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Editorial

HOW MIGHT *MYCOBACTERIUM LEPRAE* ENTER THE BODY?

It is now reasonably certain that the major exit points of *Mycobacterium leprae* from an infectious patient are the nose and mouth, and in some cases, ulcerating skin lesions, and that large numbers of leprosy bacilli are dispersed from these sites daily in untreated LL patients.[1–6] However, how *M. leprae* enters the body to cause infection is not yet known, and may not be just an academic question. It has been suggested that the route of infection is crucial in determining what type of disease will develop in a susceptible individual.[7] It is possible that intradermal inoculation leads to tuberculoid leprosy whilst inhalation of infectious droplets leads to lepromatous leprosy. Indeed, there is experimental evidence to show that intradermal injection of *M. leprae* can sensitize mice whereas intravenous injection triggers suppression.[8] Perhaps an analogy can be drawn with plague, where intradermal inoculation of plague bacilli by rat fleas usually causes the milder bubonic plague (?*cf.* tuberculoid leprosy) whilst inhalation of an infectious aerosol gives rise to a more serious disseminated and highly transmissible infection—pneumonic plague (?*cf.* lepromatous leprosy). In addition, the manner in which *M. leprae* enters the body may determine whether subclinical infection or clinical disease results. Until recently the consensus amongst leprologists was that prolonged and intimate contact was required for the transmission of *M. leprae*.[9] However, immunological evidence of exposure to *M. leprae*, with presumed subclinical infection, is very common in endemic areas.[10,11] It is therefore likely that any number of different routes of entry could allow leprosy bacilli to trigger an immune response to the antigens of *M. leprae* in healthy individuals. On the other hand, perhaps clinical leprosy occurs only when leprosy bacilli are introduced through one or two very specific routes. However, other pathogenic mycobacteria can cause disease after introduction through a variety of entry points,[12] although one particular mode of transmission is usually predominant for each species.

Until recently, information about the route of entry of *M. leprae* had come almost exclusively from clinical, bacteriological or epidemiological studies. However, in recent years much progress has been made in the development of

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animal models of leprosy.[13–17] Such models have made possible carefully controlled experimental work on the mode of transmission of *M. leprae*, and thus have allowed scientists to overcome some of the confounding problems involved in studying transmission of infection in humans. Some early experiments were done using thymectomized irradiated mice,[18] and more recently, researchers at the National Hansen's Disease Centre in Carville, Louisiana,[19] and one of the authors (RDMcD) at St Georges Hospital Medical School, London,[16] have performed a series of experiments on the mode of transmission of leprosy using congenitally athymic nude mice. In addition, some observations have been made on the transmission of leprosy in armadillos.[20,21] Rather confusingly, work with animal models has produced evidence to support several hypothetical entry sites for *M. leprae*.

Entry through the skin

Traditionally the skin has been favoured as both the route of exit and of entry of *M. leprae* in humans. Few, if any, species of bacteria can penetrate intact skin and *M. leprae* has no distinctive features to assist it to do so. However, entry through broken skin remains a possibility. There have been occasional case reports of leprosy lesions developing after various types of trauma to the skin—tattooing, smallpox vaccination, dog bites[22–25]—but these do not prove that transmission occurs in this manner. Alternative explanations for these cases include pure coincidence, an increased awareness of the skin affected by trauma, or the attraction of blood-borne *M. leprae* to scar tissue. Furthermore, even if some of these cases do represent genuine infection through the skin, this does not prove that the skin is the usual site of entry of *M. leprae* into the body. Tuberculosis affecting the skin may in rare cases follow direct inoculation of *M. tuberculosis* into the skin,[26] but no-one would suggest that this proves that the skin is the usual portal of entry of this organism.

Many epidemiological studies have been performed to determine whether the site of the first lesion in indeterminate or tuberculoid leprosy is related to the frequency of exposure of the affected area, as one would expect if *M. leprae* entered through the skin. Some early studies[9,27,28] suggested an important relation between skin exposure and first lesion site. However, more recent investigations[29–31] have failed to show any such correlation. In studies done in India[29] and Zimbabwe[30] 35 and 58% of first lesions respectively were on covered regions. In any case, as Fine points out,[7] there are many confounding variables in such studies, which severely limit their validity. Exposed areas are more easily examined by patient and doctor and are liable to be relatively cool and so favour proliferation of *M. leprae* seeded there from the blood. However, a recent study[21] has shown that the first lesion in artificially infected armadillos does indeed occur at the site of inoculation of the leprosy bacilli.

Against such a background of previous research, the investigators at Carville[19] attempted to transmit *M. leprae* infection by smearing leprosy bacilli onto both intact and abraded skin of nude mice. This method proved unsuccessful in establishing infection with *M. leprae*. However, it is worth noting that the usual, highly successful, route for the establishment of artificial *M. leprae* infection in both nude mice and captive armadillos is through intravenous or subcutaneous inoculation of the organisms.[15,16,21] One might argue that such inoculation is obviously artificial and cannot tell us anything about the transmission of *M. leprae* in humans. However, it is possible that accidental inoculation of *M. leprae* may occur if nose-blow material or contaminated fomites are introduced into the skin through lacerations or puncture wounds. Indeed, the recent finding of AFB thought to be *M. leprae* localized around a thorn in the nose of a wild armadillo,[20] together with the subsequent suggestion that wild armadillo leprosy is transmitted through infected thorns[20] lends some support to this idea, as does the existence of infections with environmental mycobacterial species, such as *M. marinum*, where inoculation into the skin is the predominant means of transmission[12].

Arthropods and transmission of *M. leprae*

Attention has in the past been focused on the possible role of biting arthropods in the transmission of leprosy.[32–37] Narayanan *et al.* have shown that *M. leprae* infection can be transmitted from lepromatous leprosy patients to mouse footpads, though only four out of 208 mouse footpads were so infected.[36] In experiments carried out at St Georges Hospital Medical School,[38] mosquitoes of the species *Aedes aegypti* were allowed to feed on the footpads of heavily infected nude mice (footpad count approximately 10^9). In some experiments the mosquitoes were allowed to complete their feeds, and were then allowed to feed on the snouts and footpads of recipient pathogen-free nude mice 4 days later. In other experiments the mosquitoes were transferred to the recipient animals before completing their blood meals. The recipient mice received between one and eight bites over a 4-month period. They were then examined for the presence of AFB some 12–20 months after the last exposure. Leprosy bacilli were found in only one mouse out of 10 so examined. Small numbers of AFB were seen in the snout of this animal 18 months after exposure. These results, together with Narayanan's findings, suggest that this route of infection is not very efficient. However, even with such a low efficiency, occasional transmission in this manner cannot yet be ruled out.

Transmission through the lungs

Rees & Meade have drawn an analogy between *M. leprae* and *M. tuberculosis* in terms of numbers of bacilli shed per day and attack rates of clinical disease among

contacts of open cases, and they have suggested that this supports a similar mode of transmission.[39] Leprosy bacilli do not produce lesions in the lungs, perhaps because the temperature is too high or because the pulmonary environment is unfavourable in other ways. However, in nude mice leprosy bacilli are readily taken up by alveolar macrophages[16] and so it is possible that in humans they may be carried within such macrophages to other sites before replicating and causing disease.

Rees & McDougall were the first to produce experimental evidence for transmission of leprosy bacilli through the lungs.[18] They were able to establish infection in thymectomized-irradiated mice by exposing the snouts of the mice to an *M. leprae*-laden aerosol. They showed that *M. leprae* delivered in artificial aerosol could be found in the lungs of four out of five mice, but could not be recovered from the nose, immediately after exposure. One third of mice so exposed showed countable numbers of AFB in either ears, nose, lungs, footpads or bone marrow between 14 months and 2 years after exposure.

Recently, attempts have been made to follow up these findings using nude mice. Direct inoculation of *M. leprae* via a tracheostomy is a technically easier and more efficient way of delivering *M. leprae* to the lungs than artificial aerosols.[16] In experiments carried out at St Georges Hospital Medical School,[16] this method proved unsuccessful in establishing infection in nude mice, in spite of the large numbers of leprosy bacilli reaching the lungs ($> 10^5$). The bacilli were gradually cleared from the lungs, to reach the limit of detectability after some 90 days. However, even after as long as 75 days, some of the *M. leprae* harvested from the lungs of one mouse were still viable, as shown by subinoculation into mouse footpads. The investigators at Carville also attempted, unsuccessfully, to establish infection in nude mice via the pulmonary route.[19] Why Rees & McDougall were successful while recent attempts have failed is not clear.

Transmission through the Gastrointestinal Tract

There have been reports of *M. leprae* being found in milk from mothers with LL,[40,41] although any epidemiological evidence to support this as a source of infection is at best marginal.[7] In addition there has been one case report of leprosy thought to have been acquired by eating wild armadillos.[42] To determine whether *M. leprae* infection could be established by ingestion of leprosy bacilli, both the London and Carville experimenters fed *M. leprae* to nude mice via gastric canuli.[16,19] No infections were established in the mice in the Carville experiments, although in London three mice out of six showed evidence of limited infection in the tailskin after ingestion of *M. leprae*.[16] It is not clear whether this represents true infection through the GI tract with subsequent dissemination to the tailskin or whether the infections arose from faecal contamination of injured tailskin. Subsequent experiments have shown that large numbers of *M. leprae* pass

straight through the GI tract and out into the faeces. In fact, viable *M. leprae*, as shown by subinoculation into nude and normal mice, can be recovered from the faeces of nude mice fed *M. leprae*[38] and this suggests that the faeces of lepromatous patients, who swallow large amounts of *M. leprae* in nasal secretions, are likely to harbour considerable numbers of viable leprosy bacilli, and should probably be considered infectious.

Entry of *M. leprae* through the nose and mouth

Barton has suggested the anterior end of the inferior turbinate as a possible site of entry of *M. leprae*.[1] It has several points in its favour—it provides ideal conditions for the growth of *M. leprae*, being cool and moist, and is the first structure likely to be encountered by inhaled material. Moreover, it is involved very early and consistently in LL. In addition, there is a significant diffe
frequency of involvement between the anterior portion of the inferior turbinate and the anterior part of the nasal septum even though both are at the same level in the nose, suggesting that coolness and moisture cannot entirely explain the early involvement of the turbinate.[1]

The same arguments that apply to entry via intact skin apply to *M. leprae*'s putative entry via the intact nasal mucosa. However, with up to 15% of the population affected by the common cold at any one time,[43] together with all those afflicted by allergic or vasomotor rhinitis, the chances of airborne *M. leprae* meeting diseased nasal mucosa are quite high. Such damaged mucosa is thought to be more susceptible in general to secondary bacterial infection than healthy tissue,[44] and could, in particular, be more susceptible to infection by *M. leprae*. Indeed, one could also speculate at this point on whether colds can appreciably increase the shedding of *M. leprae* from infectious patients. Perhaps the common cold helps *M. leprae* both to enter and leave the nasal mucosa. In addition, picking the nose has been suggested as a means whereby *M. leprae* could breach the nasal mucosa,[35] and perhaps an apt analogy can be made with rhinovirus infection, where contrary to popular belief, transmission by the hands is now thought to be more important than transmission by aerosol.[43]

The investigators both in Carville and in London attempted to transmit *M. leprae* to nude mice via the nasal and oral mucosae.[19,38] No infections occurred when *M. leprae* was placed in contact with the oral mucosa. However, in the Carville experiments[19] 10 nude mice were exposed to *M. leprae* via the nose, and infection is described in four out of five animals examined. An initial localized submucosal lepromatous lesion led eventually to generalized infection. In the London experiments,[38] where nude mice were made to sniff up 2×10^6 leprosy bacilli into the nose, all harvests were negative, but when the nasal mucosa had been lightly abraded by means of a capillary tube, one mouse out of four showed evidence of infection one year later, with more than 5×10^6 AFB in both the snout

and the ear. In another set of experiments carried out at St Georges, leprosy bacilli were introduced into the nose by means of a capillary tube, and successful transmission was achieved in two out of 10 mice.[16] These findings provide convincing evidence to support Barton's hypothesis.

Conclusions

In conclusion, while it is known that LL patients disseminate large numbers of viable *M. leprae* from the nose and mouth, and possibly also in faeces, the route, or routes of entry of the organism into the human body remain unclear. The inability to infect nude mice either through intact or abraded skin fails to support the traditional view of skin-to-skin transmission, whilst the experiments done in London and in Carville suggest that the nasal mucosa is, on balance, most likely to be the predominant entry site. However, the evidence is still inconclusive. It must be remembered that investigators working with nude mice are using a highly artificial model of human leprosy. Researchers at Carville have shown that even highly bacilliferous nude mice cannot transmit *M. leprae* infection to uninfected nude mice kept in the same cage.[19] However, this discovery may indirectly support the hypothesis of entry through damaged or diseased nasal mucosa since infective rhinitis is unlikely in pathogen-free mice and injury improbable because of the extremely small nasal apperture. Indeed, it may be that rhinitis not only contributes to the dissemination of *M. leprae* into the environment but also facilitates entry of the bacillus into the body. Leprosy bacilli could be introduced into the nose in an aerosol or in nose-blow material carried by hand-to-hand and hand-to-nose contact.

It is generally accepted that, whatever the portal of entry into the body, it is necessary in human leprosy for there to be a predisposing immunological defect,[45] which is not the same as the immunological unresponsiveness of the nude mouse.[46] Valuable information could be gained if transmission experiments, similar to those performed in nude mice, could be carried out in armadillos and primates. The Mangabey monkey[47] and the chimpanzee[48] appear to be promising candidates for primate models of leprosy. If problems in the supply of laboratory animals can be overcome,[49] then well-controlled studies on the latter species, which is probably of all existing species the one most closely related to our own, should settle the issue once and for all.

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