.) (5). Anti-DDS antibodies were demonstrated in the globulin fraction rather than the whole serum of the leprosy patients. It was observed during standardization of the technique that the DDS present in the whole serum also competed with the antibody in the SACT assay. The globulin fractions of patient sera tested were found to be devoid of DDS and so were used for the SACT.

Six (13%) of the 47 TT/BT patients were positive for anti-DDS antibodies. In the BB leprosy patients, the anti-DDS antibody could be demonstrated in 4 (29%) of the total of 14 patients; of 71 LL/BL patients, 29 (41%) were antibody positive. The highest percentage (63%) of positive cases (5/8) was observed in the neuritic type of leprosy. Anti-DDS antibodies were absent in the 30 RFC cases and in the eight normal controls (Fig. 1). The mean ID₂₅ titer in the TT/BT cases was 77, with a maximum of 200 in one of the patients. In the BB patients, the antibody titer ranged from 9 to 125. The mean antibody titers of LL/BL and neuritic patients were 194 (range ID₂₅ 8.8 to > 625) and 420 (range ID₂₅ 68 to > 625), respectively. A maximum antibody titer of > 625 was noticed in three LL/BL and two neuritic patients (Fig. 2). These antibodies were found to be of the IgG type, and had no correlation with the bacteriological status of the patients.

The variations in the levels of antibody among the different types of leprosy patients may be due to genetic factors predisposing an individual to make an antibody response to a drug or may be related to the duration

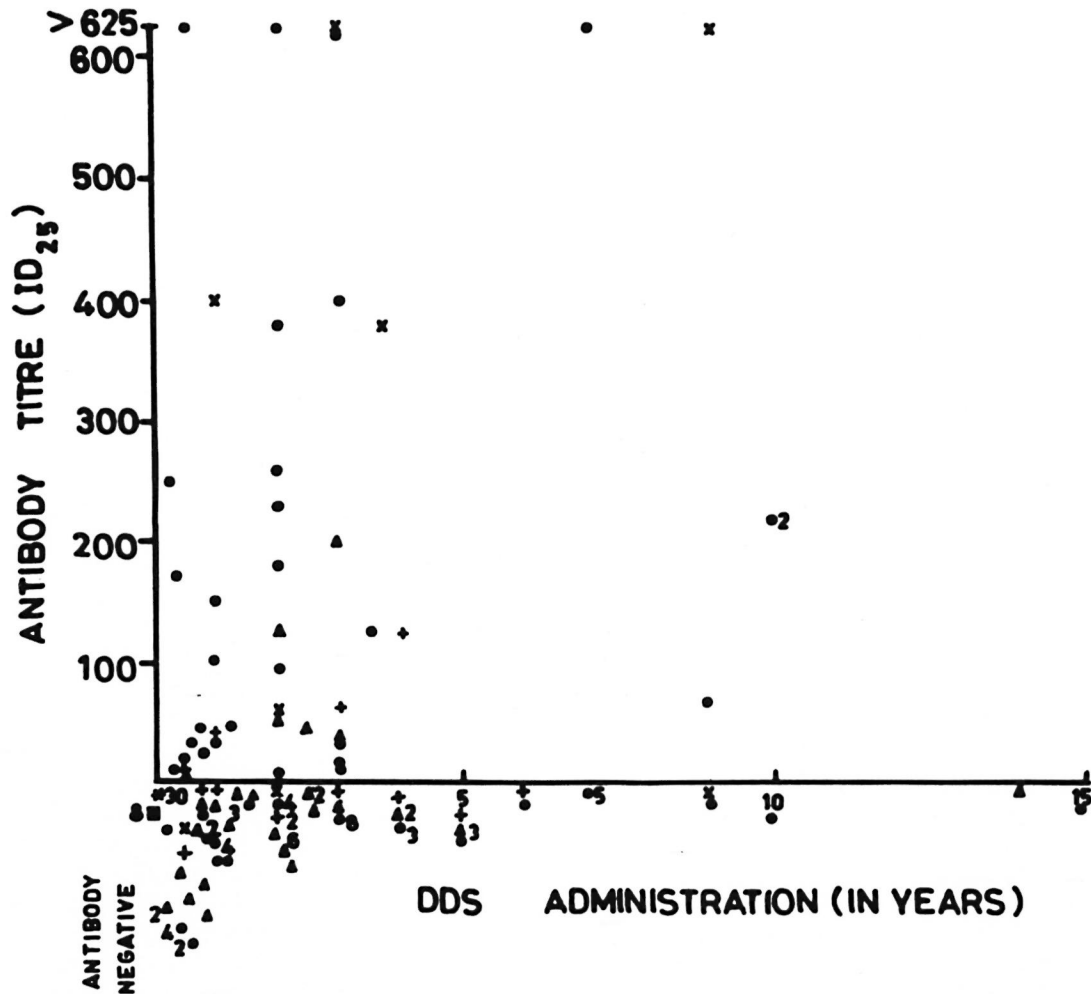


FIG. 2. Anti-DDS antibody titers (ID₂₅) in DDS-treated leprosy patients. ● = LL/BL; ▲ = TT/BT; + = BB; × = neuritic; * = released from control; ■ = normal control.

of chemotherapy. A patient not taking DDS and eight normal controls were never found to be positive for the anti-DDS antibody. Further studies are needed to find out the role of anti-DDS antibody in understanding the bioavailability of the drug, reactions, neuritis, and drug resistance in leprosy.

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Acknowledgments. We thank Dr. H. Srinivasan for providing valuable suggestions during the preparation of the manuscript. We also thank Mr. M. M. Alam and K. K. Kulshrestha for technical assistance, Mr. Hari Om Agarwal for photography, and Mr. Anil Kumar Chopra for secretarial help.

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Serum Antibodies of Normals and Leprosy Patients Show Equal Binding to Peripheral Nerve

TO THE EDITOR:

The presence of serum demyelinating factors (SDF) may lead to disseminated nerve damage in leprosy despite the infection being localized. Over the past few years several workers have detected the presence of SDFs in leprosy by using either binding assays to detect serum antibodies against peripheral nerve antigens^(4,7) or through functional assays where demyelination is observed by electron-microscopic examination of the sciatic nerve of Swiss white mice after an intraneural injection of test serum⁽⁹⁾.

Nonetheless, it is imperative to locate and characterize these SDFs in leprosy patients. The assay adopted by Shetty, *et al.*⁽⁹⁾ is obviously cumbersome for screening a large number of sera. Hence, the prescreening of sera using an ELISA was devised with the purpose of eliminating those samples having the least binding toward human peripheral nerve antigens. The serum demonstrating the highest affinity could then be used later for the functional assay. This communication describes the screening assay utilized and discusses the implications of the results obtained.

Antigen. Normal human nerve (posterior tibial and sural) was collected from a freshly amputated limb in sterile Hanks' balanced salt solution. The epineurium was removed and the nerve was incubated at 37°C for 30 min in 0.05% collagenase solution (Collagenase Type II; Sigma Chemical Co., St. Louis, Missouri, U.S.A.) prepared in Dulbecco's modified Eagle medium (GIBCO Laboratories, Grand Island, New York, U.S.A.). Thereafter the nerve was cut, finely chopped, and sonicated in 2.5% sodium dodecyl sulfate for 2 hr with 30 sec pulses. After centrifugation at 500 rpm for 10 min, the supernatant was assayed for protein content⁽²⁾. Aliquots (100 μ l) of the supernatant from a single nerve sample were stored at –20°C and used for the entire assay. The supernatant was diluted with phosphate buffered saline (PBS), pH 7.4, to give a final concentration of 500 ng per 50 μ l per well.

Antibody. Human serum was diluted 1:10 using 5% BSA (bovine serum albumin, fraction V; Loba Chemie, Bombay, India) in PBS. Leprosy patients were classified according to the Ridley-Jopling scale⁽⁸⁾. The