THE TABLE. *Detection of* M. leprae *by PCR in peripheral blood samples.*

Sample	Type	No. blood samples showing PCR amplification		Total
		+	−	
	TT	0	1	1
	BT	0	1	1
Peripheral blood	BL	3 (1)[a]	0	3 (1)
	LL	4 (4)	2	6 (4)
	Control	0	2	2

[a] Figures in parentheses designate untreated patients.

leprosy patients and two patients suffering from erythema nodosum leprosum (ENL), positive amplified PCR products were detected, indicating that *M. leprae* exist for a relatively long period in the peripheral blood of active cases (The Table).

This study assessed the applicability of PCR coupled with DNA hybridization analysis for the detection of small numbers of *M. leprae*. Two set of primers, S13 and S62, were selected on the basis of established nucleotide sequences of the 36-kDa genes to specifically detect *M. leprae* through amplification of characteristic DNA sequences. When we applied PCR on paraffin-embedded and frozen biopsy tissues from leprosy patients, the frozen section results were better than those of the paraffin-embedded sections. This was especially so in the paraffin-embedded section from a bacterial index-negative treated patient who was PCR positive. A similar result has been reported by de Wit, *et al.*([1]). When we applied PCR on peripheral blood, positive amplified PCR products were detected in five untreated leprosy patients and two patients

with ENL, suggesting that *M. leprae* exist for a relatively long period in the peripheral blood of active cases. In this report apparent identification of *M. leprae* DNA in the tissue and blood by PCR shows that this method can be an additional tool for the diagnosis of suspected cases of early leprosy and for epidemiologic study.

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Minimal Bactericidal Concentration of Clarithromycin Against *M. leprae*

TO THE EDITOR:

The new macrolide antibiotic, clarithromycin, was discovered by Franzblau and Hastings ([3]) to inhibit the metabolism of *Mycobacterium leprae in vitro* and in *M.*

leprae-infected mice to demonstrate bactericidal-type activity when administered at 0.01% in the diet. The bactericidal activity (96%) of dietary 0.1% clarithromycin for *M. leprae* was quantitated by the authors ([8]), as

THE TABLE. *Killing of* M. leprae *by various dietary concentrations of clarithromycin.*

Clarithromycin dietary concentration	No. *M. leprae* inoculated			% Killed ± S.E.	Probability that killing occurred
	5×10^1	5×10^2	5×10^3		
	No. positive foot pads/no. foot pads counted				
0.0% (control)	1/6	2/8	4/8		
0.01%	0/10	2/10	5/10	52 ± 39	0.56
0.05%	1/13	0/10	0/10	88 ± 8	0.002
0.1%	0/10	0/10	0/10	88 ± 8	0.002

well as by Ji, *et al.* ([10]), (87%–95%), wherein mice were administered 12.5–50 mg/kg clarithromycin by gavage. Because levels *in vivo* of clarithromycin that were inactive against *M. leprae* had not been studied to date, we ([6]) established for a single *M. leprae* isolate that clarithromycin fed continuously in dietary concentrations of 0.001%, 0.005%, 0.01%, 0.05%, and 0.1% consistently inhibited the growth of *M. leprae,* while 0.0001% did not, establishing a minimal inhibitory mouse serum concentration of no more than 25 ng/ml and likely as little as 2.5 ng/ml.

Because actual bactericidal activity against *M. leprae* is critical to its use in patients, this current study was designed to establish clarithromycin's minimal bactericidal dietary concentration against the same *M. leprae* isolate used by us previously to establish its minimal inhibitory concentration. For these purposes, groups of generally 10 BALB/c female mice were infected in both hindfoot pads with 50, 500, and 5000 *M. leprae* and treated for the first 2 months ([1]) with various dietary concentrations of clarithromycin [0% (control), 0.01%, 0.05%, and 0.1%]. For these studies, clarithromycin was initially dissolved in 95% ethanol and evenly distributed in mouse chow by using a Patterson-Kelly twin-drum liquid-solid blender (Patterson-Kelly, East Stroudsburg, Pennsylvania, U.S.A.). Diets were made fresh every 2 weeks and stored in a refrigerator. One year after therapy had been discontinued, a time sufficient to detect multiplication of *M. leprae* from any bacilli surviving therapy, the number of foot pads wherein *M. leprae* survived (number of *M. leprae*/foot pad ≥ 10^5) was assessed. ([1]) From these results the percentage of bacteria killed was quantitated by the method of Spearman and Kärber, described previously by Shepard ([12]), and the probability that actual killing occurred determined.

Both 0.05% and 0.1% clarithromycin were found to be bactericidal (88% ± 8%) for *M. leprae* (The Table). Furthermore, we observed that the probability that killing occurred at these two dietary concentrations was highly significant (p ≤ 0.002). Unfortunately, in this study the control inoculum itself had low viability, perhaps accounting for why these high dietary concentrations were not found to be as bactericidal as previously demonstrated for other isolates. In these studies 0.01% clarithromycin resulted in no significant killing of *M. leprae*.

It is noteworthy in this study that the growth of the *M. leprae* isolate used herein had been found to be consistently inhibited by the continuous dietary administration of clarithromycin of 0.001% and higher ([6]). Thus in this study we found a 50-fold greater concentration of clarithromycin was required to kill *M. leprae* than had been found previously to inhibit the same isolate's growth. It is further noteworthy that Walker and Shinnick ([14]) also found that for several *M. leprae* isolates, including "pan-susceptible," dapsone-resistant, and rifampin-resistant, a discrepancy between clarithromycin's minimal inhibitory dietary concentration and minimal bactericidal dietary concentration was generally demonstrable. Such discrepancies between the amount of drug necessary to inhibit and kill *M. leprae* have been demonstrated previously for active antimicrobials, dapsone ([4]), clofazimine ([5]), and ethionamide ([5]). On the other hand, the amount of minocycline required to inhibit and kill *M. leprae* was found previously to be remarkably similar ([9]). The relative activity of antimicrobials against *M. leprae* after standard doses ([2]) frequently has been compared with regard to the time in patients that inhibitory levels of the drug are maintained. Because of our general recognition now that bactericidal activity is

critical to efficacious treatment of lepromatous patients and inhibitory bactericidal levels for several antimicrobials are discordant, we believe that the actual period of time that levels above those required for bactericide would be best substituted to such considerations.

It has been found previously for dapsone ([13]) and minocycline ([7]) that sensitive *M. leprae* isolates are inhibited consistently by a very narrow range of antimicrobial concentrations. On the other hand, we previously reviewed for rifampin that there is a broad range of published results of both its minimal inhibitory concentration (MIC) and its minimal bactericidal concentration (MBC), suggesting a broader range of susceptibility among isolates ([4]). Franzblau and Hastings ([3]) found for an *M. leprae* isolate that 0.01% dietary clarithromycin inhibited its growth and was fully bactericidal. While we ([6]) found that 0.001% inhibited an isolate, it did not result in significant killing of that same isolate herein, this requiring 0.05% or greater. On the other hand, for another isolate Walker and Shinnick ([14]) found that 0.01% clarithromycin neither inhibited nor killed, killing itself for several *M. leprae* isolates being accomplished consistently only by increasing the dietary concentration to 0.1%. Thus, there appears a significant range of susceptibility among *M. leprae* isolates to the activity of clarithromycin. We ([8]) found previously that 0.1% clarithromycin was bactericidal and that this level of dietary clarithromycin results in mouse serum levels one third of that of the peak obtained in man by a very small (400 mg) dose. It is encouraging that the found range of susceptibility of several *M. leprae* isolates does not limit clarithromycin's general applicability to the treatment of leprosy patients, although the exact time/concentration level for optimal clarithromycin therapy remains to be determined. Such considerations may be especially critical to the application of clarithromycin to certain intermittent schedules, particularly once monthly ([11]), that are now being seriously entertained.

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Active Humoral Immunity in the Absence of Cell-Mediated Immunity in Murine Leprosy: Lastly an Explanation

TO THE EDITOR:

Until recently, a puzzling phenomenon in (human) lepromatous leprosy was that related to the patients' immune responsiveness to antigens of *Mycobacterium leprae*. It is a well-accepted fact that lepromatous patients show an absence of cell-mediated immunity (CMI) to *M. leprae* antigens while they retain unaltered their humoral (antibody-mediated) immunity (AbMI). This was puzzling, because the great majority of the microbial antigens belong to the so-called "thymus dependent" type, i.e., they need the participation of T-helper lymphocytes (LcTh) to generate efficient antibody responses. How, then, would the dramatic alteration in the T-cell-mediated immune competence of the host not reflect on its humoral competence?

An analogous situation of a gradual loss of CMI with an apparently unaltered AbMI has been found in mice suffering from "murine leprosy," a disease caused by *M. lepraemurium* (MLM) and characterized by the development of granulomatous lesions in the skin and viscera that highly resemble the lepromatous lesions of human leprosy.

In both mycobacterioses, several investigations point to a deficit in the function of the helper population of T lymphocytes due to the absence of antigen-reactive T cells, the lack of interleukin-2 (IL-2)-producing cells, the excess of suppression by suppressor T (CD8+ or Ly2,3+) cells, the suppression by macrophage-derived factors, etc. (for a recent review on the subject, see [11]). Those steps within the intricate net of cellular interactions that have been found altered in human lepromatous or mouse lepromatoid leprosy and that could, to a certain extent, explain the finding in both diseases of depressed (or absent) CMI to the mycobacterial antigens, are illustrated in an oversimplified manner in Figure 1.

For a long time, in the mouse as well as in humans the existence and function of only one class of helper (L3T4+/CD4+) T lymphocyte (a Th lymphocyte able to mediate cellular immunity and, at the same time, able to cooperate with B lymphocytes for antibody production) was accepted (Fig. 2). The existence of a "bifunctional" T cell, however, does not help to explain the persistence of an active humoral immunity in the absence of a helper CMI to the mycobacterial (thymus-dependent) antigens.

On the other hand, the still recent discovery of two subpopulations of helper (L3T4+) T cells in the mouse (Th1 and Th2) [2, 3, 9, 10] allows one to tentatively explain the apparent paradox mentioned above. Both Th1 and Th2 clones produce and release IL-3, GM-CSF, TNF-α, in addition to other proteins. However, while TH1 clones secrete IL-2, interferon-γ and lymphotoxin, Th2 clones secrete IL-4, IL-5, IL-6, and IL-