VOOREN, J. P., MILLAN, J. and HUYGEN, K. The major secreted antigen complex (Ag 85) from *Mycobacterium bovis* bacille Calmette-Guerin is associated with protective T cells in leprosy: a follow-up study of 45 household contacts. J. Infect. Dis. **167** (1993) 1160–1167.

10. LIMA, M. C. B. S., PEREIRA, G. M. B., RUMJANEK, F. D., GOMES, H. M., DUPPRE, N., SAMPAIO, E. P., ALVIM, I., NERY, J. A. C., SARNO, E. N. and PESSOLANI, M. C. V. Immunological cytokine correlates of protective immunity and pathogenesis in leprosy. Scand. J. Immunol. **51** (2000) 419–428.
11. PESSOLANI, M. C., PERALTA, J. M., RUMJANEK, F. D., GOMES, H. M., MARQUES, M. A., ALMEIDA, E. C., SAAD, M. H. and SARNO, E. N. Serology and leprosy: immunoassays comparing immunoglobulin G antibody responses to 28- and 30-kilodalton proteins purified from *Mycobacterium bovis* BCG. J. Clin. Microbiol. **29** (1991) 2285–2290.
12. RAVN, P., BOESEN, H., PEDERSEN, B. K. and ANDERSEN, P. Human T cell responses induced by vaccination with *Mycobacterium bovis* bacillus Calmette-Guerin. J. Immunol. **158** (1997) 1949–1955.
13. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity; a five-group system. Int. J. Lepr. **34** (1966) 255–273.
14. TERASAKI, P. I. and MCCLELLAND, J. D. Microdroplet assay of human serum cytotoxins. Nature **204** (1964) 998–2000.

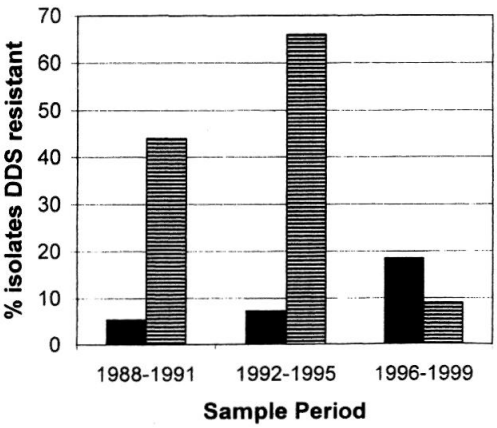
Dapsone Drug Resistance in the MDT Era

TO THE EDITOR:

The specter of drug-resistant leprosy has receded with the advent of multidrug therapy (MDT). However, there are few laboratories remaining which have the facility to measure drug resistance by the mouse foot pad method. Hence, it has been difficult to ascertain what the current state of drug resistance is and what the trends in resistance patterns after MDT implementation have been.

In Nepal, dapsone (DDS) monotherapy was introduced in 1956 and MDT in 1983, although the coverage of MDT only reached 95% more than a decade later. Since 1987, we have been testing all suitable patients for primary and secondary drug resistance using the mouse foot pad model ([3]). We have analyzed the drug sensitivities of *Mycobacterium leprae* isolated from skin biopsies from 268 new and relapsed cases and from patients re-starting treatment (The Table). In the period 1988 to 1999, there was a decline in the prevalence of acquired dapsone resistance and a rise in the prevalence of primary dapsone resistance (The Figure). There was a complete absence of rifampin (RMP) resistance among our patients.

Primary dapsone resistance arises by the infection of new persons with resistant bacteria often shed by a person with acquired resistance to dapsone. During the late 1960s and 1970s, there were alarming reports of increasing secondary and primary dapsone resistance at the end of the dapsone monotherapy era, which prompted the introduction of MDT ([2, 4]). From our data it appears that secondary resistance to dapsone does not develop under MDT. Hence, the decline in secondary resistance is due to the introduction of MDT in Nepal and to the cure of secondary dapsone-resistant cases with MDT. By contrast there was a significant increase in the prevalence of primary dapsone resistance in new, previously untreated cases (trend in proportion test $\chi = 4.98$, $p < 0.05$, The Table). All except one isolate was resistant to low-level dapsone



THE FIGURE. Prevalence of dapsone resistance in Nepal, 1988–1999. ■ = Primary resistance; ▤ = acquired resistance.

THE TABLE. *Trends in drug resistance of leprosy patients in Nepal (1987–1999).*

	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
							Primary resistance						
No. experiments	0	20	15	18	13	27	19	22	20	26	26	28	12
No. with no growth		2	2	4	3	11	2	3	5	3	5	3	4
Growth at 0.0001% DDS[a]		0	1	1	1	3	0	1	0	5	4	5	1
Growth at 0.001% DDS		0	0	0	0	0	0	0	0	0	0	0	0
Growth at 0.01% DDS		0	0	0	0	0	0	0	1	0	0	0	0
Growth at 10 mg/kg RMP[b]		0	0	0	0	0	0	0	0	0	0	0	0
							Acquired resistance—dapsone monotherapy						
No. experiments	4	11	5	8	9	8	5	2	5	3	5	3	2
No. with no growth	2	4	1	1	2	0	2	1	2	0	2	0	0
Growth at 0.0001% DDS	0	1	2	0	3	1	1	1	2	0	0	0	0
Growth at 0.001% DDS	1	0	0	1	0	0	1	0	0	0	0	0	1
Growth at 0.01% DDS	1	1	0	1	0	3	1	0	0	0	0	0	0
Growth at 10 mg/kg RMP	0	0	0	0	0	0	0	0	0	0	0	0	0
							Acquired resistance—MDT						
No. experiments	0	0	0	0	0	0	0	2	3	1	2	1	2
No. with growth								1	2	1	0	0	0
Growth at 0.0001% DDS								0	0	0	0	0	0
Growth at 0.001% DDS								0	0	0	0	0	0
Growth at 0.01% DDS								0	0	0	0	0	0
Growth at 10 mg/kg RMP								0	0	0	0	0	0

[a] DDS = Dapsone.
[a] RMP = Rifampin.

(0.1 mg) and thus sensitive to doses of dapsone used in MDT. Hence, the clinical response to MDT in these patients will not be compromised. During the monotherapy era the level of dapsone resistance increased step-wise and resistance at high levels became common ([4]). It will be important to monitor trends in the level of resistance as well as the frequency of primary dapsone resistance in the future. MDT efficacy could be severely compromised if high-level primary dapsone resistance becomes highly prevalent.

The genetic basis of dapsone resistance has recently been described ([5]). Although genetic detection of drug resistance is technically sophisticated, the application of the technology in selected leprosy-endemic areas will allow a more rapid and widespread assessment of the problem. The gene method must first be validated by paralleled mouse foot pad experiments in order to be sure what level of dapsone resistance is being detected. Although dapsone has a weaker bactericidal action than rifampin, we need to prevent the development of resistance to any component drug in MDT schedules. One could imagine a dangerous scenario developing in which high level primary dapsone resistance and poor clofazimine compliance leads effectively to rifampin monotherapy, a condition in which it is already known that rifampin resistance in leprosy can develop ([1]).

One of the unintentional but distressing effects of the leprosy elimination program has been the decline in mouse foot pad laboratories around the world. This has led to the situation where an elimination campaign based entirely on drug therapy has little or no measure of the extent of and trends in drug resistance. Our data show some important trends and should encourage the remaining mouse foot pad laboratories in the world to continue their important work of assessing drug resistance in leprosy.

—Paul W. Roche, Ph.D.
Kapil Dev Neupane
Sarah S. Failbus, M.Sc.
C. Ruth Butlin, M.B.B.S.

Mycobacterial Research Laboratory
Anandaban Leprosy Hospital
P.O. Box 151
Kathmandu, Nepal
Fax: 977-1-290-538,
email: anandaban@mailcom.np

Acknowledgment. The Mycobacterial Research Laboratory is supported by The Leprosy Mission International. The authors gratefully acknowledge the assistance of the staff of the mouse foot pad laboratory.

REFERENCES

1. GROSSET, J. H., GUELPA-LAURAS, C.-C., BOBIN, P., BRUCKER, G., CARTEL, J.-L., CONSTANT-DESPORTES, M., FLAGUEL, B., FREDERIC, M., GUILLAUME, J.-C. and MILLAN, J. Study of 39 documented relapses of MB leprosy after treatment with rifampin. Int. J. Lepr. **57** (1989) 607–614.
2. PEARSON, J. M. H., HAILE, G. S. and REES, R. J. Primary dapsone resistant leprosy. Lepr. Rev. **48** (1977) 129–132.
3. SHEPARD, C. C. and CHANG, Y. T. Effect of several antileprosy drugs on the multiplication of the human leprosy bacillus in foot pads of mice. Proc. Soc. Exp. Biol. Med. **109** (1963) 636–638.
4. WHO EXPERT COMMITTEE ON LEPROSY. Fifth report. Geneva: World Health Organization, 1977. Tech. Rep. Ser. 607.
5. WILLIAMS, D. L., SPRING, L., HARRIS, E., ROCHE, P. and GILLIS, T. P. Dihydropteroate synthase of *Mycobacterium leprae* and dapsone resistance. Antimicrob. Agents Chemother. **44** (2000) 1530–1537.

Tumor Necrosis Factor Promoter Polymorphism (TNF2) Seems to Protect Against Development of Severe Forms of Leprosy in a Pilot Study in Brazilian Patients

TO THE EDITOR:

Leprosy is a chronic infectious disease characterized by clinical forms which are associated to the immune response developed by the host against the bacteria. Polar forms are either paucibacillary (PB; tuberculoid leprosy) with a pronounced cell-mediated immune response (CMI) or multibacillary (MB; lepromatous leprosy), which lacks CMI. Believed to be an important protection mediator against infections, tumor necrosis factor-alpha (TNF-α), one of the key cytokines in CMI, is an inducible factor covering a wide range of proinflammatory and immunostimulatory activities ([3]). Moreover, depending on the quantity and the time period over which its production is sustained, TNF-α may exert a beneficial or a deleterious effect. For example, in leprosy, enhanced production of TNF-α has been associated with the development of such immunopathological states as nerve damage ([10]) and inflammatory reactional episodes ([5]) as well as with the development of the more benign tuberculoid form of the disease ([12]). High TNF-α levels were detected in the serum during reaction ([11]) and *in vitro,* following stimulation of the peripheral blood mononuclear cells, both in tuberculoid and reactional patients ([1]).

It has been suggested that development of a particular type of leprosy may be genetically determined ([2]) and could be responsible for the inter-individual differences in the immune response during the disease. Expression of TNF-α is tightly controlled at the transcriptional and post-transcriptional levels, and a particular single nucleotide polymorphism at the –308 position within the regulatory region of the TNF-α gene generates the allelic TNF2 form shown to be associated with enhanced TNF-α production ([15]) and to severe forms of some inflammatory and auto-immune diseases ([7]). In this connection, Roy, *et al.* ([9]) have recently described the significant association between TNF2 allele frequency and polar lepromatous-type leprosy in an Indian population.

In a study carried out in the Leprosy Laboratory at the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, TNF2 allele frequency was determined in 92 healthy control individuals and in 300 leprosy patients classified on the basis of their clinical and histologic features ([8]) as suffering from PB (BI negative patients, N = 90; 2 BT, 63 TT, 15 pure neural form and 10 indetermi-