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A Study of the Immunological Effects of Cimetidine in Patients with Lepromatous Leprosy[1]

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The main indication for cimetidine is in reducing gastric acid hypersecretion, but recent studies suggest that cimetidine also may act as a nonspecific stimulant of cell-mediated immunity (CMI) ([10]). Inhibition of various T cell functions ([6, 9]) and activation of suppressor cells ([8]) appear to be mediated in part via H-2 receptors. Cimetidine, an H-2 receptor antagonist, has been shown to augment cellular immune responses to Candida antigens in chronic mucocutaneous candidiasis ([5]), and delayed-type hypersensitivity (DTH) to a variety of antigens ([1]).

Whether or not cimetidine could produce immune enhancement in patients with leprosy would be of interest for two reasons. An increase in the degree of host CMI might be beneficial in the killing and clearing of bacilli. Contrariwise, enhancement of CMI might precipitate reaction phenomena. To investigate these questions, a clinical study was carried out in northern Thailand. Patients with inactive lepromatous disease were chosen for study since they are known to remain anergic to antigens of *Mycobacterium leprae* ([2]), but the reduced antigen load may allow an immunological effect to be more easily demonstrated. Moreover, the lower load of antigen places them at less risk of experiencing erythema nodosum leprosum (ENL) than active patients ([13]). A smaller group of patients with active disease were studied in a randomized, double-blind protocol.

MATERIALS AND METHODS

Study of patients with inactive lepromatous leprosy

Patients. Thirty patients who were residents of McKean Rehabilitation Institute, Chiang Mai, Thailand, enrolled in the study. They ranged in age from 24 to 60 years, and consisted of 22 males and 8 females. A single, experienced leprologist had classified all of the patients as having either lepromatous (LL, 25 patients) or borderline lepromatous (BL, 5 patients) leprosy. All patients but one had had well-documented clinical episodes of ENL while under therapy at McKean, and each had had bacterial indices (BI) of 2.0 or greater. (One exception was a patient who arrived with a BI of 1.7 after having received initial treatment at another hospital.) Twenty-two of the patients had had BIs of 3.0 or greater. Follow-up skin scrapings were performed every six months, and the BI of each patient had been 0.0 for at least one year prior to this study.

Patients were excluded from the study if they were: 1) pregnant or lactating, 2) malnourished, 3) had experienced ENL or reversal reactions in the prior three months, 4) had used glucocorticoids in the prior 30 days, 5) had bradycardia or arrhythmias, or 6) had other medical conditions known to influence immune responses. The patients were receiving dapsone monotherapy with the exception of two who had been treated with clofazamine and rifampin for one week just prior to, or during, the study. All patients were informed of the nature of the study, and gave their informed consent before entry.

Study design. In an open study, 400 mg tablets of cimetidine (provided by Smith

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Kline & French Laboratories) were given under supervision to outpatients twice a day for four weeks. Prior to starting treatment, the patients were examined, photographed, had blood drawn for *in vitro* assays, and skin tests applied and read. Cimetidine was continued until the repeat skin tests (applied after four weeks of therapy) had been read and blood collected for *in vitro* assays.

Skin testing. Skin tests, 0.1 ml of each antigen, were applied intradermally by nurses proficient in the technique. Four antigens were employed: Dharmendra lepromin of human origin (courtesy of M. Abe, National Institute of Leprosy Research, Tokyo); PPD-S (5 T.U., Connaught Labs, Toronto, Canada); candida (1:100; Hollister-Stier Labs, Atlanta, Georgia, U.S.A.); and trichophytin (1:100; Hollister-Stier). All skin tests were read by one of the authors (AB) at 48 hr. Crossed-diameters of induration were measured and averaged. When an initial test produced more than 10 mm of induration, re-testing with the same antigen was not performed.

Lymphocyte transformation test (LTT). LTTs were performed on patients before and after four weeks of treatment. Control subjects (male blood donors and project staff) were assayed concurrently. Peripheral blood mononuclear (PBM) cells were separated from heparinized blood by Ficoll-Hypaque (Litton Bionetics, Bethesda, Maryland, U.S.A.) gradient centrifugation, washed, and cultured in triplicate for five days (three days for PHA) using standard techniques ([12]). Each culture received 0.1 μCi of ^{3}H-thymidine 18 hr prior to harvesting. The mean cpm of each triplicate sample was determined and the degree of stimulation expressed as Δcpm, calculated as follows: Δcpm = mean cpm stimulated − mean cpm unstimulated.

Lymphocyte cultures were stimulated with Dharmendra lepromin, BCG (Connaught Labs), or PHA (Burroughs Wellcome Co., Tuckahoe, New York, U.S.A.). Lepromin was used in concentrations of 2×10^4, 2×10^5, and 2×10^6 bacilli/well; BCG in concentrations of 2×10^3, 2×10^4, and 2×10^5 bacilli/well; and PHA at 10 μl (125 μg/ml) per well.

Phenotyping of peripheral blood lymphocytes. PBM cells were frozen in a controlled rate freezer, and shipped in liquid nitrogen to Cincinnati. Thawed cell preparations were resuspended at a concentration of 5×10^6 cells/ml in RPMI 1640 (KC Biologicals, Lenexa, Kansas, U.S.A.). The viability of cells, as tested by trypan blue dye exclusion, was greater than 70%. Two hundred μl aliquots of cells (10^6) were placed in tubes to which was added 5 μl of one of the following monoclonal antibodies: OKT3, OKT4, or OKT8 (Ortho Diagnostics, Raritan, New Jersey, U.S.A.). After incubation for 30 min at 4°C, cells were washed twice at 4°C and further incubated at 4°C for 30 min with 100 μl of fluorescein-labeled anti-mouse immunoglobulin (Cappel Labs, Cochranville, Pennsylvania, U.S.A.). Control cells were incubated with the latter immunoglobulin only. The cells in each tube were washed twice and analyzed for surface phenotype using a fluorescence-activated cell sorter (FACS III; Becton-Dickinson, Sunnyvale, California, U.S.A.).

Statistics. Skin test results and blood and lymphocyte counts were analyzed using a comparison of paired means. Since the LTT results were not normally distributed, nonparametric analysis (Wilcoxon rank sum test) was employed ([3]).

Study of patients with active lepromatous leprosy

After giving informed consent, eight patients with active lepromatous leprosy were randomized under a double-blind code to receive either 400 mg of cimetidine (N = 3), or identical-appearing tablets of placebo (N = 5), four times per day. Patients received the study drug for one month while hospitalized, thus allowing supervised administration. Immune status was assessed before, and at the end of, the treatment period using the intradermal skin tests listed above plus mumps antigen (Eli Lilly, Indianapolis, Indiana, U.S.A.). LTT responses to lepromin, BCG, and PHA were determined before therapy and again after four weeks on drug or placebo, as described. Patients were examined by one of the authors (AB) on a daily basis to detect leprosy-related reactions and other possible side effects. To evaluate patient responses to a new antigen, contact sensitization was attempted with dinitrochlorobenzene (DNCB) by

TABLE 1. *LTT responses in patients with inactive lepromatous leprosy before and after cimetidine treatment.*

Stimulant	Bacilli per well	LTT responses[a]		
		Before cimetidine median (range) N = 28	After cimetidine median (range) N = 28	Controls median (range) N = 14
PHA		28.5 (15.6 to 39.9)	26.6 (15.7 to 38.8)	25.0 (17.4 to 36.8)
BCG	2×10^3	2.7 (−2.8 to 25.0)	2.2 (−2.3 to 18.8)	4.9 (0.0 to 22.1)
	2×10^4	3.8 (−3.4 to 14.7)	2.4 (−3.0 to 15.5)	4.3 (−0.6 to 23.5)
	2×10^5	2.9 (−1.0 to 17.9)	2.6 (−4.7 to 17.4)	4.6 (−1.4 to 27.7)
Lepromin	2×10^4	0.0 (−0.8 to 9.9)	0.6 (−2.7 to 8.6)	1.9 (−0.2 to 16.3)
	2×10^5	0.3 (−6.0 to 14.6)	0.4 (−5.3 to 17.3)	4.1 (−1.0 to 15.7)
	2×10^6	−0.2 (−8.8 to 13.1)	−0.1 (−6.3 to 8.5)	2.7 (−0.9 to 19.8)

[a] Responses given as $\Delta cpm \times 10^{-3}$.

applying 1000 μg of DNCB after two weeks of treatment with cimetidine or placebo. A challenge dose of 50 μg of DNCB was applied two weeks later.

RESULTS

Study of patients with inactive disease. The course of cimetidine therapy was completed by 29 of the 30 inactive disease patients enrolled. No patient developed either ENL or reversal reaction episodes during the study or in the following six weeks.

More than 60% of the study group had initial skin test responses greater than 10 mm with PPD, and one third of these were greater than 20 mm. (Only four of the patients had BCG vaccination scars.) Re-testing of nine patients in whom the initial PPD response was less than 10 mm showed an increase in induration from a mean of 2 mm before, to 5 mm after four weeks of cimetidine treatment ($p < 0.05$). Skin testing with antigens other than PPD demonstrated no statistically significant changes. In response to Dharmendra lepromin at 48 hr (Fernandez reaction), one patient had reactions of 5.5 mm before, and 5.0 mm ("weakly positive" [4]) after cimetidine treatment. All other Fernandez reactions were less than 5 mm, or "negative." These data are in agreement with the clinical classification of these patients as having BL/LL leprosy.

Initial LTT responses to PHA and BCG were not different from those of staff and blood bank controls; responses to lepromin were decreased ($p < 0.01$; Table 1). Repeat LTTs after four weeks of cimetidine administration revealed no significant change in the responses to PHA, BCG, or lepromin (Table 1).

The total lymphocyte counts measured in Chiang Mai were within normal limits and did not change significantly with cimetidine therapy (Table 2). In eight patients, no statistically significant changes in T cell subpopulations were observed in peripheral blood studied before and after treatment (Table 2).

Study of patients with active disease. There were no significant changes in skin test or LTT responses after therapy in eight patients with active lepromatous leprosy (Table 3). The observed difference in sen-

TABLE 2. *Lymphocyte populations in patients with inactive lepromatous leprosy before and after cimetidine treatment.*

Cell population	No.	Before cimetidine (mean ± S.D.)	After cimetidine (mean ± S.D.)
Total lymphocytes	27	2914 ± 1547/mm³	2534 ± 1266/mm³
OKT3+	8	3054 ± 1936/mm³	2433 ± 1546/mm³
OKT4+	8	1536 ± 1001/mm³	1562 ± 1106/mm³
OKT8+	8	1062 ± 789/mm³	826 ± 587/mm³
OKT4/OKT8	8	1.78 ± 0.78	1.99 ± 0.72

TABLE 3. *Responses to treatment of patients with active lepromatous leprosy given cimetidine or placebo.*

Parameter	Cimetidine group	Placebo group
Clinical events		
Leprosy-related reactions	None	Iridocyclitis × 1 ?ENL × 2
Other possible side effects	Mental depression × 1	Macular rash × 1
Skin tests (change in induration (mm))		
Lepromin	−2 (N = 3)	0 (N = 5)
Mumps	+4 (N = 3)	0 (N = 4)
PPD	+4 (N = 3)	+2 (N = 1)
Candida	+1 (N = 3)	−1 (N = 4)
Trichophytin	0 (N = 3)	−1 (N = 5)
DNCB[a]	0/3 positive	3/5 positive
LTTs (median changes in Δcpm)		
PHA	−10,376 (N = 3)	1,359 (N = 5)
BCG (2 × 10^5 bacilli/well)	528 (N = 2)	1,695 (N = 3)
Lepromin (2 × 10^5 bacilli/well)	518 (N = 2)	1,055 (N = 3)

[a] Definition of positive: 50 μg challenge dose produced induration.

sitization with DNCB between cimetidine and placebo recipients is not significant. None of three patients given cimetidine developed leprosy-related reactions. Among 5 patients on placebo, 1 developed acute iridocyclitis, 2 developed cutaneous nodules consistent with mild ENL, and 1 developed a macular rash of uncertain etiology which disappeared spontaneously.

DISCUSSION

Plaut and coworkers ([6]) demonstrated pharmacologically that mice have T lymphocytes with H-2 receptors. Mice immunized intraperitoneally with allogeneic cells developed specific, lymphocyte-mediated, cytolytic activity, inhibited more than 40% by histamine. The inhibition was reversed by antagonists of H-2 (burimamide and metiamide) but not H-1 (diphenhydramine and pyrilamine) receptors. Using guinea pigs immunized with orthochlorobenzoyl-bovine gamma globulin in complete Freund's adjuvant, Rocklin ([9]) found that histamine (10^{-3} M) reduced DTH skin reactions by 40–60%. This suppression was completely reversed by an H-2 receptor antagonist (burimamide).

Jorizzo and coworkers ([5]) reported four adult patients with chronic mucocutaneous candidiasis who were unresponsive to Candida antigen as measured by skin test, LTT, and production of leukocyte MIF. After four weeks of therapy with cimetidine, all four patients responded to Candida skin tests and 2 of the 4 produced leukocyte MIF. Four weeks after discontinuing cimetidine treatment, all four patients had reverted to their prior state of unresponsiveness. Retreatment with cimetidine was again followed by positive skin test responses and production of leukocyte MIF. Despite these changes in host responses to candida antigen, there was no significant effect on the clinical manifestations of their disease.

No studies of the effects of cimetidine, or other H-2 receptor antagonists, in leprosy have been reported. The patients in this clinical trial were on long-term treatment for lepromatous leprosy and had cleared the vast bulk of the antigen load to become clinically inactive. A limited number of active lepromatous patients with large bacterial loads of *M. leprae* were also studied. A small but statistically significant increase in PPD reactivity was found in the inactive patients studied. This is interpreted to reflect the "booster" effect of repeated PPD skin testing ([7]). No other significant immune enhancement was documented in either study group.

Immune status in leprosy has been determined functionally most often by skin test and LTT responses to *M. leprae* preparations. These techniques are somewhat imprecise but they should allow significant biological effects to be detected, if present. By these criteria, cimetidine had no im-

munological effect. However, interest in immunomodulators as adjuvant therapy in leprosy should continue since immune enhancement could play a key role in better treatment of patients with lepromatous leprosy.

The widespread use of cimetidine around the world raises concern over its safety in various subpopulations, including the estimated 12–15 million people who have leprosy ([1]). In our studies, neither patients with active disease nor those with inactive disease developed leprosy-related reactions. At highest risk for these reactions are patients with active lepromatous disease, for they possess very high antigen loads. Although three active disease patients who received cimetidine in this study did not experience reactions, administration of cimetidine to larger numbers will be required before definitive conclusions can be drawn. The sample size of patients with inactive disease allows us to predict that if cimetidine does precipitate such reactions, the incidence would be less than 4%. Thus, patients with inactive lepromatous leprosy may use the drug for peptic ulcer disease without fear of being at unusually high risk of side effects.

SUMMARY

To test the capacity of cimetidine to enhance cellular immunity in patients with lepromatous leprosy (LL), cimetidine was given for one month to 29 inactive LL patients and 3 active LL patients. Immune function was monitored with skin tests (lepromin, PPD, candida, and trichopytin), lymphocyte transformation tests (phytohemagglutinin, BCG, and Dharmendra lepromin), and quantitation of peripheral blood lymphocyte subpopulations. A small but significant "booster" response to PPD was the only change observed in the study of patients with inactive disease, and leprosy-related reactions did not occur. In the few active LL patients studied, neither immune enhancement nor leprosy-related reactions were observed. The results of this investigation suggest that cimetidine can be used safely in patients with inactive lepromatous leprosy.

RESUMEN

Para probar la capacidad de la cimetidina para incrementar la inmunidad celular en los pacientes con lepra lepromatosa (LL), se administró cimetidina durante 1 mes a 29 pacientes LL inactivos y a 3 pacientes LL activos. Las funciones inmunes se evaluaron con pruebas dérmicas (lepromina, PPD, candidina y tricofitina), con pruebas de transformación de linfocitos (PHA, BCG y lepromina de Dharmendra) y por la cuantificación de las subpoblaciones de linfocitos periféricos. En los pacientes LL inactivos sólo se observó una pequeña pero significante respuesta anamnésica con el PPD pero no se observaron reacciones relacionadas con la lepra. En los pocos pacientes LL estudiados no se observaron ni incremento en la función inmune, ni reacciones relacionadas con la lepra. Los resultados de esta investigación sugieren que la cimetidina puede usarse sin riesgos en los pacientes con lepra lepromatosa inactiva.

RÉSUMÉ

On a administré de la cimétidine pendant un mois à 29 malades atteints de lèpre LL inactive et à 3 malades LL présentant une maladie active, afin d'étudier dans quelles mesures la cimétidine peut stimuler l'immunité cellulaire des malades atteints de lèpre lépromateuse. On a suivi la fonction immunitaire par des tests cutanés (lépromine, PPD, candida et trichophytine), l'épreuve de transformation lymphocytaire (phytohemagglutinine, BCG, et lépromine de Dharmendra), et par la mesure des sous-populations de lymphocytes dans le sang périphérique. Une réponse de stimulation (booster) peu prononcée, mais toutefois significative, a été observée à la suite de l'injection de PPD, chez les malades atteints de lèpre inactive; aucune réaction associée à la lèpre n'a été enregistrée. Chez les quelques malades lépromateux actifs qui ont été étudiés, on n'a pu observer aucune stimulation immunitaire ou réaction associée à la lèpre. Les résultats de cette étude suggère que la cimétidine peut être utilisée sans danger chez les malades présentant une lèpre lépromateuse inactive.

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REFERENCES

1. Avella, J., Binder, H. J., Madsen, J. F. and Askenase, P. W. Effect of histamine H-2 receptor antagonists on delayed hypersensitivity. Lancet **1** (1978) 624–626.
2. Bullock, W. E. Immunobiology of leprosy. In: *Immunology of Human Infection, Part I.* Nah-

mias, A. and O'Reilly, R., eds. New York: Plenum, 1981, p. 374.
3. COLTON, T. Non-parametric methods. In: *Statistics in Medicine.* Boston: Little, Brown and Co., 1974, 219–227.
4. JOPLING, W. H. *Handbook of Leprosy.* London: Heinemann Medical Books Ltd., 1978, p. 46.
5. JORIZZO, J. L., SAMS, W. M., JEGASOTHY, B. V. and OLANSKY, A. J. Cimetidine as an immunomodulator: chronic mucocutaneous candidiasis as a model. Ann. Intern. Med. **92** (1980) 192–195.
6. PLAUT, M., LICHTENSTEIN, L. M. and HENNEY, C. S. Properties of a subpopulation of T cells bearing histamine receptors. J. Clin. Invest. **55** (1975) 856–874.
7. RICHARDS, N. M., NELSON, K. E., BATT, M. D., HACKBARTH, D. and HEIDENREICH, J. G. Tuberculin test conversion during repeated skin testing, associated with sensitivity to nontuberculous mycobacteria. Am. Rev. Respir. Dis. **120** (1979) 59–65.
8. ROCKLIN, R. E. Histamine-induced suppressor factor (HSF): Effect on migration inhibition factor (MIF) production and proliferation. J. Immunol. **118** (1977) 1734–1738.
9. ROCKLIN, R. E. Modulation of cellular-immune responses *in vivo* and *in vitro* by histamine receptor-bearing lymphocytes. J. Clin. Invest. **57** (1976) 1051–1058.
10. ROCKLIN, R. E. and BEER, D. J. Histamine and immune modulation. Adv. Intern. Med. **28** (1983) 225–251.
11. SHEPARD, C. C. Leprosy. In: *Harrison's Principles of Internal Medicine.* New York: McGraw-Hill, 1983, pp. 1030–1032.
12. WAITHE, W. I. and HIRSCHHORN, K. Lymphocyte response to activators. In: *Handbook of Experimental Immunology.* Wier, D. M., ed. London: Blackwell, 1978, pp. 26.1–26.10.
13. WATERS, M. F. R. and HELMY, H. S. The relationship of dapsone (DDS) therapy to erythema nodosum leprosum. Lepr. Rev. **45** (1974) 299–307.