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Susceptibility of Strains of *Mycobacterium leprae* Isolated Prior to 1977 from Patients with Previously Untreated Lepromatous Leprosy[1]

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The criterion generally used to define *Mycobacterium leprae* susceptible to dapsone (4,4'-diaminodiphenylsulfone) is the inability of the organism to multiply in mice administered dapsone incorporated into the mouse diet in a concentration of 0.0001 g per 100 g diet. This criterion emerged from early studies designed to determine the smallest concentration (minimal effective dosage; MED) of dapsone administered continuously in the diet that is capable of inhibiting multiplication of organisms inoculated into the hind foot pads of mice ([11]). These studies revealed the organism to be exquisitely susceptible to dapsone; in BALB/c mice, this dosage produces maximal plasma and tissue dapsone concentrations of approximately 3 ng dapsone per ml ([2]). Recent reports ([12, 13]) that primary resistance to dapsone has been detected with high frequency, as high as 50%, among patients with lepromatous leprosy considered to have received no previous treatment have provoked concern among those responsible for the control of leprosy. That the majority of dapsone-resistant strains of *M. leprae* encountered in surveys of the prevalence of primary resistance to dapsone have been resistant only at the MED, and that patients harboring such strains ordinarily respond well to treatment with dapsone in full dosage, suggested that the criterion by which *M. leprae* have been adjudged susceptible to dapsone should be re-examined. Perhaps the criterion has been too stringent, leading to an overestimate of the frequency of primary resistance.

During the first years of application of the mouse foot pad technique to measurement of the susceptibility to dapsone of clinical isolates of *M. leprae,* most efforts were devoted to studies of strains of the organism isolated from patients who had relapsed during treatment with the drug, almost always as monotherapy. Only a few early reports ([1, 5–7, 10]) dealt with the susceptibility to dapsone of strains of *M. leprae* that had been isolated from previously untreated patients. Yet, it is precisely the susceptibility to a drug of strains of an organism isolated from previously untreated patients before primary resistance to that drug is recognized that determines the criterion of susceptibility of the organism to the drug. Therefore, a review of the available records was undertaken in six laboratories in which measurements of the susceptibility to dapsone of strains of *M. leprae* isolated from previously untreated patients had been carried out prior to 1977, when the first report ([3]) of primary resistance to dapsone was published.

METHODS

Mice of a strain previously demonstrated to be susceptible to *M. leprae* were inoculated into one or both hind foot pads with 5000 or 10,000 organisms recovered from human biopsy specimens or harvested from mouse foot-pad tissues. One group of mice was fed a drug-free diet, and other groups were fed powdered mouse diets into which dapsone had been incorporated in concen-

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trations of 0.0001, 0.001 and 0.01 g per 100 g diet; in some instances, diets containing dapsone in other concentrations were also administered. Drug-containing diets were usually administered from the day of inoculation. In some laboratories, *M. leprae* were harvested from pooled foot-pad tissues of control mice at intervals, until the organisms were observed to have multiplied to at least 5×10^5 per foot pad, whereupon harvests were also carried out from pooled foot-pad tissues of treated mice. In other laboratories, individual harvests were performed at some predetermined interval—usually 8–12 months—on the foot pads of both control and treated mice. Acid-fast bacteria were counted, and the numbers of organisms harvested from the controls and the treated mice were compared, or the numbers of foot pads of treated mice in which *M. leprae* had multiplied were compared with those of the control mice in which multiplication had occurred. *M. leprae* were considered susceptible to dapsone at a given concentration if no multiplication occurred in the foot pads of mice administered the drug in that concentration.

RESULTS AND DISCUSSION

Information was obtained for 73 patient-strains of *M. leprae* isolated from presumably untreated patients with lepromatous and near-lepromatous leprosy before 1977, and tested for susceptibility to dapsone. The available data are summarized in The Table, in which the strains are arranged by laboratory and by year of isolation. Of the 73 patient-strains, 14 (19%) had been isolated prior to 1966; whereas 33 were isolated during the next five-year period, and 28 during the years 1971–1976. That few strains were tested during the period prior to 1966 reflects the more limited performance of the mouse foot-pad technique and its application to drug susceptibility testing during this period.

As shown in The Table, it was the practice in the London, Antwerp, and Shanghai laboratories to measure susceptibility to dapsone at the time of primary isolation; whereas these measurements were performed routinely in the course of subsequent mouse-passage in the Atlanta and San Francisco laboratories. Justification of the latter practice, which is more economical, depends upon the demonstration that the susceptibility of *M. leprae* to dapsone does not change in the course of repeated passage. As shown in The Table, the susceptibility to dapsone of nine strains had been tested in the course of two passages in Atlanta and San Francisco, and had been found not to have changed. In the case of two of the strains, the two passages were the fourth and eighth, and the sixth and twenty-fifth, respectively. In fact, that it had been a practice in the Atlanta laboratory to maintain a large number of strains in mouse-passage made possible the retrospective determination of susceptibility to dapsone of strains that had been isolated during the late 1950s and early 1960s, in the course of developing the mouse foot-pad technique ([8, 9]), and before the first reported isolation of *M. leprae* resistant to dapsone ([4]).

The 73 patient-strains had originated in 19 countries or dependencies; however, the countries were not equally represented. Thus, only seven of the strains originated in Africa, and only 13 in the Americas, whereas 47 (64%) had originated in Asia. In fact, two Asian countries—Malaysia and the Philippines—accounted for almost half of the patients, reflecting the fact that these data had not resulted from systematic surveys, but rather were by-products of long-sustained research activities sponsored by the British Medical Research Council in Sungei Buloh and the U.S. Leprosy Panel in Cebu. Despite the nonrepresentative sampling embodied in these data, it is evident that all 73 patient-strains of *M. leprae* were inhibited from multiplying by administration to the mice of dapsone in a concentration of 0.0001 g per 100 g diet. Moreover, 16 (44%) of the 36 strains tested had been found susceptible to 0.00001 g per 100 g mouse diet.

During the period prior to 1977, there may have been exceptions to this pattern of universal susceptibility to 0.0001 g dapsone per 100 g diet. For example, an early report ([6]) from the London laboratory described *M. leprae* isolated from previously untreated patients which were said to have multiplied in small proportions of the foot pads of mice administered dapsone in concentrations of 0.01, 0.025 or 0.1 g per 100 g

THE TABLE. *Susceptibility to dapsone of patient-strains of* M. leprae *isolated prior to 1977 from previously untreated lepromatous patients.*

Laboratory	No. of strains	Year of isolation	Country of origin	Dapsone concentration[a] (g per 100 g diet)		
				0.00001	0.00003	0.0001
Shanghai	3	1973–1974	China	N	N	S
Cebu	1[b]	<1971	Philippines	N	N	S
	1	1975	Philippines	N	N	S
Antwerp	1	1963	Zaire	N	N	S
	1	1965	Rwanda	S	NA	NA
	1	1967	Cambodia	S	NA	NA
	1	1973	Morocco	S	NA	NA
San Francisco	1[b,c]	1965	Mexico	R	S	NA
	8[b]	1971–1973	Philippines	S	NA	NA
	1[b,c]	1971–1973	Philippines	S	NA	NA
	5[b]	1971–1973	Philippines	R	S	NA
	1[b,c]	1971–1973	Philippines	R	S	NA
	2[b,c]	1971–1973	Philippines	R	R	S
London	5	1966–1967	Malaysia	R	N	S
	2	1967	Malaysia	S	NA	NA
	4	1967	Malaysia	N	N	S
	1	1967	India	S	NA	NA
	1	1967	India	R	N	S
	1	1967	Samoa	R	N	S
	4	1969	Ethiopia	N	N	S
	1	1972	Malaysia	N	N	S
	1	1973	Australia	N	N	S
	1	1974	India	N	N	S
Atlanta	1[b]	1958	Philippines	N	N	S
	1[b]	1961	Mexico	N	N	S
	1[b]	1962	Viet Nam	N	N	S
	1[b]	1963	Cuba	S	NA	NA
	1[b]	1964	Nicaragua	N	N	S
	1[b,c]	1964	U.S.A.	N	N	S
	1[b,c]	1964	Okinawa	R	N	S
	1[b]	1964	Philippines	R	N	S
	1[b]	1965	Philippines	N	N	S
	1[b,c]	1965	Mexico	R	N	S
	1[b]	1965	Unknown	N	N	S
	1[b]	1966	Mexico	N	N	S
	1[b,c]	1966	Guam	R	N	S
	1[b]	1967	Samoa	N	N	S
	1	1968	India	N	N	S
	3[b]	1968	India	N	N	S
	3[b]	1968	Mexico	N	N	S
	1[b]	1969	U.S.A.	N	N	S
	1[b]	1969	Philippines	N	N	S
	1[b]	1970	Philippines	N	N	S
	1	1974	Mexico	N	N	S
	1[b]	1975	Trinidad	N	N	S

[a] N = not tested; NA = not applicable; R = resistant; S = susceptible.
[b] Susceptibility tested on passage.
[c] Susceptibility unchanged on two passages.

diet. Unfortunately, the data that served as the basis of the earlier report were not found in the course of collecting data for this present analysis; moreover, it is not now possible to ascertain the criteria of multiplication employed in the earlier report, or the numbers of strains accounted for by the foot pads demonstrating multiplication of *M. leprae* in the earlier report. On the other hand, it appears likely indeed that the vast majority of the strains, the susceptibilities of which were described in the earlier re-

port, are among those presented in The Table as having been studied and found fully dapsone susceptible in the London laboratory. Nevertheless, even if there were instances of primary resistance to dapsone among the strains tested before 1977, the prevalence of primary resistance to dapsone must have been very low, and certainly smaller than the proportions of 30 to 50 per 100 patients at risk currently encountered. Thus, the frequency of strains of *M. leprae* isolated from previously untreated patients that are capable of multiplying in mice treated with dapsone appears to have increased during the period 1977–1985, and one may justifiably consider the strains currently isolated to be primarily resistant to dapsone.

SUMMARY

Because of the recent spate of reports of primary resistance to dapsone among patients with lepromatous leprosy, largely to small concentrations of the drug, a survey was made of the results of dapsone-susceptibility testing of strains of *Mycobacterium leprae* isolated before 1977 among six laboratories which employed the mouse foot pad technique for this work prior to that time. Data have been found for strains that had been isolated from 73 patients, representing 19 countries and dependencies, with previously untreated lepromatous leprosy; all 73 strains were inhibited from multiplication by dapsone administered to mice in a concentration of 0.0001 g per 100 g mouse diet. These data suggest that the properties of *M. leprae* isolated from previously untreated patients with respect to susceptibility to dapsone have changed since the years preceding 1977.

RESUMEN

Debido a la reciente avalancha de reportes sobre la resistencia primaria a la dapsona entre los pacientes con lepra lepromatosa que reciben pequeñas concentraciones de la droga, se hizo un análisis de los resultados de las pruebas de susceptibilidad a la dapsona de varias cepas de *Mycobacterium leprae* aisladas antes de 1977 en 6 laboratorios que emplearon la técnica del cojinete plantar en el ratón. Se encontraron los datos correspondientes a las cepas aisladas de 73 pacientes, representantes de 19 paises y dependencias, los cuales tenían lepra lepromatosa sin tratamiento previo. Los registros indican que la dapsona administrada a los ratones en una concentración de 0.0001 g por 100 g de alimento, inhibió la multiplicación de las 73 cepas. Comparando los datos anteriores a 1977 con los actuales, see deduce que han ocurrido cambios en la susceptibilidad a la dapsona en los *M. leprae* aislados de pacientes sin tratamiento previo.

RÉSUMÉ

Par suite de la publication récente d'innombrables rapports relatant une résistance primaire à la dapsone chez des malades atteints de lèpre lépromateuse, et principalement une résistance à des concentrations faibles du produit, on a passé en revue les résultats des épreuves de susceptibilité à la dapsone, menées sur des souches de *Mycobacterium leprae* isolées avant 1977 dans six laboratoires qui employaient déjà l'épreuve du coussinet plantaire de la souris dès avant cette époque. On a retrouvé des données concernant des souches qui avaient été isolées de 73 malades, sans traitement préalable pour la lèpre lépromateuse, et originaire de 19 pays et territoires. Dans ces 73 souches, la multiplication était inhibée par la dapsone administrée aux souris à la concentration de 0,0001 g par 100 g dans la ration alimentaire. Ces données suggèrent que les propriétés de *M. leprae* isolé chez des malades non-traités au préalable, ont changé, en ce qui concerne la susceptibilité à la dapsone, depuis les années antérieures à 1977.

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