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.

Cimetidine Inhibits Suppressor Factor Production in Ethiopian Lepromatous Leprosy Patients

TO THE EDITOR:

Brown, *et al.* ([5]) reported in the December 1985 JOURNAL a study on "The Immunological Effects of Cimetidine in Patients with Lepromatous Leprosy" in Chiang Mai, Thailand. The patients had well documented episodes of erythema nodosum leprosum (ENL). Cell-mediated immunity (CMI)—as measured by lymphoproliferation *in vitro* (LTT), helper : suppressor cell ratios, and responses to four skin test antigens—was tested before and after cimetidine treatment. We have approached the question of the immunological effects of cimetidine in a group of patients across the leprosy spectrum *in vitro*. Our findings did show effects on CMI in lepromatous patients, and may be at variance with the conclusions of Brown's group in Chiang Mai.

Our study was undertaken in light of the findings that serum levels of histamine were found to be increased in lepromatous leprosy patients in India ([16]), as well as numerous reports in the literature concerning the ability of H-2 receptor antagonists, such as cimetidine, to reduce or reverse immunosuppression caused by endogenous or exogenously added histamine ([3, 9, 15, 21]). We studied 6 lepromatous (1 BL, 5 LL) and 6 tuberculoid (BT) leprosy patients who had long-term-treated disease without history of reaction and who were characterized clinically and, in some cases, histopathologically by experienced leprologists at the ALERT Leprosy Control, Addis Ababa, Ethiopia. One patient in each group was a newly diagnosed patient at the ALERT Hospital.

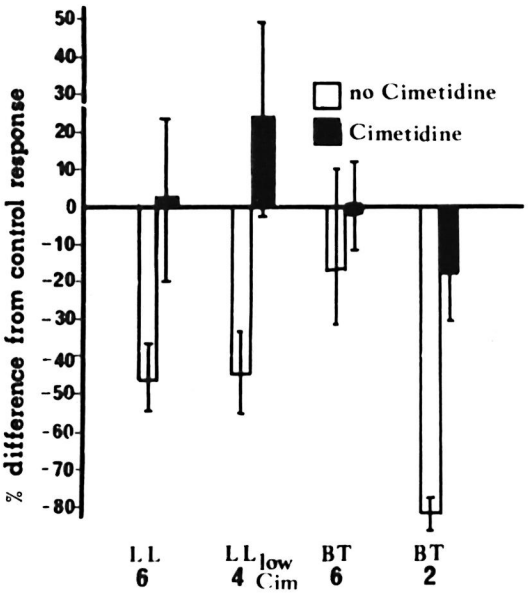
Thirty ml of blood was taken from each patient and defibrinated by gentle rotation with glass beads. Mononuclear (PBM) cells were isolated by standard Ficoll-Isopaque techniques. PBM (2×10^6) were cultured with different doses of cimetidine (2.5, 5.0, and 25.0 μg/ml) and with a low concentration of *Mycobacterium leprae* (2.5×10^5) in 24-well culture plates in a volume of 2.0 ml in 20% normal human serum and RPMI 1640 with penicillin/streptomycin. Cimetidine was a kind gift of Dr. N. G. Clarke of Smith, Kline and French, Welwyn, England. Supernatants were harvested after 24 hr, filtered (0.2 μ, Millipore) and stored at −20°C until ready for testing. Other cells were cultured with cimetidine and *M. leprae* in 96-well microtiter plates to assess lymphocyte proliferation (LTT) in a 7-day assay. Cimetidine did not cause any changes in the LTT with or without antigen in the 12 patients in this study or, in fact, in 6 other patients where this function was also assessed, even in the presence of 1.0 μg/ml indomethacin which blocks *de novo* synthesis of prostaglandins which suppress CMI ([3, 21]).

The 24-hr supernatants were tested for the presence of suppressor factors (SF) given that SF have been consistently found in lepromatous leprosy by all investigators who have looked into this phenomenon ([17–19]).

Cells (10^5/well) from a healthy individual whose cells were known to proliferate in response to *M. leprae* antigens *in vitro* were cultured with batch CD-46 organisms (provided by Dr. R. J. W. Rees through the World Health Organization Immunology of Leprosy Program) and an equal volume (100 μl) of supernatant in microtiter wells in triplicate. Supernatants from 3 lepromatous and 3 tuberculoid patients were tested simultaneously; the supernatants from the other 6 patients were tested simultaneously on another occasion. After 6 days of culture ^{3}H-thymidine was added to each well, and the cultures were harvested onto filter paper the following day. After the paper had dried, the samples were counted in scintillation fluid in a beta counter. Suppression or reduction of responsiveness based on the variation observed in control cultures without supernatant was arbitrarily defined as: >40% = high or significant; 25%–40% = moderate; <25% = low or insignificant.

It was found that cells from 6 of 6 lepromatous leprosy patients in response to *M. leprae* produced factors in the culture supernatants that caused moderate (3) or high (3) levels of suppression of the response of the normal individual's lymphocytes to leprosy bacillus antigens. In the tuberculoid patient supernatants, 2 caused high levels of suppression, 2 very low, and 2 appeared to produce enhancing factors—1 low level enhancement and 1 high level. At the high dose of cimetidine (25.0 μg/ml), almost no effect on the level of suppressor factor (SF) production was observed in any of the 4 patients, 2 BT and 2 LL, tested. At the lower doses (2.5 and 5.0 μg/ml), significant effects were observed in all patients who produced SF.

The Figure shows that on average lepromatous patient supernatants caused a 46% reduction of the normal individual's response to *M. leprae*. In the presence of cimetidine, the suppression was eliminated. In fact, on average there was a slight enhancement (3%) of the response. However, in the four lepromatous patients whose cells were treated with a low dose of cimetidine (low Cim), the suppression of 49% was reversed to a net enhancement of 24%. The third pair of bars shows that as a group tuberculoid patients produced only low levels



THE FIGURE. The effect of supernatants from *M. leprae*-stimulated mononuclear-cell cultures on the proliferative response of a healthy responder individual. The counts per minute (cpm) of ^{3}H-thymidine after 6 days of culture in the presence of *M. leprae* from the healthy responder were 11,782 ± 2837. Background cpm in cultures without antigen were 746 ± 163. "0" on the horizontal axis represents the healthy responder's cpm in the presence of *M. leprae* and culture medium. The mean percent difference from the control response in the presence of patient culture supernatants with or without cimetidine is represented in the bars.

of SF, and in the presence of cimetidine, there was on average no effect of the cimetidine in culture. However, while studies on cellular responses *in vitro* have shown little variation in the lepromatous groups, except in responsiveness to interleukin-2 (e.g., [7, 10, 14]), marked variation has often been observed within the BT group ([4]). Two of the six BT patients in the present study did indeed produce high levels of SF in their supernatants in response to *M. leprae*. The cultures from these patients that had been treated with 5.0 μg/ml cimetidine reduced the observed suppression to moderate or insignificant levels.

The low dose of cimetidine used in our study was comparable to that used by others in *in vitro* studies, and the dose is comparable to the concentration obtained in patients treated for ulcers or hypogammaglob-

ulinemia (refs. in [3, 21]). The twice-daily dose given to the Thai patients ([5]) is nearly half that given to the patients in the studies referred to above, and should be considered in evaluating the apparent lack of effect on CMI found in these lepromatous leprosy patients. Brown, *et al.* ([5]) treated patients *in vivo* with cimetidine, and found that there was no change in the LTT responses after 1 month of treatment. We found that adding cimetidine to lymphocyte cultures likewise failed to reverse the well-documented, antigen-specific T-cell anergy in lepromatous disease. However, we did examine a different parameter—suppressor factor production—that is nearly as well documented and found that the drug did indeed have an effect that reduced or even reversed immunosuppression *in vitro*.

The patients in the two studies were different. In the Chiang Mai report, the large group (N = 29) consisted of treated patients with inactive disease and a clear history of ENL. The smaller group of eight patients had active lepromatous leprosy (whether these patients had had ENL was not indicated by the authors); only three of the patients in this group received cimetidine. In contrast, the present study involved six tuberculoid and six lepromatous patients; none of the latter had any history of ENL. Patients with ENL are known to have had at least one episode where immunological alterations have occurred. These alterations include a reversal of immunosuppression as measured by the reduction of ConA responses in the presence of lepromin ([11]), an increase in the helper : suppressor T-cell ratio both in lesions ([11]) as well as in peripheral blood ([1, 12, 20]), an enhanced response to mitogens ([1, 12]) and, compared to other BL/LL patients, to PPD ([12]). There is, thus, clearly some difference in immunoregulation in patients with and without a history of ENL. Patients without ENL sustain an unremitted state of suppressed immunity, at least to *M. leprae* antigens.

Production of SF was found in all lepromatous patients in this study, and likewise by Salgame ([18]) and Sathish ([19]), but only rarely in tuberculoid patients. Susceptibility to histamine-induced suppression is variable in humans ([3]). It could be expected that this variability may also exist in LL patients. Susceptible patients may respond to cimetidine for removal of at least part of their immunological defect.

Cimetidine did appear to block SF production in lepromatous patients in Ethiopia. Presumably, SF in supernatants was detected due to the presence of endogenous histamine in the mononuclear cell cultures which could be reversed by cimetidine. Barsoum, *et al.* ([2]) found that cimetidine significantly increased schistosomiasis patient responses to specific parasite antigens. Moreover, they cite the evidence of Hofstetter, *et al.* ([8]), who found that basophils contaminate most peripheral blood mononuclear cell populations. In the latter study, it was demonstrated that parasite antigens at least triggered the release of suppressive levels of histamine. Removal of nylon wool adherent cells also removed 74% of basophils and enhanced the responsiveness to parasite antigen. This effect was reversed by adding back histamine to these cultures.

Such phenomena may partially explain our own data as well as the results of others ([6, 10, 13]) who also found that removal of monocytes or adherent cells enhanced responsiveness to mycobacterial antigens. The presence of basophils in Ficoll-purified blood of leprosy patients is currently under investigation in our laboratory.

A poorly explored area is the question of interactions of cimetidine with drugs used in leprosy treatment. However, it is possible that the use of cimetidine or other immunopharmacological agents may be a useful adjunct in the treatment of leprosy perhaps concomitantly with immunostimulatory products.

—Paul J. Converse, Ph.D.
—R. M. Mshana, M.D., Ph.D.
—G. J. Bjune, M.D., Ph.D.

Armauer Hansen Research Institute
P.O. Box 1005
Addis Ababa, Ethiopia

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Dr. Nelson, *et al.*'s Response

To the Editor:

We are interested in learning of the intriguing experiments reported by Converse, *et al.* ([2]), which examine the effects *in vitro* of various doses of cimetidine on lymphocyte transformation (LT) responses to *Mycobacterium leprae* and the generation of suppressor factors by lymphocytes stimulated with *M. leprae* in the presence or absence of cimetidine.

The *in vivo* studies we reported ([1]) are in agreement with the experiments reported by