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AIRBORNE INFECTION WITH MYCOBACTERIUM LEPRAE IN MICE

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While our extensive bacteriological and pathological studies (1,2) on the nose in lepromatous patients underline the importance of nasal secretions as a source of M.leprae in the transmission of leprosy, they do not necessarily clarify the route of infection. However, patients with M.leprae loaded nasal mucus could produce and project infected aerosols and droplets during the process of talking, coughing and sneezing. Likewise, from shed and dried nasal secretions in handkerchiefs or on the ground, M.leprae infected particles could be formed and released into the air. Because of the similarities in the nature and excretion of leprosy nasal secretion and sputum of patients with open tuberculosis, it was tempting to consider also a respiratory route of infection for leprosy. Therefore studies were undertaken to determine whether mice could be infected with M.leprae by the respiratory route.

METHODS

The larger scale experiment to be described was started in 1972 based on techniques developed in 1967 for airborne infections with M.leprae when we were studying a wide range of infection routes. We were encouraged to proceed with the airborne route because in our pilot experiment 3 mice were infected when killed 15-23 months after exposure to M.leprae; unfortunately the majority of the mice had been killed earlier and all were negative.

A Henderson apparatus (3) was used to produce clouds of M.leprae containing particles following exactly the physical condition which had been used successfully for infecting the lungs of mice with known numbers of viable units of M.tuberculosis. Calculations on the basis of M.tuberculosis showed that each mouse retained 7 ml of the cloud in

1 minute and that from a reservoir containing 5×10^6 viable units M.tuberculosis/ml a cloud of 1.0×10^4 viable units/litre/min was produced. We aimed to infect the lungs of each mouse with 1.0×10^5 M.leprae, and assuming the same conditions held for M.leprae with an available pool of 45 ml at 1.2×10^8 M.leprae/ml the calculated cloud-exposure time was 60 min, given in six 10 min separate exposures. The M.leprae suspension was obtained from 4 pooled skin biopsies from previously untreated lepromatous patients, homogenised in 0.1% bovine albumin in water, the morphological index was 10. A sample of M.leprae from the pool before and after being used in the Henderson was inoculated into the footpads of mice.

Forty-eight female CBA mice previously immunologically suppressed by thymectomy followed by 5 exposures to cobalt irradiation 200R (T/R) at fortnightly intervals were used. Immediately following exposure to M.leprae aerosols 5 mice were killed and their right and left lungs and nose tips were individually homogenised for acid-fast bacillary (AFB) counts. Deaths prior to 14 months following aerosol exposure were discarded, but from then onwards remaining mice were killed, or those that died, were dissected, taking both ears and foot pads, nose tip and right and left lungs. For mice dying all sites were homogenised and counted for AFB; from a proportion of the killed mice whereas all representative sites were counted, some of the duplicate sites were submitted for histology as was a deeper part of the nasal tissue.

RESULTS

Of the 5 mice killed after exposure to airborne infection, the individual AFB counts on homogenates from the whole lungs were 36, 8.4, 6.0, 3.2 and $<2.0 \times 10^4$ whereas no AFB were counted from homogenates from the 5 individual nose tip homogenates ($<2.0 \times 10^4$). Therefore the aerosol technique had enabled M.leprae to reach and be retained within the lungs but not to be retained within the nose. Moreover, the number of M.leprae retained in the lungs was in the order of that calculated from M.tuberculosis, on which

the conditions of the apparatus had been set. Because the earlier pilot experiment had failed to show infections in mice before 15 months, no mice from the present experiment were investigated before 14 months following aerosol exposure. At 14 months 30 of the original 43 remained alive and all of these as they died or were killed in the ensuing 10 months (i.e. up to 2 years after exposure) were examined for the presence of AFB in homogenates from their ears, foot pads, nose and lungs. From the results summarised in Table 1, it can be seen that 33% (10/30) of the mice showed countable numbers of AFB at one of the sites; the AFB morphologically resembled M.leprae and in the 3 isolates passaged to foot pads of normal mice their growth curves resembled M.leprae. It is of interest that in the last surviving mouse killed 24 months after aerosol exposure, at a time when we were routinely examining the bone marrow of M.leprae infected mice, AFB were present in the pooled bone marrow suspension from both femurs as well as a countable AFB yield from a foot pad. None of the representative tissues submitted for histology showed unequivocal evidence of M.leprae infection.

DISCUSSION AND CONCLUSIONS

These results show that immunologically suppressed mice can be infected with M.leprae by the respiratory route when exposed to aerosols that deliver bacilli down to the lungs. The distribution of the subsequent infection in distant parts such as the ear, foot pad and nose are consistent with a systemic infection originating from M.leprae entering via the lung. The long incubation period and small proportion of takes is consistent with extremely small numbers of viable M.leprae escaping from the lung, probably via blood vessels or lymphatics, and subsequently coming to rest at a site where they eventually multiplied.

From these positive results we conclude that leprosy in man could result from an airborne infection.

TABLE 1.

TEN ISOLATES OF M.LEPRAE BY SITE, YIELD OF ACID-FAST BACILLI (AFB) AND MONTHS AFTER EXPOSURE OF 30 MICE TO 10^5 M.LEPRAE AEROSOL INFECTION

TIME (MONTHS)	NUMBER OF MICE		SITE/AFB x 10^4)			
	TOTAL	POSITIVE	EAR	FOOTPAD	NOSE	LUNG
14	2	1	-	(12)	-	-
16	4	2	(24)	-	-	-
			-	-	-	(12)[P]
17	3	1	(70)[P]	-	-	-
18	6	0	-	-	-	-
19	2	0	-	-	-	-
20	2	1	(40)	-	-	-
21	7	3	-	-	-	(6)
			-	-	(20)	-
			-	-	(10)	-
22	1	1	-	-	(300)[P]	-
23	2	0	-	-	-	-
24	1	1[BM]	-	-	(20)	-
TOTAL	30	10	3	1	4	2

[P] Passage to further mice

[BM] AFB also present in bone marrow from femurs

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