

**SESSION II. TEST MODELS FOR THE
EFFECTIVE CONTROL OF CHEMOTHERAPY
FREE COMMUNICATION**

Chairman **A M DHOPLE (USA)**

The use of rodent models in assessing antimicrobial activity against *Mycobacterium leprae*

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Prior to the landmark discovery in 1960 of 'The Experimental disease that follows the injection of human leprosy bacilli into footpads of mice,'¹ the only means of searching for drugs active against human disease was to conduct clinical trials. Because clinical improvement of lepromatous patients is both very slow and variable, because the number of AFB (BI) in the skin falls extraordinarily slowly despite adequate therapy, and because the viability of solid-staining bacilli (MI) was not appreciated, early short-term clinical trials were difficult to conduct and the results even harder to interpret. Of the earlier studies on dapsone only the study of Lowe² followed a stable population until bacteriological negativity, finding 32 of 39 (83%) negative at 5 years, 31 of 35 (89%) negative at 6 years, and 34 of 35 (97%) smear-negative at 7 years.

The earliest studies on the effect of antimicrobial agents on *Mycobacterium leprae*-infected mice utilized primarily drugs known to be effective against *M. tuberculosis*. These first studies utilized constant treatment from the time of mouse footpad infection, generally with 5×10^3 *M. leprae*/footpad, either by incorporation of drug into mouse chow or daily (actually usually five times weekly) intraperitoneal injections. By these means Shepard³ found dapsone, clofazimine, isoniazid, para-aminosalicylic acid, streptomycin, and cycloserine active and ethambutal and pyrizinamide inactive. By similar means Gaugas⁴ in 1967 found the same drugs active, and notably, as well, rifampicin and cephaloridine. This method of drug testing, termed the 'continuous method', does not distinguish between purely bacteriostatic and bactericidal activity. Since it has been well established in bacterial endocarditis and a number of other infectious diseases where protective local or systemic host defence mechanisms are inadequate (osteomyelitis, meningitis, and Gram negative bacteremia in the neutropenic patient) that bactericidal therapy is crucial to a salutary outcome and that the key to effective short-course chemotherapy for pulmonary tuberculosis is the inclusion of two or more bactericidal agents,⁵ actual bactericidal activity against *M. leprae* is likely to be similarly important in the therapy of lepromatous

leprosy. Since it was recognized, even before the importance of bactericidal therapy was determined to be important for most of these other infectious diseases, that purely bacteriostatic drugs were not likely candidates to be used in the therapy of leprosy, the 'continuous method' has been abandoned in more recent times.

Shepard,⁶ recognizing the inherent problem with the 'continuous method', developed the 'kinetic method' to distinguish bactericidal drugs from those that are merely bacteriostatic. By this method mice are treated from day 60 to 150 after mouse footpad infection. Activity of a drug is measured in terms of the delay between 'plateau' in treated and control mice. A delay no longer than the period of time during which drug is administered represents bacteriostasis, whereas a longer delay which cannot be explained by drug accumulation represents bactericide. One pitfall in such studies is that drugs must be screened at maximal tolerated doses in order to determine whether a drug is truly inactive or merely bacteriostatic. Unfortunately, for many prospective agents such dosage information is not readily available. Utilizing the 'kinetic technique' Shepard⁷ reported the following active drugs to be bactericidal: rifampicin, cephaloridine, dapsone, clofazimine, and ethionamide, and these others to lack such activity: isoniazid, gentamicin, cycloserine, PAS, clindamycin, streptomycin, and thiambutosine.

Colston⁸ developed a procedure termed the 'proportional bactericide technique' which utilizes the mouse footpad analogously to an *in vitro* tube dilution and provides incontrovertible evidence of bactericide. In this procedure groups of mice are inoculated in both feet with 10, 100, 1000, and 10,000 *M. leprae* and treated for the initial 60 days; single feet are harvested and *M. leprae* enumerated 1 year after the conclusion of therapy, a time sufficient for regrowth of *M. leprae* from one or more *M. leprae* surviving the therapy. The percent bactericide can then be quantified by a most probable number calculation or the Spearman-Kärber technique. The latter appears preferable because it allows for direct confidence limits to be applied to the results,⁹ is considerably more precise,¹⁰ and does not assume a Poisson distribution,⁹ i.e. a random dispersion of *M. leprae*, which from the work of Shepard¹¹ appears not to be the case. By this technique Colston has established the following: percentage bactericide dapsone 72%, 78%; clofazimine 96%, 98%; rifampicin 99.99%, 100%; ethionamide 97%, 99%; thiacetazine 42%; thiocarline, thiambutosine 0%.

The mouse footpad can be utilized to establish the minimal effective dietary concentration for a drug and by analysis of the resultant plasma concentration the minimal inhibitory plasma concentrations.^{6,12-14} Minimal inhibitory dietary concentration: dapsone 0.0001%, rifampicin 0.0003-0.01%. Minimal inhibitory plasma concentration: dapsone 3, 4 ng/ml, rifampicin 0.1-3.0 µg/ml

Some of our own work over the past few years has been involved in screening, primarily by the kinetic technique, new agents in mice for their activity against *M. leprae*. For promising agents we try to establish a minimal inhibitory dietary and plasma concentration and evaluate their bactericidal activity and efficacy when

combined with established agents. These efforts are based on the obvious problem in leprosy chemotherapy that the armamentarium of useful drugs is small, intolerance to the established agents is not uncommon, drug resistance to all those agents utilized is appreciated and on the rise, and no new drugs have been brought to the patient since rifampicin in 1970.

Minocycline is a commercially available oral tetracycline which has proven safe on chronic administration. Our studies demonstrate that amongst the tetracyclines minocycline is unique in being active against *M. leprae*. We have established that the minimal inhibitory concentration of minocycline for *M. leprae* is exceedingly low and considerably less than levels easily obtained in plasma and tissues of patients treated with customary doses. Furthermore, in our studies minocycline has proven to be consistently bactericidal for *M. leprae*.

Previously, others^{4,7} have found tetracycline itself inactive against *M. leprae*. In our studies doxycycline (0.02% in mouse chow) also was inactive. In three separate studies we found minocycline bactericidal against *M. leprae*-infected mice: in two studies by the kinetic technique, 0.04% dietary minocycline resulted in prevention of *M. leprae* multiplication for 270 days and for at least 180 days (study still in progress) after therapy was discontinued, and, in another study by the proportional bactericide technique, 0.04% dietary minocycline was found to be 99% bactericidal. Of the drugs used to treat leprosy only rifampicin has proved more bactericidal. This impressive activity found for minocycline is most likely to be the result of its being at neutral pH the most lipid-soluble tetracycline derivative, allowing for its penetration of *M. leprae*'s largely lipid outer capsule and cell wall to its ribosomal site of action. We have established minocycline's minimal inhibitory dietary concentration for *M. leprae* in mice to be 0.01% and, by analysis of resultant mouse plasma by an agar disk diffusion method utilizing the minocycline sensitive *Bacillus cereus* strain ATCC 1178, established the mouse minimal inhibitory plasma level to be $\leq 0.2 \mu\text{g/ml}$. Also, we have demonstrated increased activity against *M. leprae* with dietary concentrations from 0.01% to 0.04%, which yield plasma concentrations from 0.5 $\mu\text{g/ml}$ to 0.9 $\mu\text{g/ml}$. It is noteworthy that in man following usual therapeutic doses 2–4 $\mu\text{g/ml}$ plasma levels are attained, skin levels exceed plasma levels, and minocycline appreciably penetrates nerves. Furthermore, we found minocycline additive or synergistic with dapson, rifampicin, and kanamycin against *M. leprae*.

Draper¹⁵ in an editorial extolled the virtues of development of drugs for leprosy that work at a cell wall locus. Since bacterial cell walls contain moieties that do not occur in animal cells, interference with bacterial cell wall function or inhibition of cell wall synthesis offers an excellent locus for selective toxicity without harm to the host. The extraordinary worth of the beta lactam antibiotics (penicillins and cephalosporins) certainly attests to the value of such a strategy. Cycloserine, being a structural analogue of D-alanine, acts as a competitive antagonist of the enzymes which link D-alanine molecules in the bacterial cell wall. Shepard¹⁶ previously found that 0.5% cycloserine in mouse chow by the

continuous method was only very weakly active, only delaying and partially suppressing multiplication of *M. leprae*. Because we¹⁷ found reason to suspect cycloserine to be unstable in diet, we prepared diets every 2 weeks, changed mouse feeders 2 times weekly, and stored diets refrigerated. Though in these studies other hydroxamic acid derivatives were found inactive, in two separate experiments cycloserine 0.5% and 2% by the kinetic technique resulted in growth delay greater than a year and often for more than 2 years. The minimal inhibitory dietary concentration was established at between 0.1% and 0.5%, since lower levels in mouse chow, 0.1%, 0.025%, and 0.0025% were inactive. Studies are currently in progress with cycloserine at 0.5% and 2% by the proportional bactericide method in order to quantify actual killing of *M. leprae* with cycloserine alone and combined with dapson.

Beta lactam antibiotics may also prove useful in leprosy and are known to act on cell wall synthesis and to be synergistic for certain Gram positive cocci with other agents working at a ribosomal level, particularly aminoglycoside antibiotics. As we discussed previously, Shepard⁶ and Gaugas⁴ found cephaloridine by the kinetic technique to have bactericidal activity against *M. leprae*. Unfortunately, it required injection and proved nephrotoxic, thus being removed from the US market. We screened a number of cephalosporins and cephamycins; all were inactive, except cephradine at 0.5% in mouse chow. *M. leprae* counts were 2.62×10^4 at the completion of therapy but 1.4×10^6 60 days later, which suggests that only bacteriostatic behaviour was observed. Also clavulanic acid, a penicillinase inhibitor, together with amoxicillin (100 mg/kg), appeared active, however again merely bacteriostatic. Unfortunately, clavulanic acid is most unstable in solution and diet, but both tablets containing amoxicillin and clavulanic acid and an injectable including ticarcillin and clavulanic acid are currently marketed. The minimal activity we observed against *M. leprae* may be enhanced if larger doses are employed. Certain infections in mice, particularly klebsiella, require 800 mg/kg.¹⁸ Both ticarcillin + clavulanic acid and amoxicillin + clavulanic acid are currently being studied in our laboratory both by the kinetic and the proportional bactericide technique. It is noteworthy that a number of mycobacteria have been found to contain a penicillinase. Prabhakaran *et al.* 'Beta lactamase: An induced enzyme in *Mycobacterium leprae*?' American Society of Microbiology Annual Meeting, March 23–28, 1986) has found penicillinase activity in *M. leprae*.

Previously, Pattyn *et al.*¹⁹ found by the continuous method that the minimal effective dose of streptomycin was 50 mg/kg weekly. These studies found no profound difference whether the streptomycin was given once, twice, or three times weekly. Pattyn *et al.*¹⁹ found (by the proportional bactericide technique) that streptomycin 100 mg/kg twice weekly resulted in 93 and 81% killing respectively if therapy was begun 2 and 22 days after footpad infection. We²⁰ previously reported that daily intraperitoneal kanamycin (100 mg/kg), streptomycin (150 mg/kg), and amikacin (100 mg/kg) resulted in impressive killing

(respectively 99.7%, 97%, and 96% bactericidal), while gentamicin (20 mg/kg) and tobramycin (20 mg/kg) were inactive. Because these very high doses and frequencies of administration might be toxic for man and are certainly impractical, we attempted to see whether reduced dosage or frequency of administration was effective. The results suggest that for streptomycin both reduced frequency of administration, at least down to once weekly, and reduced dosage, as low as 12.5 mg/kg/day, are associated with significant bactericidal activity. The synergism of rifampicin and streptomycin previously found for *M. kansasii* and *M. intracellulare* infections in mice²¹ appears also to hold for *M. leprae*. It is noteworthy that once-monthly rifampicin plus streptomycin was more active than either drug alone and extraordinarily potent, 99.96% \pm 0.02% bactericidal. Perhaps streptomycin could be applied to certain once-monthly rifampicin regimens.

Published studies utilizing the proportional bactericide technique to assess killing of *M. leprae* by dapsone and rifampicin are limited and confined to constant and high dietary concentrations against only two strains of *M. leprae*. Colston *et al.*⁸ found dapsone 0.01% in mouse chow to be 78% bactericidal for one strain and 72% bactericidal for the other, while rifampicin 0.003% and 0.01% were found 99.99% and 100% bactericidal respectively against these two strains. The antimicrobial therapy of leprosy in man results in a range of bioavailability quite different from the relatively constant levels found in such mouse studies. We thus studied the killing potential of dapsone and rifampicin over a wide range of mouse dietary concentrations that result in the broad range of levels which may actually be experienced by leprosy patients in the course of therapy.

The strain studied herein had been extensively studied previously in this laboratory and was known to be consistently inhibited by 0.0001% dapsone and 0.00003% dapsone, but not by 0.00001% dapsone.²¹ In the present study 0.00001% and 0.0001% dietary dapsone produced no measurable lethal consequences for the bacillus. Thus we observed a discordance between the minimal inhibitory concentration and minimal bactericidal concentration for this strain of *M. leprae*. The ability of higher dietary dapsone concentrations actually to kill *M. leprae* is modest and similar to that reported by others. Low-level sulfone therapy, including 1 mg dapsone/day, sulphetrone,²² and DADDS²³ maintains plasma levels above the minimal inhibitory concentration but near those required for bactericidal activity for the strain herein studied. Such therapy has resulted in treatment failure. This further suggests the possible importance of bactericidal and not just bacteriostatic therapy in the successful therapy of lepromatous leprosy. Conversely, the significant killing of *M. leprae* by both 0.001% and 0.01% dapsone in mouse chow, which resulted in levels maintained in man by the usual 100 mg dapsone therapy, might also serve to explain why patients resistant to 0.0001% dapsone but not to higher dietary levels when treated with full dosage (100 mg) dapsone daily improve clinically and bacteriologically.²⁴

The highest studied dietary concentration of rifampicin (0.01%) resulted in

considerable bactericidal activity, again in agreement with previous studies of Colston *et al.*⁸ using the same methods. On the other hand, lower levels of dietary rifampicin showed a progressively diminished ability to kill *M. leprae*. While Colston⁸ found 0.003% dietary rifampicin to be 99.99% bactericidal, we found rifampicin 0.005% to be 90% \pm 6% bactericidal and rifampicin 0.003% to be only 50% \pm 18% bactericidal (not sufficiently different from untreated controls). Such major differences between these two studies suggest that differing strains of *M. leprae* vary considerably in their susceptibility to the lethal effects of rifampicin. This is not surprising as previously Holmes¹³ had demonstrated, amongst different strains of *M. leprae*, a range of minimum inhibitory dietary concentrations of rifampicin from 0.0003% to 0.003%. Furthermore, where Rees¹⁴ found the minimal inhibitory dietary concentration of rifampicin for *M. leprae* to be 0.0025%, Shepard⁶ required 0.01% for his strain. Thus *M. leprae* strains appear to exhibit a range of susceptibility both to the inhibitory and bactericidal activity of rifampicin. In this respect the efficacy of monthly 600 mg rifampicin as advocated by the WHO raises serious questions as to whether sufficient duration concentration of drug at the active site of drug action is maintained, especially for certain relatively insensitive strains of *M. leprae*, such as that studied in this report.

Previously Levy²⁶ reported 0.0001% clofazimine in mouse chow to be purely bacteriostatic. We found 0.0001% clofazimine to have significant lethal activity against *M. leprae* and higher levels to be progressively more bactericidal. Though the pharmacokinetics of clofazimine in mouse and man are highly complex²⁶ and extrapolating from mouse to man hazardous, perhaps lower-dose clofazimine therapy, especially for certain people troubled by clofazimine discoloration, might be considered.

Because dapsona works in the folate pathway and sequential blockage of this pathway has proved synergistic against a number of aerobic bacteria, exploiting such potential for *M. leprae* appears attractive. Previously Shepard⁷ found that trimethoprim was not active against *M. leprae* alone nor did it potentiate the activity of dapsona. For the past 10 years we have evaluated a number of dihydrofolate reductase inhibitors.^{27,28} Many were found inactive alone and unable to potentiate the activity of dapsona, while certain ones were definitely active and potentiated the activity of 0.0001% dapsona but combined with dapsona were fundamentally bacteriostatic. A rational approach to generating potentially useful dihydrofolate reductase inhibitors for use in leprosy has been spearheaded by Professor Seydel.²⁹ Seydel has been interested in a number of dihydrofolate reductase inhibitors, especially brodimoprim for its potential use in leprosy because of:

1 Its increased binding to the isolated dihydrofolate reductase of *M. lufu* and *M. leprae*. *M. leprae* is uniquely sensitive to dapsona MIC 1 ng/ml; the nearest mycobacterial sensitivity to dapsona is found in *M. lufu*, 40 ng/ml.

- 2 Brodimoprim's demonstrably profound synergism (more than with TMP) with dapsonе against *M. lufu*.
- 3 Its potency in even low concentrations in combination with dapsonе against highly dapsonе-resistant *M. lufu*.
- 4 Its pharmacokinetic similarities to dapsonе in man (half-life approximately 30 hours; TMP's, one hour).
- 5 Its safety (brodimoprim does not bind to mammalian dihydrofolate reductase and is safe in chronic toxicity studies).

We screened brodimoprim (0.03%) and TMP (0.1%, as previously studied by Shepard) alone and in combination with dapsonе (0.0001%) against *M. leprae* infection of the mouse footpad by the kinetic technique. Both TMP and especially brodimoprim appeared active in combination with dapsonе. Brodimoprim plus dapsonе resulted in a growth delay of about 120 days. In a second experiment, both dapsonе and brodimoprim and SE-SC-60, another dihydrofolate reductase inhibitor were active but only marginally superior to dapsonе alone. It is noteworthy, however, that peak *M. leprae* counts in the mice treated with dapsonе + 2 levels of SE-SC-60 never attained levels reached by controls or other treatment groups. Particularly because mouse staphylococcal infections though quite sensitive *in vitro* to sulfamethoxazole/TMP are not ameliorated by such therapy, these relative inactivities that we found in mice may be a function of certain peculiarities in the mouse folate pathway as opposed to an inherent lack of *M. leprae* sensitivity. Because the two studies utilizing brodimoprim yielded somewhat conflicting findings, brodimoprim and SE-SC-60 alone and together with dapsonе are being assessed again, this time by the proportional bactericide technique. The ability of SE-SC-60 and brodimoprim to inhibit growth of a fully dapsonе-resistant strain of *M. leprae* is also being assessed.

Newer quinolone antibiotics, unlike the prototype drug naladixic acid, have a very broad bacterial spectrum of activity and provide not just significant levels in the urine but effective systemic levels. Cultivable mycobacteria are uniformly sensitive to ciprofloxacin (personal communication, Seydel). In our laboratory by the kinetic technique, at 0.1% in mouse chow, ciprofloxacin appeared active but only bacteriostatic. Recent industry pharmacokinetic data suggests that such dietary administration, even at much higher concentrations, results in poor gastrointestinal absorption and peak plasma concentrations of $< 1 \mu\text{g}/\text{mg}$; we are thus reevaluating this agent following gavage at doses which ought to yield considerably higher levels.

An animal model analogous to human lepromatous leprosy, wherein bacterial numbers approach 10^9 or more and which is similarly immunosuppressed, might allow a model of bacterial persistence and the examination of effective chemotherapy to eradicate persisters. Because clinical trials in leprosy are expensive and require many years to allow proper interpretation of outcome, an animal model suitable for chemotherapeutic experiments would greatly

facilitate the development and screening of candidate regimens. The normal mouse permits the number of *M. leprae* to reach a ceiling of only 10^6 and is immunologically intact, therefore not providing such a suitable model. The thymectomized irradiated bone marrow reconstituted mouse described by Rees³⁰ permits the development of larger bacterial populations in an immunologically compromised animal, but attempts to reproduce such a model in the United States have not been successful, owing to early animal mortality. The neonatally thymectomized Lewis rat (NTLR), however, does survive well and permits the development of large populations of *M. leprae*. Fieldsteel *et al.*³¹ utilized this rodent in a number of experiments for this express purpose. First he established the minimal effective dose of dapsone which prevented multiplication of 5×10^3 bacilli in rat footpads, which was 5×10^{-5} g%, this resulted in the establishment of a plasma minimal inhibitory concentrations for dapsone in rats of 4 ng/ml.³¹ Next he conducted a number of experiments so as to evaluate the killing potential of 5×10^{-5} g% dapsone in mouse chow and at 100-fold greater concentrations. Five $\times 10^{-5}$ g% dapsone resulted in no killing of bacilli, while 5×10^{-3} g% resulted in killing of bacteria at a rate similar to that found following dapsone therapy in man. However, neither dapsone alone (up to 0.005%) nor single doses of rifampicin (1 mg/kg, 5 mg/kg, 10 mg/kg, or 20 mg/kg) eliminated persisters.^{32,33} Similarly, the combination of a single dose of rifampicin, 10 mg/kg, on a background of 5×10^{-5} g% dapsone in rat chow was not fully effective either. Finally, single doses of rifampicin (10 mg/kg) together with continuous dapsone, 5×10^{-3} g%, and continuous dapsone 5×10^{-5} g% together with up to 10 doses of rifampicin resulted in viable bacilli still being present and detectable upon mouse of NTLR subpassage.³³ Thus a model of persisting *M. leprae* in an immunologically compromised rodent capable of permitting very high levels of bacilli has been created which cannot be sterilized by at least some reasonably potent multidrug therapy. The stage was hence set to try other and perhaps more efficacious therapy in an attempt to eliminate these persisting bacilli. Thus we decided to study more intensive regimens of dapsone and rifampicin in heavily infected NTLR.

Because of the large groups of animals required and the consequent expense, there have been only limited trials in experimental animals on the potential for synergistic antimicrobial therapy. However, Shepard³⁴ has tested in mice 30 combinations of two to three of the four clinically utilized bactericidal drugs (dapsone, rifampicin, clofazimine, and ethionamide). Twenty-three of these combinations showed a significantly increased killing of *M. leprae* over that of the most potent single agent of the combinations. However, only combinations containing clofazimine + rifampicin or dapsone + ethionamide in no instance demonstrated antagonism. Thus, these combinations are being studied in order to assess their potential for sterilizing established *M. leprae* infections in NTLR. Because rifampicin and ethionamide appear to be the most bactericidal agents established and in use in therapy of leprosy, this combination also will be

assessed. Thus in all, six therapeutic regimens in NTLR are being studied: 1, dapsone 0.005% + 10 doses of rifampicin 10 mg/kg by gavage; 2, dapsone 0.05% + rifampicin 0.01%; 3, rifampicin 0.01%; 4, clofazimine 0.01% + rifampicin 0.01%; 5, dapsone 0.005 + ethionamide 0.2%; 6, rifampicin 0.01% + ethionamide 0.2%.

In 1983 and 1984 four batches resulting in 100 NTLR (Charles River Labs) were inoculated in both hind footpads with 5×10^3 *M. leprae*. In order to provide an accurate baseline, at one year three NTLR from each batch were harvested and their hind footpads counted individually. All six control NTLR footpads from two batches that were harvested thus far one year following infection with *M. leprae* were shown to be uniformly infected with massive numbers of *M. leprae*, $> 5 \times 10^7$ per footpad.

Table 1. Number of *M. leprae* per footpad at 1 year.

NTLR	left foot	right foot
1	7.64×10^7	1.30×10^8
2	3.23×10^8	5.17×10^8
3	7.96×10^7	5.96×10^7
4	6.48×10^7	5.37×10^7
5	6.51×10^7	5.00×10^7
6	2.64×10^8	7.91×10^8

Because in recent years NTLR have not been found uniformly immunosuppressed and hence highly susceptible to massive multiplication and dissemination of *M. leprae*, these results are encouraging insofar as a uniformly highly infected NTLR model, simulating levels of infection of lepromatous leprosy, has been established in our laboratory. Beginning at one year groups of 15 rats were given four months of therapy as previously described: harvests on at least three of these treated NTLR are being performed at 2, 4, 6, 8 months and upon demise. Since it was decided to treat for 4 months, later harvests (6 and 8 months) from NTLR will enable determination of regrowth from any remaining 'persisters' after completion of therapy. The viability of *M. leprae* obtained from the footpads of all treated NTLR will be assessed in mice following inoculation of 5×10^3 bacilli per footpad and more sensitively assessed in NTLR by passage of 10^5 to 10^7 bacilli per footpad. If growth in passage rats is equivocal (< fourfold increase), *M. leprae* from these will be passed in turn as previously described to hind footpads of mice. Unfortunately, results are too fragmentary at this juncture to reach any conclusions. Immunotherapy of *M. leprae*-infected NTLR with interleukin 2,

gamma interferon etc., alone and combined with antimicrobials, is also being initiated in our laboratory.

The ability of various rodent systems to monitor chemotherapy trials in lepromatous leprosy and, in particular, to identify persisters is another issue of some interest. Though T/R mice were in part utilized to detect persisters in Malaysia following long-term dapsone³⁵ and rifampicin³⁶ therapy, it is not clear from those studies whether they proved more sensitive than normal mice in detecting persisters. Fieldsteel, in the normal mouse, utilizing larger inocula, 10^5 or 10^6 heat killed *M. leprae* with 10 live bacilli, by the ploy of subpassage was able to detect a smaller percentage of viable *M. leprae* than when utilizing a more customary inoculum size.

In two of our own publications^{37,38} we demonstrated that the neonatally thymectomized Lewis rat provides a more sensitive monitoring system for detecting viable *M. leprae* in skin biopsies of lepromatous patients undergoing initial chemotherapy. This rat model allows for larger inocula (10^5 – 10^7) than the mouse as usually employed (5×10^3) but remains superior in detecting persisters to mice receiving even larger inocula (10^4 – 10^6). Furthermore, the neonatally thymectomized Lewis rat appeared superior in this respect, for unclear reasons, than the congenitally athymic or nude rat. Though not statistically significant in this small trial monitored by the NTLR, daily dapsone 100 mg plus a single dose of rifampicin 1500 mg appeared more effective than daily 100 mg dapsone plus weekly rifampicin 900 mg. A regimen, unlike the ones just reviewed, which could be found regularly to prevent multiplication of *M. leprae* from skin biopsies in the NTLR might be the most likely candidate for preventing persisters and allowing for safe discontinuation of therapy. On the other hand it may be, as is emerging from the WHO trials, that a minimum of 10–15% of the time even the most potent regimens leave persisters. Perhaps such patients are a subgroup of lepromatous patients, albeit a minority, with absolute anergy to *M. leprae*. Perhaps such patients will require lifelong antimicrobial therapy or in addition immunotherapy to effect a cure.

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