AIRBORNE INFECTION WITH *MYCOBACTERIUM* *LEPRAE* IN MICE

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ALTHOUGH our bacteriological and pathological studies on the nose in patients with lepromatous leprosy (Davey and Rees, 1974; McDougall *et al.*, 1975) underline the importance of nasal secretions as a major source of *Mycobacterium leprae* in the transmission of leprosy, they do not necessarily clarify the route of infection. However, patients whose nasal mucus is loaded with *M. leprae* might be expected to produce and to project infected aerosols and droplets during talking, coughing and sneezing. Similarly, *M. leprae*-infected particles from shed and dried nasal secretions in handkerchiefs or on the ground could become airborne. A comparison of the modes of spread and incidence of pulmonary tuberculosis and leprosy (Rees and Meade, 1974) in comparable populations in South India has indicated that the attack rates for the two diseases in family or household contacts are similar, as are also the numbers of *M. tuberculosis* excreted in sputum and of *M. leprae* excreted in nasal discharges by patients with pulmonary tuberculosis and lepromatous leprosy respectively (Rees and Meade, 1974). Thus it was reasonable to consider a respiratory route of infection for leprosy, and the present study was undertaken to determine whether mice were susceptible to airborne infection with *M. leprae*.

MATERIALS AND METHODS

The present larger scale experiment was based on results obtained in 1967 when one of us (R. J. W. R.) was investigating the different routes for infecting mice with *M. leprae*. By airborne infection the only three mice successfully infected were killed 15–23 months after exposure to *M. leprae*; unfortunately the 17 other mice exposed to infection had been killed earlier and all were negative. Therefore in the present study the outcome of airborne infection was confined to mice killed 14 months or more after exposure to *M. leprae*.

Methods for production of and exposure to airborne infection

The apparatus used was based on that described by Henderson (1952) and Druett and May (1952), modified for 10 mice at a time to be exposed to clouds of dry bacteria-containing particles of 1–2 μm diameter. In this apparatus, only the head of the mouse is exposed to the infective cloud.

The physical conditions of the apparatus for the delivery of clouds containing *M. leprae* were adjusted to those that had been established for the quantitative delivery of known numbers of viable particles of *M. tuberculosis* reaching the lungs of mice (S. Peacock, personal communication). It was estimated that the lung retention per mouse was 7 ml of the bacteria-containing cloud per min. and that from a reservoir containing 5×10^6 viable units of *M. tuberculosis* per ml, a cloud of 1×10^4 viable units per litre per min. was produced. Therefore the average rate of lung challenge with *M. tuberculosis* per mouse was 70 viable

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units per min. Under these same conditions the whole-head challenge per mouse was estimated to be 4 ml per min., i.e., 40 viable units of *M. tuberculosis* per min. Although no data are available for the anatomical distribution of the bacilli within the head of the mouse, a proportion could have been retained in the nasal passages.

Preparation and standardisation of M. leprae for airborne infection

The pilot study in 1967 indicated that mice could be systemically infected with *M. leprae* by airborne infection that gave a lung retention of 1×10^5 bacteria. Therefore, from the data for *M. tuberculosis* with the Henderson apparatus, it was calculated that a lung retention of 1×10^5 *M. leprae* per mouse would require a cloud-exposure time of 60 min. from a spray suspension containing 1.2×10^8 *M. leprae* per ml. From this, the whole-head retention of *M. leprae* was calculated to be 5.7×10^4 . To avoid tiring the mice, they were given six separate 10-min. exposures to the *M. leprae* cloud.

The suspension of viable *M. leprae* was obtained from biopsies of skin from four patients with active and previously untreated lepromatous leprosy. The bacteria were extracted from skin by homogenisation in 0.1% (w/v) bovine albumin in water, followed by differential centrifugation, and the concentration of *M. leprae* determined by measuring the number of acid-fast bacilli (AFB) by the methods of Rees (1964). The suspensions were pooled and diluted to yield 45 ml of *M. leprae* in 0.1% bovine albumin in water containing 1.2×10^8 AFB per ml. The morphological index (mean percentage of solidly-stained bacilli on Ziehl-Neelsen staining) of the bacteria was 10, indicating a 10% viability (Rees, 1965).

Samples of *M. leprae* from the pooled suspension, before and after use in the Henderson apparatus, were inoculated at a concentration of 1×10^4 AFB into both hind foot pads of six mice in order to determine and compare their respective viabilities.

Assessment and sampling of mice

Six-week-old female CBA mice were used in all the experiments. For the main airborne infection, 48 mice previously immunologically suppressed by thymectomy followed 2 weeks later by five exposures to irradiation (200 R ^{60}Co) at fortnightly intervals were used, since such mice ("T/R mice") were more susceptible to infection with *M. leprae* than normal animals (Rees, 1966). Immediately after exposure to aerosols of *M. leprae*, five mice were killed and their lungs and nose tips individually homogenised for enumerating AFB. Mice dying less than 14 months after aerosol exposure were discarded, but from then onwards remaining mice were killed, or those that died were dissected, taking from each both ears, foot pads and right and left lungs separately and the nose tip. All of the tissues taken from dying mice were homogenised and the AFB content determined; from a proportion of the killed mice, tissues from all representative sites were subjected to AFB counts and some of the duplicate samples were also examined histologically, as was a deeper part of the nasal tissue.

Normal, not T/R, mice were used for determining by foot-pad inoculation the viability and growth characteristics of the bacteria prepared for the airborne infection and of bacteria harvested from the lung, ear or nose of three individual mice 16, 17 or 22 months, respectively, after airborne infection. For each of these assessments, 1×10^4 AFB were inoculated into the hind foot pads of six mice, and the yield of AFB per foot pad determined in individual mice killed from each group 5 months after inoculation and at monthly intervals thereafter (Rees, 1964).

RESULTS

Viability of M. leprae used for airborne infection

There was no quantitative difference in the rate or pattern of growth of *M. leprae* in mice after foot-pad inoculation of 1×10^4 AFB from the reservoir of *M. leprae* sample before and after completion of the airborne infection.

TABLE

Recovery of Mycobacterium leprae by site and yield of acid-fast bacilli (AFB) 14-24 months after exposure of 30 mice to airborne challenge with 10⁵ M. leprae organisms

Time after challenge (months)	Number of mice		Yield of AFB* (10 ⁴) from tissue of			
	Total	Positive	Ear	Footpad	Nose	Lung
14	2	1	...†	12	...	...
16	4	2	24	...	...	...
17	3	1	...	...	...	12‡
18	6	0	70‡	...	...	...
19	2	0	...	...	...	...
20	2	1	40	...	...	...
21	7	3	...	...	...	6
			...	...	20	...
			...	...	10	...
22	1	1	...	...	300‡	...
23	2	0	...	...	...	...
24	1	1§	...	...	20	...
At any time	30	10	3	1	4	2

* AFB = acid-fast bacilli; counts given are in tens of thousands.

† Yield <2 × 10⁴.

‡ Multiplication observed when inoculated into foot pad of normal mice.

§ AFB also present in bone marrow from femurs.

Retention of M. leprae in the lung and nose

Of the five mice killed immediately after exposure to airborne infection with *M. leprae*, the individual AFB counts on homogenates from the whole lungs were 36, 8·4, 6, 3·2 and <2 × 10⁴ respectively, whereas no AFB (<2 × 10⁴) were counted in homogenates from the noses of any of the five animals.

Airborne infection with M. leprae

Because the earlier pilot experiment had failed to show systemic infection in mice until 15 months after airborne infection with *M. leprae*, no mice from the present experiment were examined before 14 months post-infection. At 14 months, 30 of the original 43 were 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 AFB in homogenates from their ears, foot pads, nose and lungs. From the results summarised in the table it can be seen that 33% (10 of 30) of the mice had countable numbers of AFB (>2 × 10⁴) at one or more of the sites. The AFB morphologically resembled *M. leprae*. Although only an isolated observation, it is of interest that in the last surviving mouse killed 24 months after aerosol exposure, at a time when the bone marrows of *M. leprae*-infected mice were being examined as a routine, AFB were seen in the pooled marrow suspension from both femurs in addition to the countable AFB yield from a foot pad.

None of the other representative tissues of this last mouse showed convincing histological evidence of *M. leprae* infection.

AFB harvested from a lung, ear and nose of three individual mice, respectively 16, 17 and 22 months after airborne infection, multiplied when inoculated at a dose of 1×10^4 into the foot pads of normal mice. Moreover, their rates and patterns of multiplication were similar to those of *M. leprae*.

DISCUSSION

These results have established that immunologically suppressed (T/R) mice can be infected with *M. leprae* by airborne infection, thus confirming the evidence from an earlier and smaller experiment. Although a different type of apparatus for producing the infection was used in the two studies, both were based on a lung retention of 1×10^5 *M. leprae*. The Henderson apparatus as used in the present study, was adjusted to expose only the head of the mouse to clouds of dry bacteria-containing particles of 1–2 μm diameter, which favours lodgement in the lung rather than in the nose. The infecting dose of 1×10^5 *M. leprae* was obtained by using the apparatus under conditions established in quantitative pulmonary infections with *M. tuberculosis*. This proved to be satisfactory, for a mean lung retention of 1.1×10^5 *M. leprae* (counted as AFB) was obtained from five mice killed immediately after airborne infection. Under the same conditions the total head retention of *M. tuberculosis* was 57% of that retained in the lung, giving a calculated total head retention for *M. leprae* of 5.7×10^4 AFB. In fact, none of the nasal tissues from the same five mice sampled immediately after airborne infection yielded countable AFB's ($< 2 \times 10^4$); thus the nasal tissues retained $< 35\%$ of the calculated total head retention of *M. leprae*.

It seems that within the murine respiratory tract the main lodgement of *M. leprae* is in the lungs, making this site the probable portal of entry. However, the nose is not completely excluded as a portal of entry; nor is the gastrointestinal tract excluded as a secondary portal for *M. leprae* swallowed by the mice. Such bacteria might initially be held in nasal secretions or might be carried up from the lung via the trachea or licked off the head. However, there is no direct evidence that the gastro-intestinal tract in the mouse is a portal of entry for *M. leprae*, since no animals have become infected when fed with suspensions of *M. leprae* by stomach tube (R. J. W. Rees, unpublished data).

Fourteen to 24 months after airborne infection with *M. leprae*, small but significant numbers of AFB were harvested from selected sites in 33% (10 of 30) of the mice. Identification of these AFB as *M. leprae* was based on their morphological characteristics, their inability to multiply in conventional mycobacterial media and the rate and pattern of growth in the mouse foot pad of inocula from three of the animals exposed to *M. leprae* aerosol.

The unusually long incubation period and small proportions of animals infected after aerosol exposure suggest that only small numbers of viable *M. leprae* have escaped from the lungs, probably via blood or lymphatic vessels, and subsequently came to rest at sites favourable for their multiplication. After airborne infection of 10 mice, four had significant numbers of *M. leprae* in the nose and three in the ear. These favoured sites for multiplication of *M. leprae* after airborne infection are also favoured after intravenous

inoculation or systemic spread from foot pad inoculation of *M. leprae* (Rees, McDougall and Weddell, 1974). This suggests that infection in the nose, as in the ear, arises from systemic spread rather than from small numbers of *M. leprae* retained during the airborne infection.

The importance of the nose and nasal secretions in leprosy, although noted long ago (Goldschmidt, 1891; Sticker, 1897; Schäffer, 1898), attracted little comment until Muir (1921; 1929) and Browne (1966) drew attention to its clinical and epidemiological significance. Pedley (1973) described the enormous numbers of apparently viable bacilli issuing from the nasal cavity of untreated lepromatous patients, and emphasised the rarity of bacilli on the intact skin surface (Pedley, 1970). Histopathological studies of the septum and turbinates (Job, Karat and Karat, 1966; McDougall *et al.*, 1975) have amply confirmed the density of bacilli in these tissues, the numerous routes of escape of bacilli from the stroma into the nasal cavity, and the high morphological index of the many bacilli seen in sections. Despite all this, there is no direct evidence that the nose is a portal of entry in either lepromatous or non-lepromatous forms of the disease. Indeed, with the doubtful exceptions of intra-nasal infections with staphylococci and with *Klebsiella rhinoscleromatis* in man, there are no known bacteria that enter and also initiate a primary chronic infection in the nose, in man or in animals. However, by analogy with tuberculosis and with the epidemiological evidence already quoted (Rees and Meade, 1974), the lung is a likely portal of entry in leprosy. Nevertheless, involvement of the lung in clinically established lepromatous leprosy has never been convincingly described in patients, and only rarely in post-mortem material (Junarkar, 1957; K. V. Desikan, personal communication). On the other hand, in the thymectomised-irradiated mouse (Rees *et al.*, 1967) and in the armadillo (Kirchheimer, Storrs and Binford, 1972), lung parenchyma is indeed involved after experimental infection with *M. leprae* from man. This suggests that further studies should be undertaken on the lungs of patients with lepromatous leprosy. Meanwhile from the positive results of our studies in the mouse, we conclude that leprosy in man may also result from an airborne infection entering through the lung, although other routes of infection are not excluded.

SUMMARY

Although the portal of entry and mode of spread of *M. leprae* in human leprosy are still uncertain, it is widely held that direct person-to-person skin contact is important. This assumption has ignored the fact that patients with highly bacilliferous leprosy have nasal as well as dermal infection and that, since *M. leprae* is shed predominantly from the nose, leprosy might be an airborne infection. The present study was designed to investigate this possibility with mice exposed to airborne infection with *M. leprae*. The conditions are described in which thymectomised-irradiated CBA strain mice exposed to *M. leprae* aerosols sustained an immediate lung retention of 1×10^5 bacteria. Fourteen to 24 months later, 33% (10 of 30) of the mice had countable numbers of acid-fast bacilli ($>2 \times 10^4$) with the characteristics of *M. leprae* in one or more homogenates prepared from ears, foot pads, nose or lungs. Evidence

is presented from the distribution of *M. leprae* that the infection had arisen from systemic spread of bacilli initially entering the lungs rather than from multiplication of organisms locally retained there, or in the nose, at the time of airborne infection. The relevance of these results to the possible route of infection of leprosy in man is discussed.

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