Mycobacterium leprae and Mycobacterium lepraemurium infections in domestic and wild animals

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Summary

*Mycobacterium leprae*, the aetiological agent of leprosy in humans, gives rise to a chronic granulomatous disease that affects primarily the skin and peripheral nerves, and secondarily some internal organs such as the testis and the eye; viscera are seldom involved. Depending on host resistance, leprosy may present as a benign disease (tuberculoid leprosy) or as a malignant disease (lepromatous leprosy), with a spectrum of intermediate stages appearing between the two. Immunity against leprosy depends on the cell-mediated immunity of the host, and this is severely compromised in the malignant (lepromatous) form of leprosy. Although culture of *M. leprae* has never been achieved in artificial media, the bacterium may be grown in several experimental animals, including the armadillo, non-human primates, and to a certain extent, rodents. Naturally acquired leprosy has been reported in wild nine-banded armadillos (*Dasypus novemcinctus*) and in three species of non-human primates (*Pan troglodytes*, sooty mangabey monkeys (*Cercocebus atys*) and cynomolgus macaques (*Macaca fascicularis*)), thus qualifying leprosy as a zoonosis.

Murine leprosy is a leprosy-like disease of rats and mice, caused by *Mycobacterium lepraemurium*. The disease affects primarily viscera and the skin, and very rarely peripheral nerves. Depending on the host strain, rodent leprosy may also evolve as 'lepromatous' or 'tuberculoid' leprosy, and strains of mouse that develop intermediate forms of the disease may exist. Growth of *M. lepraemurium* on conventional media for mycobacteria is not successful, but the bacterium has been cultured on an egg yolk-based medium. Naturally acquired murine leprosy has been observed in rats, mice and cats, but not in humans or any other species. Thus, in contrast to human leprosy, murine leprosy is not a zoonosis.

Keywords

addition to hushtha in India (600 BC) and as lāi-ping in China (AD 281-AD 341). The name leprosy seems to appear for the first time in the Hebrew Bible in the year 300 BC and is synonymous with tsaraath.

Leprosy is described in medical treatises from India and China dating from approximately the 5th Century BC. The earliest evidence of bone involvement due to leprosy was found in a mummy in Egypt from the 2nd Century BC, and the first clear description of leprosy appears to be that found in Sushruta Samita, an ancient Indian book of medicine, where the disease is described as vāt-rākta, vatasonita and hushtha by the year 600 BC. While vāt-rākta and vatasonita are used to describe the disease characterised by hyperaesthesia, anaesthesia and deformities, hushtha described the disease characterised by skin ulceration, loss of fingers and sinking of the nose. Complementary descriptions of leprosy, recognising loss of eyebrows, development of nodules, absence of sweating, distortion of the ears and fingers, dim or blurred vision and blindness, appeared in medical treatises from China and other countries, between 600 BC and the beginning of the 6th Century AD.

Leprosy appears to have been introduced into Italy in the 1st Century BC, by Roman soldiers returning to Pompeii after fighting in Egypt. The disease spread throughout Europe during the Middle Ages, reaching a peak at the time of the crusades. Leprosy victims were so numerous that the disease was thought to be highly contagious, leading to the establishment of hundreds of leprosaria to protect the population from infection. The first leprosaria were founded in Nottingham, England (AD 625-AD 638), St Gallen, Switzerland (AD 720), Palencia, Spain (AD 1067), Bergen, Norway (1400), Culion, the Philippines (1906), and several locations in Japan (1907); many others followed. Commitment to a leprosarium was for life, and leprosy became known as the living death. In France, for instance, such was the fear surrounding the disease that people were buried alive, burnt at the stake, or simply sent out with a bell and sometimes a candle. Philip IV (1285-1312), King of France, ordered that all persons with leprosy be gathered together and burned, and that the practice should continue until the disease was eradicated.

Leprosy seems to have reached the Americas between the 16th and 18th Centuries, via the European conquistadors and the slave trade.

For reasons not well understood, but probably related to improvement in health services and education, leprosy began to decline in Europe in the 15th Century AD. However, at the end of the 19th Century, leprosy was still endemic in Norway and in some areas of the Mediterranean basin. It was in Norway, in 1873, that Dr Gerhard Armauer Hansen discovered the bacillus that causes leprosy (96, 97).

The disease

Leprosy or Hansen's disease, is a chronic, granulomatous disease of humans, caused by a slow growing acid-fast bacillus, Mycobacterium leprae. The disease affects primarily peripheral nerves and the skin, and secondarily other organs and tissues such as the eyes, the mucosa of the nasal and upper respiratory tract and the testes. In peripheral nerves, the key targets of M. leprae are the Schwann cells. However, leprosy does not affect the central nervous system, perhaps because of the higher temperature at this location. Damage to sensory nerves leads to anaesthesia and when these nerves supply the extremities of hands and feet, the latter become vulnerable to burns and other injuries that can often result in the loss of fingers and toes, and sometimes hands and feet. When cranial nerve involvement occurs, sensorial damage to the eye may produce corneal anaesthesia and even blindness. When motor nerves are involved, various forms of paralysis such as 'dropped foot', 'dropped wrist', 'clawed hand' and lagophthalmos (inability to close the eyes) can result. Nerve damage may also lead to loss of hair (mainly alopecia of the eyebrows and eyelashes) and dysfunction of the sweat and sebaceous glands, which causes drying and cracking of the skin. Lesions in the skin may also lead to secondary infections.

Skin-testing using lepromin has often been used to diagnose this infection. The lepromin test is a skin reaction elicited by the intradermal injection of lepromin, an autoclaved suspension of bacilli-containing tissue prepared from lepromatous nodules. A positive test appears as an indurated inflammatory reaction larger than 5 mm in diameter, maximally developed within three to four weeks of injection of lepromin. The test indicates the ability of the subject to mount a specific cell-mediated immune response to the antigens of M. leprae.

Depending on host resistance, leprosy presents a broad spectrum of clinical manifestations, ranging from one or a few lesions to widespread multiple nodules. All forms of leprosy appear to develop from a mild infection called indeterminate leprosy (283).

Indeterminate leprosy

Indeterminate leprosy (IL) consists of single or multiple slightly hypopigmented or faintly erythematous and indefinite macules on the skin. Sensation, sweating and hair growth in the affected area are unaffected, as are peripheral nerves. Slit-skin smears are mostly bacilli-negative and the lepromin test may be either positive or negative. Indeterminate leprosy is usually self-healing, but may progress to other forms of leprosy.

Tuberculoid leprosy

Tuberculoid leprosy (TT) presents as a single or a few asymmetrical, reddish or hypopigmented lesions on the skin that appear to be dry and well demarcated by a raised edge; sensory loss is also reported in the area of the lesions (Fig. 1). A few bacilli may be found in skin biopsies, but slit-skin
A well-defined, raised, erythematous, scaly plaque of tuberculoid leprosy on the face

Photo: courtesy of Dr. R. Arenas, Hospital Dr Gea González, Mexico

smears are usually negative. In some cases, a lesion may affect a cutaneous nerve leading to loss of feeling, pain, tingling and muscle weakness or even paralysis. Patients with TT are positive to lepromin, indicating a functional and efficient cell-mediated immunity.

Borderline leprosy

Borderline leprosy may be subdivided into borderline-tuberculoid (BT), mid-borderline (BB) or borderline-lepromatous (BL) leprosy. Depending on the position in the borderline spectrum, macular and other lesions vary greatly in number, size and shape. Hence, when the disease is nearer to the lepromatous leprosy (LL) end of the spectrum, the lesions are more numerous, shiny, bacilliferous and less anaesthetic. Bacilli are frequently found in the lesions, and numbers increase from very few in the BT lesions to many in the BL lesions. The lepromin test is generally negative, which indicates depressed cell-mediated immunity. The most severe damage to nerves is observed in this type of leprosy.

Lepromatous leprosy

Lepromatous leprosy is a malignant form of the disease which may present three varieties, namely: macular, nodular or diffuse (Fig. 2). Macules are small, multiple and symmetrical, with smooth shiny surfaces and very indistinct margins, they are faintly erythematous, not anaesthetic and contain bacilli.

The infiltrated skin of diffuse leprosy is thickened, erythematous and shiny, with slight or insignificant anaesthesia in the infiltrated areas. Bacilli are abundant, and alopecia of eyebrows is a common finding.

Nodular lesions of LL leprosy appear on the ears, face, extremities, trunk, and occasionally on genitalia. The lesions may be erythematous or skin-coloured, small or large, fairly hard and bacilliferous.

Lepromatous patients are lepromin-negative and specifically anergic to M. leprae in lymphoproliferative assays in vitro. Cellular anergy to M. leprae in LL leprosy accounts for the systemic dissemination of the disease; M. leprae is found throughout the body, in lacrimal secretions, nasal mucus, sputum, breast milk, blood, semen and faeces.

Thus, resistant individuals develop paucibacillary (TT and BT) leprosy, while poorly resistant persons develop multibacillary (LL and BL) leprosy. Resistance or susceptibility to leprosy depends on several known and unknown factors, including genetic and immune mechanisms (as discussed below).

Leprosy is regarded as the least contagious of all of the infectious diseases, and the period from initial infection to onset of disease lasts from two to ten years. The shortest incubation time, of two months, was reported in a two-month-old girl who developed IL leprosy (83).

Epidemiology

Leprosy continues to be a significant problem in the developing world, with approximately 1.15 million cases registered in 1998, compared to over 2.4 million in 1994, 5.5 million in 1991 and 10-12 million in the mid-1980s. During the mid-1980s, the World Health Organization (WHO) recommended fixed multidrug therapy (MDT) for the treatment of leprosy (188, 190, 281). Leprosy still affects approximately eighty countries in Asia, Africa, Latin America and Oceania, but is relatively rare in Europe. The disease is highly prevalent in South-East Asia (including India, the Philippines and the Republic of Korea), in the south of the People's Republic of China, Papua New Guinea and some Pacific islands. Most cases reported in the Americas originate in five countries, namely: Argentina, Brazil, Colombia, Mexico and Venezuela, but other less populated countries of Central and South America are also affected. By 1990, India had the largest leprosy problem in the world, with nearly 2.4 million
people affected (165), followed by Africa (the WHO African region), which had the second highest prevalence, at one per 1,000 people. The global number of registered cases in the WHO African region was close to 480,000 (48).

Aetiology
For centuries, leprosy was regarded as divine punishment for the sin of inappropriate behaviour. Leprosy was also considered a hereditary dyscrasia induced by eating hot food, pepper, garlic and the meat of diseased pigs and fish. The infectious nature of leprosy was recognised in 1749 in the Chinese medical classic Golden Mirror of Medicine (240), which cited contact with those affected by leprosy, or with the houses or belongings of these people as infectious causes. However, in Norway, the renowned leprologist Danielssen, who discovered the ‘brown bodies’ characteristic of leprosy (now recognised as ‘globi’ or conglomerations of leprosy bacilli) regarded leprosy as a non-contagious ‘hereditary dyscrasia sanguinis’. This was probably as a result of his several failed attempts, performed between 1844 and 1856, to transmit leprosy by inoculating himself and several volunteers with leprous material (see below). The infectious nature of leprosy was finally established in 1873 in Bergen, Norway, when Hansen discovered the ‘rod-shaped bodies’ of the leprosy bacillus (96, 97). Hansen was aware that evidence for an infectious agent, based on microscopic observations in tissues, would be significantly strengthened by the isolation of the germ in culture and transmission of the disease by inoculating humans and animals with material from the tissues of leprosy patients (152).

The leprosy bacillus
Based on biochemical, morphological and bacteriological characteristics, the leprosy bacillus was finally classified as *M. leprae*. When stained by the Ziehl-Neelsen procedure, *M. leprae* is an acid-fast, slightly curved bacillus, 0.3 μm-0.4 μm × 4 μm-7 μm in size, sometimes showing a metachromatic granule either near to a pole or in the centre. Within histiocytes, leprosy bacilli frequently aggregate in globi, the organisms being ordered into parallel bundles. The acid-fast character of the bacillus has been shown to be related to the cell wall, in particular to mycolic acids. One of these lipids, phenolic glycolipid-1 is regarded as specific for *M. leprae* and has been used in assays to diagnose the disease, to identify household contacts with incipient disease and to monitor patients undergoing chemotherapy (104, 221). Arabinogalactan, arabinomannan, lipoarabinomannan and peptidoglycan, are among the other components present in the thick cell wall of *M. leprae*.

Transmission of *Mycobacterium leprae*
Untreated LL patients may discharge many millions of leprosy bacilli from nasal secretions every day, and the bacilli may survive for several days. The inhalation of bacilli-laden droplets is regarded as the most likely mode of entry of leprosy bacilli; bacilli have been detected in the nasal mucosa of household contacts of multibacillary patients and damage to the nasal mucosa is a facilitating factor (274, 279). Skin is also an important source of infection. In 1997, Namisato et al. reported the case of a thirty-five-year-old LL patient showing typical lepromatous skin lesions and many scar-forming lesions, some with erosive surfaces (183). Excretions from these lesions contained many viable *M. leprae*, thus representing a potential source of leprosy transmission. In a more extensive study of the skin of LL patients, Job et al. detected high numbers of bacilli in the superficial keratin layer of the skin, which were shed into the environment in large numbers (110). Job et al. concluded that direct skin-to-skin contact with such patients is more likely to transfer viable *M. leprae* from a patient than any other route.

In vitro culture of *Mycobacterium leprae*
Culture of the leprosy bacillus in vitro has been attempted since the discovery of the bacillus in leprosy lesions by Hansen in 1873. Surprisingly, *M. leprae* has never been undisputedly cultured in artificial media of any sort, although many attempts have been made and several ‘successful results’ have been reported. For instance, in 1975, Skinsnes et al. reported in vitro cultivation of *M. leprae* in approximately six weeks, in a medium based on hyaluronic acid with yeast extract, bovine albumin and glycerine in phosphate buffer (medium LA-3) (241). The growth of *M. leprae* on medium LA-3 incorporated in agar (LA-3P) yielded small orange-yellow colonies in two to three weeks. Identification of the cultured bacilli as *M. leprae* was based on several weak observations, including similar isolations from different patients, failure to grow on the usual media for mycobacteria, and reactivity of the harvested micro-organism with fluorescein isothiocyanate-coupled anti-*M. leprae* antibody.

In 1988, Dhople et al. observed limited in vitro multiplication of *M. leprae* in a conditioned medium used for the growth of mouse (*Mus musculus*) dorsal root ganglion, after sixteen weeks of incubation at 34°C (59). The harvested bacilli were able to grow in mouse footpads, but subculture of the micro-organism in the same artificial medium was not achieved.

In 1989, Biswas reported development of a visible colony of *M. leprae* on the surface of Dubos-Lowenstein-Jensen medium supplemented with thyroxin, after sixteen weeks of incubation at 37°C (22). Inoculation of the bacterial suspension into the footpad of a cortisone-treated mouse produced a lesion with infiltration of nerves by lepra cells.

Successful growth of *M. leprae* was also reported in 1990 by Ishaque, after eighteen to twenty-four weeks of culture on an artificial medium, in a gas mixture containing 2.5% O₂ and 10% CO₂ (105). The cultivated micro-organism was able to grow in mouse footpads, but lost viability after thirty-six weeks of culture. Three years later, Ishaque and Sticht reported that addition of palmitic acid to the above medium allowed the growth of *M. leprae* after sixteen to twenty weeks of incubation at 37°C, even in the absence of the gas mixture (106).
Dhople and Lamoureux, in 1991, were able to grow *M. leprae* *in vitro*, in the artificial Dhople-Hanks (DH) medium, in the presence of reduced concentrations of air (oxygen), and also found that *M. leprae* was microaerophilic (60). The same year, Dhople reported that addition of carboxylated sulphhydril compounds and dithiothreitol to DH medium would allow maximum multiplication of *M. leprae* (58). However, no proof was supplied to authenticate that the micro-organism grown *in vitro* was *M. leprae*.

In a more recent paper, Dhople and Lamoureux reported successful multiplication of *M. leprae* in DH medium supplemented with either cell-free extracts of armadillo-derived *M. leprae* or extracts from irradiated livers and spleens of *M. leprae*-infected armadillos (61). However, this multiplication was not achieved when the medium was supplemented with liver and spleen extracts from normal armadillos, and the authors suggested the existence of a growth factor in armadillo-grown *M. leprae*.

Despite these and other claims of successful cultivation of *M. leprae* *in vitro*, growth of *M. leprae* in a convincing and reproducible manner has never been achieved.

**Immunology**

**Basic mechanisms of immunity**

Protective anti-mycobacterial immunity depends on co-ordinated lymphocyte and macrophage activity. Upon phagocytosis, macrophages initiate the killing and degradation of ingested mycobacteria and the synthesis and secretion of cytokines, such as interleukin (IL)-1, IL-6, IL-12 and tumour necrosis factor alpha (TNF-α). Macrophages conclude the microbial processing by exposing a diversity of microbial epitopes on their cell membrane, in the context of major histocompatibility complex (MHC)-I or MHC-II molecules. These membrane-exposed epitopes are recognised by reactive T cells (178), which under the influence of macrophage-derived factors, proliferate and secrete a wide array of new cytokines. Depending on the reacting T cell, the resulting response may be humoral, cell-mediated, cytotoxic or suppressive immunity (Fig. 3). Activation of T helper (TH)1 cells induces proliferation and secretion of gamma interferon (IFNγ) and IL-2, among other cytokines (35, 173, 175). *In vitro*, IFNγ suppresses the antigen-induced

**Fig. 3**

Anti-mycobacterial immunity: activation of several sets of cells

The cells activated include macrophages and other phagocytic cells that internalise and process the bacilli into a variety of epitopes, which are ultimately presented on the cell surface. While recognition of epitopes by TH2 cells and B cells leads to humoral (antibody-mediated) immunity, epitope recognition by TH1 cells triggers the development of cell-mediated immunity. Activation of Ts cells down-regulates the response of TH and B cells, and activation of Tc cells mediates the killing of infected macrophages and extracellular bacilli.
proliferation of TH2 cells (78), while IL-4 and IL-10 inhibit the antigen-induced proliferation of TH1 cells (174) (Fig. 4).

Macrophage-derived IL-12 is a key co-stimulatory molecule for TH1 cells. This cytokine stimulates TH1 cells to synthesise IFNγ and other cytokines (144). In turn, IFNγ stimulates the synthesis of TNF-α (a cytokine) in macrophages. This TNF-α autocrinically stimulates macrophages to produce nitric oxide, a potent cytotoxic metabolite, which is able to kill ingested and intracellular micro-organisms, including M. leprae (144) (Fig. 5). In contrast, activation of TH2 cells induces the synthesis of IL-4, IL-6, IL-10 and other cytokines by these cells. These cytokines stimulate B cells to produce high levels of antibodies, specifically the immunoglobulins IgG1 and IgE (41). The TH1 cells, through IFNγ, also weakly induce B cells to synthesise immunoglobulins, in this case IgG2a (243). Humoral immunity is generally protective against extracellular micro-organisms, but is not protective against intracellular mycobacteria.

The role of TH1 and TH2 cells and cytokines in leprosy

The balance between TH1 and TH2 cell functions determines the outcome of infection with M. leprae. The most obvious contrasting feature between LL and TT leprosy is the lack of M. leprae-specific cell-mediated immunity in LL leprosy and the fully operating cellular immunity in TT leprosy. Since 1970, extensive work has been performed to discover the reason for the cellular anergy in leprosy. At present, attention
is focused principally on the role of TH1 and TH2 cell subpopulations (166, 167). These cells originate from a common precursor cell named TH0, a CD4+ lymphocyte (CD: cluster of differentiation), which under undeciphered routes of differentiation, is transformed into either a TH1 or a TH2 lymphocyte. Activation of TH1 cells promotes cell-mediated immunity, whereas activation of TH2 cells reinforces humoral immunity (34). Despite the lack of specific cell-mediated immunity, LL patients present a strong humoral antimycobacterial response. However, antimycobacterial antibodies are not protective, on the contrary, these antibodies have been involved in the development of type-2 (acute inflammatory downgrading) leprosy reactions, including erythema nodosum leprosum and necrotising vasculitis or Lucio’s phenomenon (231, 283).

Several research groups have analysed the cytokine patterns expressed by T cells in both the lesions and blood of TT and LL leprosy patients. Cytokines are believed to play immunoregulatory roles both in host protection and immunopathogenesis of the disease. On this basis, recombinant cytokines have been used for the experimental upregulation of the malignant disease (LL), with some success (239). The TH1 cells and TH1-type cytokines (IL-2 and IFNγ) are generally associated with resistance to infection (TT

Fig. 5

Destruction of intracellular mycobacteria by activated macrophages

Upon phagocytosis (a), macrophages process and present mycobacterial antigens to antigen-reactive TH1 cells, and through signals transduction (b), macrophages activate the genes responsible for synthesis of several cytokines such as IL-6, CK, TNF-α and IL-12. The IL-12 activates TH1 cells (c) and in response, these cells produce IFNγ (d), among other TH1 cytokines. The IFNγ upregulates the synthesis of TNF-α by mycobacteria-laden and normal macrophages (e) and this cytokine, in turn, upregulates the synthesis of iNOS (f) within these cells. This enzyme promotes arginine metabolism from which citruline and the toxic metabolite nitric oxide are produced (g). Nitric oxide, acting both autocrinally and paracrinally, mediates the killing of intracellular bacteria.
leprosy), whereas TH2 cells and TH2 cytokines (IL-4 and IL-10) are associated with progressive (LL) disease (166, 226, 235, 236).

In response to M. leprae, T cells from TT patients produce high levels of IFNy and low levels of IL-4 (95). The same result is obtained in the reverse transcriptase-polymerase chain reaction (RT-PCR) products from ribonucleic acid (RNA) extracted from the lesions of TT or LL leprosy. Higher expression of genes for IFNy and IL-2 is observed in TT leprosy, while preferential expression of genes for IL-4, IL-5 and IL-10 is observed in LL leprosy (164, 282).

In other studies, injection of recombinant IFNy or IL-2 into the lesions of LL leprosy induced local reversion of the anergy to M. leprae and promoted the healing of the lesions. In 1986, Nathan et al. intradermally injected 1 µg-3 µg of recombinant (r)IFNy, over a period of three days, into single skin lesions of six patients with LL leprosy, and six days later collected biopsies for histopathological analysis (185). The rIFNy induced local changes characteristic of a cell-mediated immune response, including mononuclear cell infiltration, a decline in the number of epidermal Langerhans' cells, increased expression of the human leucocyte antigens HLA-DR, and in some cases, a reduction in the number of intracellular bacilli. In a similar study of seven LL patients in 1997, Villahermosa et al. injected several doses of rIL-2 into five lesions on the back. Twenty-one days later, the development of delayed-type hypersensitivity reactions was observed, including erythema and induration, infiltrates rich in CD4+ cells, monocytes and Langerhans' cells, and epithelioid granulomas at the sites receiving the highest doses (150 µg) of rIL-2 (268). Some patients showed favourable shifts in histological classification or bacterial load. With the same idea, Kaplan et al., in 1988, intradermally injected twelve LL patients with a single dose of 5 IU of purified protein derivative (PPD) and twenty-one days later collected biopsies from injected and control sites (116). Eight out of ten patients that gave a positive PPD reaction (12 mm-21 mm) showed a marked mononuclear cell infiltrate, an increased number of CD4+ T cells compared to CD8+ T cells, and extensive destruction of the previously parasitised macrophages. A comparable response to the injection of rIL-2 was observed by Giedlin and Zimmerman in 1993 (82), who observed that relatively low doses of IL-2 reversed the course of LL leprosy towards the benign TT pole. Thus, leprosy is a disease in which distinct M. leprae-responsive T cell subsets play an important role, both in host defence and in the clinical and immunological manifestations of the disease. Whereas TH1 cells predominate in TT leprosy and are associated with strong M. leprae-specific cell-mediated immune responses, in LL leprosy, TH2 cells predominate, are associated with a strong antimycobacterial humoral immunity, and probably play a role in the cellular energy to M. leprae.

Although several aberrations may contribute to the anergy to M. leprae, a dysfunction of the M. leprae-responsive TH1 lymphocyte appears to be the most probable cause. A failure at this level would affect the specific cell-mediated immunity without affecting the specific humoral (TH2-dependent) immunity.

Lymphoid cells other than macrophages, TH1 and TH2 cells, may also play a role in anti-leprosy immunity. These cells include CD4+ cytotoxic T cells, Tyô cells, CD4+ CD1-restricted T cells, dendritic cells, CD8+ cytotoxic T cells, killer (K) cells and natural killer (NK) cells (Fig. 6).

Cytotoxic CD4+ T cells

In addition to endowing macrophages with the capacity to kill mycobacteria (150, 193), activated CD4+ T cells also kill mycobacteria-containing macrophages, and the protective role of these cells can be induced by bacillus Calmette-Guérin (BCG) vaccination (112, 113, 177, 179). Furthermore, activated cytotoxic CD4+ T cells release granulysin and perforin, proteins which are able to directly kill extracellular mycobacteria (252).

Cytotoxic CD8+ T cells

Activated CD8+ T cells are MHC-I-restricted cytotoxic cells (120) whose principal role in anti-mycobacterial immunity appears to be to kill macrophages in granulomas that still contain bacilli (72). Like cytotoxic CD4+ T cells, CD8+ T cells are also able to kill extracellular mycobacteria through the secretion of granular proteins (121). The CD8+ T cells display a peripheral distribution in tuberculoid granulomas and an intermixed distribution in lepromatous granulomas (168), this might suggest that these cells have a bacilli-confining role in TT leprosy and reflect the lack of organised immune response in LL granulomas. Alternatively, CD8+ T cells might be cytotoxic (protective) cells in TT granulomas and suppressor T (anergy-sustaining) cells in LL granulomas.

Gamma/delta receptor T cells

Over 95% of T cells carry the alpha/beta T cell receptor (TCRαβ), and the remaining 3%-5% carry the gamma/delta T cell receptor (TCRγδ). Furthermore, Tyô cells expand considerably during the acute phase of tuberculosis as a result of interaction with small phosphorylated mycobacterial antigens (230, 263). Upon activation, Tyô cells release pro-inflammatory cytokines that provide the host with additional protective immunity (52). A protective role for Tyô cells in leprosy is suggested by the fact that TT patients...
Mycobacteria are captured and processed by macrophages and other antigen-presenting cells so that antigen epitopes become exposed on the surface of these cells. These epitopes are presented to major histocompatibility complex (MHC) II-restricted CD4^+ TH1 and TH2 cells, to cytotoxic MHC I-restricted CD8^+ Tc and Td cells, and to non-MHC-restricted NK cells. Interactions between these cells give rise to an increase in anti-mycobacterial humoral, cellular and cytotoxic immunity. While TH1-dependent cellular immunity is responsible for macrophage activation and the killing of intracellular micro-organisms, cellular cytotoxicity (which depends on the activation of CD8^+ Tc, CD4^+ Td cells and NK cells) accounts for the killing of bacilli-containing macrophages, and also for the extermination of extracellular micro-organisms. Some of the micro-organisms escape from damaged macrophages only to be engulfed by younger macrophages, or killed by granulysin, a toxic enzyme produced by cytotoxic cells.

Two other cell populations, γδ cells and CD1^+ Td cells, although a minority within the lymphoid cell population, play an important role in anti-mycobacterial immunity. These cells recognise 'unusual' mycobacterial antigens such as small phosphorylated molecules and lipids, and respond with strong cell-mediated and cytotoxic anti-mycobacterial immunity.

CD1-restricted T cells

CD1-restricted CD4^+ T cells recognise unusual (lipid) mycobacterial antigens presented by antigen-presenting cells (mostly dendritic, CD83^+ cells), in the context of CD1 molecules (15, 16). The CD1 molecules are strongly expressed in dermal TT granulomas, but are only weakly expressed in LL granulomas. However, TT and LL patients harbour comparable levels of circulating CD1-restricted T cell precursors than LL patients (12, 99). Also, stimulation of blood mononuclear cells with M. tuberculosis results in a greater increase in the number of γδ cells in TT (32%) than in LL (9%) patients (2). In addition, early, two-day lepromin reactions in BL patients contain low numbers of these cells (± 4.4%), which increase more than three-fold (to ± 16%) at the time of maximal skin-reactivity (twenty-one days) (75, 76, 169).
precursors (237). The CD4+ CD1-restricted T cell lines-derived from the lesions of leprosy patients are able to recognise mycobacterial antigens and, in response, release IFNγ and granulocyte-macrophage colony-stimulating factor, but no detectable IL-4. *Mycobacterium leprae*-reactive CD4+ CD1-restricted T cells that produce a TH1 cytokine pattern may play an important role in immunity to leprosy (238).

**Dendritic cells**

Dendritic cells are regarded as highly efficient, antigen-presenting cells (237), which are able to induce long-lasting, TH1-dependent anti-mycobacterial immunity. This immunity is achieved through the sustained secretion of IFNγ, a cytokine known to activate macrophages (and NK cells) (62). To date, no conclusive studies exist regarding the quantities or function of these cells in LL or TT leprosy (Fig. 6).

**Natural killer cells**

The role of NK cells in anti-mycobacterial immunity has been deduced from the following observations:

a) NK cells are able to kill heavily parasitised macrophages while simultaneously stimulating the microbicidal activity of those macrophages with smaller parasitic loads (170)

b) cytotoxic activity of NK cells in TT leprosy can be induced by the stimulation of peripheral blood mononuclear cells with mycobacterial antigens (112, 113), perhaps through the TH1-derived secretion of IFNγ and IL-2

c) lepromatous patients have circulating NK cells whose cytolitic activity is stimulated by *M. leprae* in the presence of IL-2 (36).

**Immunology: a summary**

Thus, clinical and experimental evidence indicate that cellular immunity to *M. leprae* is functional and is efficiently expressed in TT leprosy. Tuberculoïd patients respond strongly to intradermally injected lepromin (Mitsuda reaction), but harbour low levels of anti-*M. leprae* antibodies. This suggests that the following is true of TT leprosy:

a) cellular immunity is more efficiently induced than humoral immunity

b) activation of TH1 cells requires lower antigenic doses (a few bacilli) than activation of TH2 cells, as has been demonstrated with attenuated *M. bovis* (BCG) in mice (199)

c) activated TH1 cells inhibit activation of TH2 cells.

In contrast, for LL leprosy, specific cell-mediated immunity is completely absent, while humoral immunity is highly elevated. The reason for this cellular anergy in LL leprosy is not clear. However, several possibilities may be envisaged, as follows:

a) preferential activation of suppressor T cells

b) preferential activation of TH2 cells over TH1 cells

c) *M. leprae*-induced aberrations in the CD4+ T cell population that render these cells unable to produce IL-2 or IFNγ

d) sequential activation first of TH1 cells and then of TH2.

The activity described in d) is predominant once the disease has definitively become established.

Supposing the existence of a single strain of *M. leprae* (not proved because of the inability to grow this micro-organism in vitro), the outcome of the disease as TT or LL leprosy would probably depend on the participation of host-specific genetic factors.

**Susceptibility/resistance: genetic factors**

Genetic factors linked to the major histocompatibility complex (*human leucocyte antigen*)

Attempts to identify genetic factors responsible for susceptibility to leprosy have been underway for many years. Some authors have suggested the existence of a HLA-linked genetic influence on susceptibility to leprosy. For instance, Todd et al. (261) discovered that HLA-DR2 and HLA-DQw1 antigens were equally associated with both the LL and the TT forms of leprosy (relative risks: 2.65 for DR2 and 2.73 for DQw1). Others have found no direct link between HLA genes and susceptibility to leprosy per se, although significant associations have been reported between HLA-DR3 and TT leprosy, and HLA-DQw1 and LL leprosy (57). A marked association of DR2 with TT leprosy was reported by Dessoukey et al. in a study with leprosy-affected siblings (56). An excess of DR2/DR2 homozygous individuals was found among TT siblings and the authors concluded that although susceptibility to leprosy per se may be due to non-HLA linked genes, DR2-homozygous individuals may be at a relatively high risk of developing leprosy or TT leprosy.

A positive association between DR2 specificity and TT leprosy (frequency: 56.3%; relative risk: 4.2), but not the LL, borderline or IL forms, was also found in a population in south Brazil by Visentainer et al. in 1997 (269). In a study in Indonesia, while susceptibility to multibacillary (LL/BL) leprosy was found to be associated with HLA-DRB1*02 (attributable risk: 41.5%), resistance to the disease was associated with HLA-DRB1*12 (246). In a similar study with leprosy patients from north India, a strong association was observed between the DRB1*1501 allele (a subset of the serologically defined DR2) and LL leprosy (203). The much stronger association of DRB1*1501 with LL rather than TT leprosy suggests a possible role of this allele in the differential immune response to *M. leprae*. Thus, DR2 alleles or subtypes appear to be closely linked to susceptibility to leprosy in general, and to TT leprosy in particular. In association with HLA-DQ1 antigens, HLA-DR2 antigens may play a critical role in the entrance of mycobacteria into macrophages and/or in macrophage-dependent peptide presentation to TH1 cells.
Genetic factors not linked to the major histocompatibility complex

In the mouse, resistance or susceptibility to infection with mycobacteria seems to be controlled by the natural resistance associated macrophage protein 1 (NRAMP1) gene. This gene codes for an endosomal/lysosomal protein of the macrophage, which is rapidly incorporated into the phagosome membrane upon phagocytosis (88). The function of this protein has been related to the acidification of the phagosome (93), to metal ion transportation (288), and to the transport of nitrogen (and nitric oxide) into the phagosomal milieu (30, 267). Although recent genetic studies have suggested that allelic variants at the human NRAMP1 locus are associated with susceptibility to leprosy (1), other studies have not supported this theory (215, 224). Susceptibility to leprosy is likely to be determined by several polymorphic genes that remain undefined at present.

Vaccines

Many attempts have been made to produce a vaccine against leprosy. The first reports refer to the use of M. bovis BCG, a vaccine known to be effective in at least some trials. ‘Unfortunately, it has been found to be less effective in just those areas of the world where a vaccine is most needed’ (248). Although molecular biology offers the prospect of alternatives, no genetically engineered vaccine for field application has yet been produced, therefore the use of BCG is currently the only option (248). Attempts to improve the efficiency of BCG against leprosy include mixing with heat-killed M. leprae (HKML), or administration in combination with MDT. Apart from BCG, other putative vaccines against leprosy are Mycobacterium vaccae, M. vaccae heat-killed M. (HKML), or administration in intact vaccines are discussed below.

Vaccines based on bacillus Calmette-Guérin

The first vaccine used to protect against leprosy was BCG. The BCG vaccine was used in five historic trials, with very variable results (189). In the Uganda trial (started in 1960), 16,150 leprosy-contact children received the vaccine and 80% protection was reported eight years after vaccination. In the trial in Papua New Guinea (initiated in 1962), over 5,000 people were vaccinated, and protection of nearly 46% was reported after twelve years of follow-up. In the trial started in Burma in 1964, 28,220 children under fourteen years of age were vaccinated, and a minimal protection of 20% was reported after twelve years of follow-up. In the trial in Malawi (trial started in 1974), 79,801 children under fifteen years of age received the vaccine and 57% protection was reported after twelve years of follow-up.

A recent study performed in Nagpur, India, indicates that protection against leprosy by BCG is more effective during the first decade of life, among females, and in the lower socio-economic strata (287).
Bacillus Calmette-Guérin combined with heat-killed Mycobacterium leprae

Convit et al. reported a study that commenced in 1983, in which 29,113 contacts were randomly allocated vaccination with BCG alone or BCG combined with HKML (45). The BCG alone conferred substantial protection against leprosy (vaccine efficacy: 56%) and several doses of BCG possibly offered additional protection. However, in the first five years of follow-up of this trial, no evidence was obtained that BCG with HKML offered substantially better protection than BCG alone. Similar results were obtained in 1992 by Gupta et al. who conducted a study of 997 individuals randomly allocated vaccination with one dose of BCG or BCG with variable doses of HKML (91). No significant differences in the lepromin results were observed within the different groups tested.

In another study, a second vaccination with BCG appreciably increased the rate of protection against leprosy conferred by a single dose of BCG (119). Addition of HKML did not improve the protection afforded by a primary BCG vaccination and the rate ratio for BCG with killed HKML versus BCG alone, among scar-negative individuals, was 1.06 for all ages.

Bacillus Calmette-Guérin combined with multibacillus treatment

Majumder et al. administered MDT for two years combined with one to six doses of a mixed vaccine containing HKML and BCG, to thirty far-advanced, lepromin-negative, LL patients (151). Twenty similar patients were treated with MDT and BCG, and twenty control patients were only given MDT. The overall results indicated that within two years, all patients receiving MDT plus the vaccines, and nineteen of the twenty patients receiving only MDT, became clinically inactive and bacteriologically negative. Although the results were essentially similar in both groups of patients, those patients receiving the mixed vaccine therapy showed a more rapid clinical cure and clearance of bacilli than the patients in the other groups. In addition, conversion to lepromin positive responses was observed in 63% of the patients in the group given MDT, HKML and BCG, in 15% of the group given MDT and BCG and in 5% of the MDT group. In another study, Chaudhury et al. (32) randomly allocated four groups of lepromin-negative BL patients to treatment with the following:

a) MDT, BCG and HKML
b) MDT and BCG
c) MDT and HKML
d) MDT alone.

Follow-up of patients was undertaken every three months for two years. All patients within the four groups showed clinical cure and were bacteriologically negative within two years. However, immunological potentiation, assessed by lepromin testing and the leucocyte migration inhibition test, was better in patients receiving treatment a) compared to patients from the other groups.

Vaccines based on the Indian Cancer Research Centre bacillus

The ICRC bacillus is a micro-organism grown in vitro, originally isolated from a case of LL leprosy, and presently classified within the M. avium-intracellulare complex. This bacillus shares with M. leprae epitopes able to elicit cross-reactive cell-mediated immunity. The first attempt to protect against leprosy using the ICRC bacillus was performed by Deo et al. in 1990 (55). Seventy-one LL patients, eleven BL patients, twelve leprosy contacts and twenty healthy non-leprosy contacts, all negative to lepromin, were inoculated with 10⁹, 10⁸, 10⁷ and 10⁶ killed ICRC bacilli, respectively. After ten months, a positive lepromin skin reaction was observed in 38% of LL patients, 91% of BT patients, 92% of leprosy contacts and 45% of non-contact individuals.

In another study which commenced in 1991 (92), 171,400 healthy people from a leprosy-endemic region were randomly allocated into five groups that were vaccinated with one of the following:

a) BCG
d) ICRC vaccine
c) Mycobacterium w
b) BCG with HKML
e) saline solution.

c) Mycobacterium w

A survey in 1998 indicated protection rates of 64% (BCG with HKML group), 65.5% (ICRC group), 23.7% (Mycobacterium w group) and 34.1% (BCG group). The ICRC vaccine and the combination of BCG and HKML fulfilled the requirement for public health use and deserve further consideration. In a more recent study, Bhatki and Chulawala treated eight highly bacilliferous (bacterial index [BI] >4+) LL patients with a single dose of ICRC vaccine at the start of MDT (18). Eight similar patients received MDT alone. After two years of follow-up, an average reduction of BI was reported, from 4.4+ to 1+ in the ICRC-vaccinated group and from 4.7+ to 2.6+ in the group given MDT alone, a finding consistent with the expected response to MDT.

Vaccines based on Mycobacterium w

Mycobacterium w is a non-pathogenic, rapidly growing mycobacterium that shares a number of antigenic determinants with M. leprae and M. tuberculosis, and for this reason has been used as an alternative vaccine for these diseases. In one representative study, autoclaved Mycobacterium w and MDT were administered to fifty-four multibacillary, lepromin-negative patients (258). Thirty-seven similar patients received MDT and placebo injections. Vaccination was repeated every three months, and the effects were assessed after one year of follow-up. Bacterial clearance and clinical improvement were observed to be more rapid in the vaccinated patients. None of the multibacillary patients from the placebo group became bacteriologically negative during the study. After four doses of the vaccine, 100% of BB, 85.7% of BL and 61.5% of LL patients became...
lepromin-positive. In a similar study, Kar et al. administered the *Mycobacterium* w vaccine to sixty-eight lepromin-negative multibacillary leprosy household contacts and registered the rate of conversion to lepromin positive responses (118). A conversion rate of 98.5% (67/68) was reported, and conversion was determined to be permanent. Similar results were reported in 1993 by Zaheer et al. who applied the vaccine to a group of BL and LL patients treated with MDT (286). Patients receiving the vaccine underwent rapid clinical improvement with an upgrading in the disease spectrum and accelerated bacteriological clearance of the granuloma. Of the BL and LL patients who received both the vaccine and MDT, 42% were bacteriologically negative after two years of treatment (versus 20% in the unvaccinated group). Lepromin conversions were observed in 80% of the patients in the vaccinated group and in only 14.3% of the unvaccinated patients.

Thus, as an adjunct to MDT, the vaccine expedites bacterial clearance and accelerates clinical regression of lesions. Vaccination significantly shortens the treatment period required, is effective in inducing a fall in the bacterial index in multibacillary patients and promotes the conversion of over 60% of LL, 71% of BL and 100% of BB patients from a lepromin negative to a lepromin positive status. A significant number of vaccinated patients show histopathological upgrading and eventually the attainment of a state of non-specific infiltration without dermal granulomas (257). Similar properties have been recognised for *M. vaccae* by Stanford et al. (249), and intradermal injection of heat-killed *M. vaccae* has been found to promote cell-mediated immunity to antigens common to all mycobacteria, including *M. leprae* and *M. tuberculosis*.

**Experimental transmission to humans**

In 1844, deliberate attempts to infect humans with leprosy were first recorded by Danielssen. However, these attempts were unsuccessful, as were others performed at that time (207). Danielssen repeatedly attempted to transmit leprosy by inoculating himself and nine volunteers with leprous material, without success. In 1868, Profita and Cagnina inoculated themselves and eight volunteers with leprous material without causing leprosy (240). In 1879, Hansen inoculated material taken from a lepromatous nodule into the eye of a thirty-three-year-old female patient suffering from a mild form of leprosy, without her consent. Although LL leprosy did not result, the experiment was impugned and the episode ended in a court of law, with the dismissal of Hansen from his post as a physician at the Leprosy Hospital, Bergen, Norway. However, because of the paramount importance of leprosy in Norway, Hansen retained his position as Chief Medical Officer for Leprosy (23). In Hawaii, in 1884, Aming inoculated a convicted murderer (with consent) with a freshly excised leproma; twenty-five months later, the subject showed disseminated nodular leprosy. In 1916, Mouritz reported attempts to infect fifteen consenting leprosarium assistants with leprous material without producing leprosy (207). Effective accidental infection by finger prick was reported by Langen in 1933 (137) and also by Marchoux in 1934 (155), while Porritt and Olsen in 1947 reported two men who developed leprosy in America, three years after being tattooed in the Philippines (197). Deliberate self-inoculation leading to leprosy infection was also reported by Lagoudaky in 1937 (133).

**Experimental transmission to animals**

The experimental transmission of leprosy has been researched in over fifty animal species. The following is a selected account of the many attempts so far performed.

**Rabbits**

Rabbits appear to be the first species sought as a possible animal model for leprosy, and the anterior eye chamber is the most utilised inoculation site, perhaps because this route of inoculation had proved successful for transmission of tuberculosis (Table I).

**Monkeys**

Attempts to experimentally infect several species of monkeys with leprosy were first attempted at the same time as those in rabbits (Table II).

**Rodents**

The first report on transmission of leprosy to mice appears to be that of Sugai in 1909 (111). Many other reports followed, including those by Shepard, who introduced the footpad model (233, 234), Rees et al. with thymectomised and irradiated mice (206, 209), and Colston and Hilson (43) with congenitally athymic nude mice (Table III).

**Armadillos**

The nine-banded armadillo (*Dasypus novemcinctus*), one of several species of armadillo, was first sought as a model for the study of leprosy by Storrs in 1971 (253). The species is an inhabitant of the Americas and is found throughout South America and Central America and up to the south-east of the United States of America (USA). The rationale for considering armadillos as a potential leprosy model was the low body temperature (32°C-35°C) and long life span, estimated to be fifteen years. Production of monozygotic quadruplet breeds was an additional bonus of this animal, as this offered the possibility of conducting genetic studies on the disease. This and other attempts to transmit leprosy to armadillos are summarised in Table IV.

**Other species**

In addition to rabbits, monkeys, rodents and armadillos, many other animal species have been used as prospective models of leprosy. Some of these are listed in Table V.

**Experimental transmission: a summary**

Experimental transmission of leprosy has been reported in most of the species tested, although birds, reptiles and amphibians have been clear exceptions. However, the definition of success has varied widely and whereas most
### Table I
Experimental leprosy in rabbits

<table>
<thead>
<tr>
<th>Author</th>
<th>Inoculum (route)</th>
<th>Number (infected/inoculated)</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neisser, 1881</td>
<td>Leprous nodules (NG)</td>
<td>232/24</td>
<td>Local response around the inoculation sites</td>
<td>111</td>
</tr>
<tr>
<td>Damsch, 1939</td>
<td>Leprous tissue (io)</td>
<td>2/2</td>
<td>Slight multiplication of AFB after 139-219 days</td>
<td>111</td>
</tr>
<tr>
<td>Aning, 1885</td>
<td>Leprous material (NG)</td>
<td>2/8</td>
<td>Did not infect</td>
<td>111</td>
</tr>
<tr>
<td>Melcher, 1885</td>
<td>Leprous material (io)</td>
<td>2/24</td>
<td>Dissemination of AFB to internal organs</td>
<td>111</td>
</tr>
<tr>
<td>Wessner, 1907</td>
<td>Leprous material (io)</td>
<td>2/8</td>
<td>Dissemination of AFB to internal organs</td>
<td>111</td>
</tr>
<tr>
<td>Vossius, 1889</td>
<td>Leprous tissue (io)</td>
<td>2/4</td>
<td>Confirmed results of Damsch</td>
<td>111</td>
</tr>
<tr>
<td>Wnoukou, 1953</td>
<td>Leprous material (sr)</td>
<td>14/20</td>
<td>Localised 'tuberculosis' lesions</td>
<td>111</td>
</tr>
<tr>
<td>Thrivis, 1905</td>
<td>Leprous material (id)</td>
<td>8/30</td>
<td>Leprous lesions hours later</td>
<td>111</td>
</tr>
<tr>
<td>Bayon, 1913</td>
<td>Leprous material (io)</td>
<td>22/24</td>
<td>Coronal lesions after 5 months</td>
<td>111</td>
</tr>
<tr>
<td>Linoussin, 1924</td>
<td>Nasal leprous mucus (io)</td>
<td>1/1</td>
<td>Disseminated infection after 22 months</td>
<td>111</td>
</tr>
<tr>
<td>Naar, 1928</td>
<td>Leprous tissue (io)</td>
<td>8/30</td>
<td>Localised granulomas at the implantation site</td>
<td>107</td>
</tr>
<tr>
<td>Taninura, 1930</td>
<td>Leprous nodules (ic)</td>
<td>2/2</td>
<td>Localised lesions in the brain</td>
<td>107</td>
</tr>
</tbody>
</table>

* only the first author is cited

AFB: acid-fast bacilli

### Table II
Experimental leprosy in monkeys

<table>
<thead>
<tr>
<th>Author</th>
<th>Inoculum (route)</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kohner, 1982</td>
<td>Leprous material (sc)</td>
<td>Lesions in internal organs at autopsy (Java monkey)</td>
<td>107</td>
</tr>
<tr>
<td>Nicolle, 1906</td>
<td>Leprous material (sc)</td>
<td>Nodules with AFB at the injection site after 2 months</td>
<td>111</td>
</tr>
<tr>
<td>Babes, 1906</td>
<td>Leprous material (sc)</td>
<td>Nodule at the inoculation site after 1 month</td>
<td>107</td>
</tr>
<tr>
<td>Marchoux, 1906</td>
<td>Leprous tissue (sc)</td>
<td>Nodule at the implantation site after 3 months</td>
<td>111</td>
</tr>
<tr>
<td>Kitasato, 1909</td>
<td>Leprous material (io)</td>
<td>Lesions at the inoculation (cornelia) site (orangutan)</td>
<td>111</td>
</tr>
<tr>
<td>Veratti, 1914</td>
<td>Leprous material (sc)</td>
<td>Nodule at the inoculation site, no further details</td>
<td>111</td>
</tr>
<tr>
<td>Bradley, 1919</td>
<td>Leprous tissue (sc)</td>
<td>Lesions with AFB at the inoculation site after 2 months</td>
<td>111</td>
</tr>
<tr>
<td>Reenstierna, 1928</td>
<td>Leprous extract (sc)</td>
<td>Nodular lesions persistent for 40-62 days (macaques)</td>
<td>111</td>
</tr>
<tr>
<td>Roffo, 1927</td>
<td>Leprous material (sc)</td>
<td>Localised leprosy lesions (African monkeys)</td>
<td>111</td>
</tr>
<tr>
<td>Franchini, 1939</td>
<td>Leprous material (id)</td>
<td>Local nodule and hind limb paralysis after 39 months</td>
<td>111</td>
</tr>
<tr>
<td>Schöbi, 1930</td>
<td>Leprous material (id)</td>
<td>Local lesions that eventually healed</td>
<td>111</td>
</tr>
<tr>
<td>Selliers, 1956</td>
<td>Leprous material (ic)</td>
<td>No evidence of intracranial infection</td>
<td>111</td>
</tr>
<tr>
<td>Cochrane, 1940</td>
<td>Leprous nodule (graft)</td>
<td>No infection in splenectomised monkeys</td>
<td>111</td>
</tr>
<tr>
<td>Dharmendra, 1944</td>
<td>Leprous material (gi)</td>
<td>No signs of infection on autopsy in splenectomised monkeys</td>
<td>111</td>
</tr>
<tr>
<td>Obadionofie, 1939</td>
<td>Leprous material (sc)</td>
<td>Nodules at the injection site of sapotoxin-fed animals</td>
<td>111</td>
</tr>
<tr>
<td>Collier, 1940</td>
<td>Leprous material (sc)</td>
<td>Nodules with AFB and long nerve involvement</td>
<td>42</td>
</tr>
<tr>
<td>Chausinand, 1941</td>
<td>Leprous material (sc)</td>
<td>Progressive resistance to dissemination of leprosy</td>
<td>111</td>
</tr>
<tr>
<td>Sato, 1949</td>
<td>Leprous material (ic)</td>
<td>No evidence of infection (Japanese monkeys)</td>
<td>111</td>
</tr>
<tr>
<td>Lai, 1965</td>
<td>Leprous nodules (sc)</td>
<td>Spread of the disease in 7 of 17 macaque monkeys</td>
<td>134</td>
</tr>
<tr>
<td>Gundes, 1958</td>
<td>Leprous material (im)</td>
<td>Nodules all over the body after 11 months (chimpanzees)</td>
<td>89</td>
</tr>
<tr>
<td>McFadzen, 1961</td>
<td>Leprous material (ac)</td>
<td>Localised nodules in the skin of IX-irradiated macaques</td>
<td>149</td>
</tr>
<tr>
<td>Sengupta, 1962</td>
<td>Leprous material (ac)</td>
<td>A local lesion on the forehead, free of AFB</td>
<td>232</td>
</tr>
<tr>
<td>Turanov, 1973</td>
<td>M. leprae bacilli (cc)</td>
<td>Tuberculosis lesion after 10 years (chimpanzees)</td>
<td>264</td>
</tr>
<tr>
<td>Waters, 1978</td>
<td>M. leprae bacilli (im)</td>
<td>Disseminated lepromatous leprosy after 15 years (gibbon)</td>
<td>276</td>
</tr>
<tr>
<td>Wolf, 1983</td>
<td>M. leprae bacilli (iv)</td>
<td>Successful transmission (hessus and African monkeys)</td>
<td>278</td>
</tr>
<tr>
<td>Martin, 1985</td>
<td>M. leprae bacilli (iv)</td>
<td>Successful systemic transmission (mangabeys)</td>
<td>158</td>
</tr>
<tr>
<td>Baskin, 1991</td>
<td>M. leprae bacilli (iv)</td>
<td>Borderline lepromatous leprosy and intraneural ENL (mangabeys)</td>
<td>14</td>
</tr>
<tr>
<td>Cho, 1993</td>
<td>M. leprae bacilli (im)</td>
<td>Clinical leprosy and nerve involvement after 36 months</td>
<td>37</td>
</tr>
<tr>
<td>Gornus, 1995</td>
<td>M. leprae bacilli (iv)</td>
<td>Neuritic and ENL leprosy after 4-12 years (mangabeys)</td>
<td>66</td>
</tr>
<tr>
<td>Baskin, 1997</td>
<td>M. leprae bacilli (id)</td>
<td>Borderline lepromatous leprosy in 3 African green monkeys after 5 years</td>
<td>13</td>
</tr>
<tr>
<td>Gornus, 1996</td>
<td>M. leprae bacilli (iv)</td>
<td>Indeterminate to lepromatous leprosy after several years (hessus monkeys)</td>
<td>97</td>
</tr>
</tbody>
</table>

* only the first author is cited

AFB: acid-fast bacilli

ENL: erythema nodosum leprosum

graft: implanted nodule

ic: intracranial
### Table III
Experimental leprosy in rodents

<table>
<thead>
<tr>
<th>Author*</th>
<th>Inoculum (route)</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugai, 1939</td>
<td>Leprous material (ip)</td>
<td>Lesions with AFB in peritoneum after several weeks (mice)</td>
<td>111</td>
</tr>
<tr>
<td>Duval, 1911</td>
<td><em>M. leprae</em> (intranasal)</td>
<td>Successful transmission of leprosy (mice)</td>
<td>111</td>
</tr>
<tr>
<td>Bayon, 1913</td>
<td><em>M. leprae</em> bacilli (sc)</td>
<td>Cutaneous nodules at the injection sites (rats)</td>
<td>111</td>
</tr>
<tr>
<td>De Souza, 1931</td>
<td>Leprous material (ip)</td>
<td>Successful transmission (mice, rats and guinea-pigs)</td>
<td>111</td>
</tr>
<tr>
<td>Cantacuzene, 1932</td>
<td>Leprous material (ip)</td>
<td>Successful transmission after 5 months (rats)</td>
<td>111</td>
</tr>
<tr>
<td>Ota, 1932</td>
<td><em>M. leprae</em> cultures (iv)</td>
<td>Visceral and cutaneous lesions (rats, guinea-pigs)</td>
<td>111</td>
</tr>
<tr>
<td>Sellards, 1936</td>
<td>Leprous material (ic)</td>
<td>No intracranial infection 2 years later (mice)</td>
<td>111</td>
</tr>
<tr>
<td>Shiga, 1936</td>
<td>Leprous material (ic)</td>
<td>Successful infection in 1 of 1,000 (mice)</td>
<td>111</td>
</tr>
<tr>
<td>Adler, 1937</td>
<td>Leprous nodules (sc)</td>
<td>Successful disseminated infection (Syrian hamsters)</td>
<td>111</td>
</tr>
<tr>
<td>Burnet, 1938</td>
<td>Leprous nodules (sc)</td>
<td>Successful infection in 1 of 14 (splenectomised hamsters)</td>
<td>111</td>
</tr>
<tr>
<td>Dubois, 1940</td>
<td>Leprous material (ip)</td>
<td>Transmission unsuccessful (hamsters)</td>
<td>111</td>
</tr>
<tr>
<td>Dhamdham, 1940</td>
<td>Leprous nodules (sc)</td>
<td></td>
<td>111</td>
</tr>
<tr>
<td>Nagina, 1939</td>
<td>Leprosy bacilli (sc)</td>
<td>Infection at 2 months (mice treated with human placenta)</td>
<td>111</td>
</tr>
<tr>
<td>Yamamoto, 1939</td>
<td>Leprous material (sc)</td>
<td>Inflammatory changes in lungs, with a few AFB (mice)</td>
<td>111</td>
</tr>
<tr>
<td>De Souza, 1941</td>
<td>Leprous material (sc)</td>
<td>Infection in 2 out of 3 rats after 15 months</td>
<td>111</td>
</tr>
<tr>
<td>Barman, 1945</td>
<td>Leprous material (sc)</td>
<td>Systemic infection 1 year after inoculation (rats)</td>
<td>111</td>
</tr>
<tr>
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* only the first author is cited

# Abbreviations
- AFB: acid-fast bacilli
- ALS: anti-lymphocyte serum
- ifp: intrafootpad
- ic: intracranial
- iv: intravenous
- it: intratesticular
- ip: intraperitoneal
- sc: subcutaneous

*Notes:* All authors are cited only once, except for the first author of each entry. Where available, the reference number is provided at the end of each entry.
**Table IV**

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* only the first author is cited
id : intradermal
iv : intravenous
sc : subcutaneous

**Table V**

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<th>Outcome</th>
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<td>Kober, 1982</td>
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<td>Arning, 1985</td>
<td>Leprous material</td>
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<td>Ota and Sato, 1940</td>
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<td>Nomaka, 1940</td>
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<td>Lobo and Carvalho, 1940</td>
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<td>Klingmuller, 1979</td>
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* several routes used: intramuscular, intraperitoneal, intravenous, subcutaneous
AFB: acid-fast bacilli

researchers considered generalised infection to be proof of transmission, many others simply felt that localised lesions were sufficient evidence (111). Genuine transmission of leprosy has been achieved in five non-human primates (white-handed gibbons [Hylobates lar], rhesus monkeys [Macaca mulatta], African green monkeys [Cercopithecus aethiops], mangabeys [Cercopithecus atys] and chimpanzees [Pan troglodytes]), in three species of armadillos (D. novemcinctus, D. hybridus and D. sabanicola) (Fig. 7), in normal mouse footpads, in congenitally athymic nude mice and rats, in Korean chipmunks (Eutamias sibiricus asiaticus), and in experimentally immunosuppressed mice and rats.

**Naturally acquired disease in wild animals**

Chimpanzees

Donham and Leininger reported a leprosy-like disease in a chimpanzee in 1977 (64). This primate was obtained from Sierra Leone, Africa, for a study on the susceptibility of chimpanzees to bovine leukaemia virus. Two months after inoculation with the virus, lesions suggestive of leprosy appeared on the face and ears of the chimpanzee. Biopsies taken from the lesions contained acid-fast bacilli that could not be cultured in conventional mycobacterial media. However, these bacilli produced a limited infection in mice, similar to that produced by *M. leprae* of human origin. Further studies undertaken over a twenty-one-month period on the same animal determined that the nodular lesions were characterised by diffuse dermal infiltration, foamy pale histiocytes containing acid-fast bacilli, loss of dermal collagen, except for a thin zone just beneath the epidermis, and affection of small dermal nerves, which appeared to be surrounded by histiocytes and dense collagen. Acid-fast bacilli were observed in the nerve and the cytoplasm of infiltrating histiocytes. These clinical, histopathological and bacteriological findings were all compatible with LL leprosy (139). This animal died thirty-three months after the first
been housed together with the first mangabey, in which naturally acquired leprosy was diagnosed in 1979. Clinical symptoms of leprosy appeared in the second mangabey almost seven years after detection of leprosy in the first monkey. The second mangabey may have contracted leprosy from the first monkey, or both animals may have contracted the disease from a common third source, perhaps a patient with active leprosy. This finding suggests a potential zoonosis in wild mangabeys that may serve as a reservoir for the disease in areas where leprosy is endemic.

**Cynomolgus macaques**
A case of naturally acquired leprosy was reported by Valverde et al. in 1998, in a macaque imported from the Philippines (266). The macaque developed a positive skin test to tuberculin after three years of captivity in the California Regional Primate Research Center, USA, and showed cutaneous lesions suggestive of mycobacterial infection. Biopsies of the lesions were taken and subjected to histopathological examination and to molecular diagnoses by the polymerase chain reaction (PCR) specific for *M. leprae*. The clinical features of the disease and the results from the histopathological, PCR and serological studies, were all compatible with BB leprosy.

**Armadillos**
A naturally acquired leprosy-like disease was detected in seven nine-banded armadillos captured from the wild in Southern Louisiana. These animals were found to be afflicted with a disseminated disease involving several organs and nerves. Lesions in the diseased tissues (skin, nerves, lymph nodes, spleens and livers) contained macrophages full of acid-fast bacilli that did not grow on standard mycobacterial media (Löwenstein-Jensen and Middlebrook 7H10) and were suspected to be *M. leprae* (270). After this first report, forty-nine additional armadillos, captured in Louisiana, were reported to have the disease (272). The histopathological and microbiological study of these animals revealed that the lesions were composed of macrophages containing numerous bacilli, similar to those observed in armadillos experimentally inoculated with *M. leprae*. Cultures on mycobacterial media of lymph node and spleen specimens were negative for twenty-four of thirty-two armadillos, but *M. avium* and *M. intracellulare* were cultivated from the remaining eight armadillos. The acid fastness of the bacilli in all tissues was pyridine-extractable and the bacilli were dihydroxophenylalanine (DOPA) oxidase positive. Thus, the histopathological and microbiological studies of these armadillos suggested that the infecting micro-organism was *M. leprae* (21).

Smith et al. confirmed a leprosy-like disease of wild nine-banded armadillos (243). Of twenty armadillos captured in the wild in French Acadia, Louisiana, USA, two had a leprosy-like disease that was indistinguishable from the disease experimentally produced in armadillos by inoculation with *M. leprae* (20, 106). The disease was also similar to that of...
previously described in wild armadillos by Walsh et al. in 1975 (271), and by Meyers et al. in the same year (159).

More recent surveys of naturally occurring leprosy-like disease in wild armadillos have indicated a very significant global incidence of the disease, ranging from 1.0% to 15% in several areas investigated. Recent surveys were performed by Folse and Smith (73), on the Gulf Coast of Texas, on 451 animals (4.66% infected), by Truman et al. (262) in Louisiana with 216 armadillos (2.7% infected), and finally by Job et al. in Louisiana with 494 armadillos (2% infected) (108), and later with thirty-nine armadillos (3%-53.3% infected) (109). This latter study was of particular interest because the several methods used to detect the leprosy infection in armadillos produced very different results; the histopathological study of ears revealed infection in 3% of the animals, the study of granulomas in the inguinal lymph node revealed infection in 6.7%, autopsy of the whole animal revealed a 13.3% infection, and the PCR study of inguinal lymph nodes indicated a rate of infection of 53.3%.

These findings of naturally acquired leprosy in armadillos strongly suggest that the disease in this animal is a true zoonosis. However, although the possibility that naturally infected armadillos could represent a source of infection for humans has been considered, definitive proof of this is still lacking. In a study to determine the association between contact with armadillos and the presence of leprosy in humans, contact between armadillos and people with indigenous leprosy in Louisiana was compared to the contact between matched healthy controls and armadillos. No difference in the nature or frequency of contact was found (69). However, extensive contact with armadillos has been implied by other observers to be the cause of leprosy in several patients in Texas (244).

*Mycobacterium leprae* in sphagnum mosses

While leprosy patients undoubtedly constitute the most common source of contagion and transmission of the disease, naturally infected animals or soil can also harbour the aetiological agent. Acid-fast bacilli containing *M. leprae*-specific phenolic glycolipid-I have been isolated from *Sphagnum* mosses from a former leprosy-endemic region of Norway (124). These acid-fast bacilli multiplied in a limited manner in the footpads of normal mice, and multiplied by nearly ten-fold in the footpads of nude mice at sixteen weeks. The limited multiplication of the sphagnum-derived *M. leprae*-like mycobacteria, in both normal and nude mice, could be due to low pathogenicity of the micro-organism compared to the higher pathogenicity of the *M. lepraemurium*, the causative agent of murine leprosy (147,216). Infection has also been experimentally established in armadillos, mangabeys, rhesus monkeys, African green monkeys and white-handed gibbons, following intravenous and intradermal inoculation of *M. leprae*. The experimental disease, in each case, strongly resembles leprosy in humans clinically, histopathologically and immunologically. Therefore, several non-human primates may serve as a zoonotic source of *M. leprae* (158,162,163, 273).

Murine leprosy —  
*Mycobacterium lepraemurium*

Since the 1940s when murine leprosy was abandoned as a model for human leprosy, few further studies of this leprosy-like disease have been performed. Hence most of the current knowledge of the disease is derived from studies performed between 1903 and 1940. However, due to the fact that the mouse is the most popular laboratory animal, basic research on murine leprosy has remained a very productive activity. Murine leprosy is still being used as a study model to understand those cellular, molecular and genetic factors that render a host susceptible or resistant to infection by intracellular mycobacteria similar to *M. leprae* (147, 216).

**History**

Murine leprosy was first described in 1902 by V.K. Stefansky (250), who found the disease in rats while working on a rabies eradication campaign in the city of Odessa in the Ukraine. The following year, the disease was reported in England by Dean (50) and within a few years, cases were reported from other parts of the world (153). The first descriptions of the disease by Stefansky (251) and Dean (50, 51) recognised a striking similarity between murine and human leprosy, both in terms of the causative organisms and in terms of the clinical and histopathological manifestations of the disease. These findings led to the belief that human and murine leprosy were identical or very closely related diseases (154, 156, 270), and for several years, this was the major motivation for the study of murine leprosy. As a result, considerable attention was afforded to murine leprosy, both in clinical journals and public health reports (46, 47, 153). The similarity between the two mycobacterioses was supported by the following:

a) studies demonstrating serological cross-reactivity between *M. leprae* and *M. lepraemurium*, the causative agent of murine leprosy (51,229)

b) reports that human leprosy had been transferred from humans to mice (111)

c) reports on murine leprosy in humans (154)

d) microbiological reports claiming that *M. leprae* and *M. lepraemurium* were identical or almost identical (270).

However, as a result of improved knowledge of both the disease and the micro-organism, the idea that murine leprosy was a reservoir for human leprosy was gradually abandoned.
Murine leprosy as a model for the study of human leprosy

Despite the recognition that murine leprosy and human leprosy were not the same disease, the need to create a model for human leprosy persisted (210). Experimental infection with *M. leprae* in the mouse footpad was first described in 1960 (233), but until then the only laboratory model for leprosy was murine leprosy. In contrast to *M. leprae*, *M. lepraemurium* has the clear advantage of being adequate for experimental use in the mouse as it is a natural pathogen in this species (129, 135). In addition, Stefansky and Dean had already described polar forms of murine leprosy, with a broadly continuous spectrum in between the two poles (51, 251). The observation that experimental murine leprosy in inbred strains of mice displayed the spectral pattern reported in human leprosy greatly strengthened the value of the murine leprosy model (39, 122, 123, 211).

Murine leprosy and human leprosy are both naturally occurring chronic granulomatous diseases of mammals caused by acid-fast bacteria. The macroscopic and microscopic similarities between murine and human leprosy are striking and both illnesses are 'spectrum diseases'. Both *M. leprae* and *M. lepraemurium* were until recently regarded as non-cultivable and only since 1970 has *M. lepraemurium* been grown *in vitro* (191). Both mycobacteria multiply very slowly in the host, with a doubling time of one to three weeks (26, 233). Both are found predominantly in an intracellular location and appear to be essentially non-toxic to host cells (4, 51, 70, 153). The two mycobacteria display structural similarities (51, 77) and show immunological cross-reactivity, as detected with antibodies (131) and in skin tests (186). In both human and murine leprosy, a specific depression of cell-mediated immunity appears to occur, but no depression of humoral immunity (10, 40, 180, 216).

However, several differences exist between human and murine leprosy. *Mycobacterium leprae* and *M. lepraemurium* are clearly different species of bacteria (198), and chemical, immunological and molecular DNA studies indicate that *M. leprae* and *M. lepraemurium* are as closely related to other mycobacteria as they are to each other (5, 132). *Mycobacterium leprae* and *M. lepraemurium* are intracellular parasites of macrophages, but again show important differences. Both micro-organisms are captured and initially enclosed within phagocytic vesicles, but *M. leprae* disrupts the phagolysosomal membrane and escapes into the cytoplasm where the bacterium proliferates safely (171). In contrast, *M. lepraemurium* resides within the phagolysosome (98) and seems to require a high concentration of lysosomal enzymes to multiply (4, 25). Within macrophages, both *M. leprae* and *M. lepraemurium* appear to be surrounded by electron transparent zone material (ETZ), which in *M. leprae* is composed of spherical droplets of lipid material that is always liquid at body temperature. In *M. lepraemurium*, however, the ETZ is composed of ribbon-like or membranous structures that are solid or crystalline at the body temperature of mice (187).

Unlike *M. leprae*, *M. lepraemurium* does not show great affinity for peripheral nerves, although both human and murine leprosy are granulomatous diseases affecting the skin (220, 259) (Fig. 8). Nevertheless, depending on the host strain, a small percentage (1%-3%) of *M. lepraemurium*-infected mice develop bilateral paralysis of the rear limbs as a likely manifestation of nerve involvement (223).

**Fig. 8**
Murine lepromatous leprosy: nodular lesions (lepromas) under the skin of a BALB/c mouse bearing a six-month infection with *Mycobacterium lepraemurium* (left). Similar nodular lesions on the arm of a patient with lepromatous leprosy (right)

*Mycobacterium lepraemurium*

Like *M. leprae*, *M. lepraemurium* is a slender rod-like acid-fast bacillus measuring 2 μm-7 μm in length and 0.3 μm-0.4 μm in width (Fig. 9). For many years, growth of *M. lepraemurium* *in vitro* was not successful, and therefore this micro-organism was traditionally propagated by animal passages after isolation from infected wild rats or mice (a technique that is still in use). Some differences between strains of *M. lepraemurium* have been reported, both with regard to general virulence and extent of skin involvement in infected animals (6), but no antigen differences have been observed (247).
Transmission

Under natural circumstances, transmission of murine leprosy is very likely to occur through abrasions in the skin and through the mucosal respiratory surfaces, as in human leprosy (110, 183, 276, 279). Infection through the digestive surfaces is another possibility, as cannibalism is common among rats and mice. The thick and complex lipid coat of *M. lepraemurium* is very likely to resist the digestive effect of gastric juice.

Natural and experimental disease

Few differences have been reported between natural and experimental infection with *M. lepraemurium*. Skin involvement appears to be more common in natural rather than experimental disease, and greater involvement of the viscera appears to occur in experimental, compared to natural disease (130). However, these differences could be due to the larger inocula used in experimental infection.

The natural mode of transmission of murine leprosy has not been definitely established. However, the disease appears to start in the skin, mainly in those regions most exposed to scratches and bites (145). The disease may also be transmitted by the respiratory route, just as in humans; the successful transmission of leprosy through nasal mucosa has been reported in the nude mouse. Attempts to transmit the disease experimentally by insects have been unsuccessful (145). Infection through the digestive surfaces is another possibility, as cannibalism is common among rats and mice. The thick and complex lipid coat of *M. lepraemurium* is very likely to resist the digestive effect of gastric juice.

Infections usually undergo a stage in which the primary defence barriers are broken, and the initial immune reactions will occur at a peripheral site. Experimental infection with small doses of bacteria administered subcutaneously would therefore be expected to closely mimic natural infection. A difference between strains of *M. lepraemurium* with regard to skin involvement was reported by Badger and Fite in 1940 (6), but no further data have been reported.

The species of experimental animal may influence development of the disease. Murine leprosy affects approximately 5% of mice, and about the same percentage of rats (156). Spontaneous musculocutaneous murine leprosy has been described in a wild mouse specimen (129). Using bacteria from this mouse, experimental disease in mice and rats presented only minor differences between the species; some experimentally inoculated mice developed disseminated skin lesions resembling those in spontaneous disease (130). Certain strains of rat are relatively resistant to visceral infection, and develop leprosy that is more like human leprosy, with skin involvement, whereas other strains have a low tendency to affect the skin (94). Nude mice (92) and hairless mice (194) develop extensive skin involvement after experimental *M. lepraemurium* inoculation, which suggests that the immune system and possibly other genetic factors are important in determining whether involvement of the skin develops. Hairless euthymic (194) and hypothyricic (222) mice are also susceptible to infection with *M. lepraemurium*. Intradermal or intraperitoneal inoculation of these animals with suspensions of *M. lepraemurium* produced a disseminated disease, similar to that observed in control hairy mice.

**Murine leprosy in inbred mouse strains**

Since the 1950s, inbred strains of mice have dominated work on experimental murine leprosy. Comparative observations on the development of murine leprosy in various inbred strains of mice (C57Bl, BALB/c and C3H) demonstrated that experimental mouse leprosy can be classified as one of three clinical types, depending on the strain, namely: benign (C57Bl), intermediate (BALB/c) and malignant (C3H) (122).

The highly inbred homozygous state is excellent for the identification of isolated genetic factors influencing resistance or susceptibility to infection and for obtaining reproducible *in vivo* experimental results (146). However, as emphasised by Rees and Weddell (210), this is an artificial state and the use of outbred or even-wild mice may be required for the final test of the validity of experimental findings.

**Immunology**

Mouse inbred strains are not equally susceptible to infection by *M. lepraemurium*; CH3 mice are more susceptible than BALB/c mice, and the latter are more susceptible than C57BL mice (38). Murine leprosy infection in susceptible mice induces a progressive loss of non-specific immune responses, both cell-mediated (skin response to oxazolone or picryl...
chloride) and humoral (antibodies to sheep erythrocytes) (29, 201, 217). Differences in the M. lepraemurium-specific cell-mediated immune response of susceptible and resistant strains of mice have also been observed. Early in the infection (two to four weeks), both susceptible and resistant mice develop delayed type hypersensitivity to M. lepraemurium antigens that correlates with the capacity of the splenocytes to proliferate in vitro in response to the microbial antigens (24). Differences between susceptible and resistant strains become evident at four weeks after infection. Susceptible animals start to show progressive loss of the capacity to produce IL-2 or proliferate in response to M. lepraemurium, whereas resistant animals continue these activities (101). In agreement with these data, lymphoid cells from M. lepraemurium-infected C57BL/6 (resistant) mice secrete more IFNγ than cells from CBA (susceptible) animals (213). In addition, progression of murine leprosy in resistant mice is accompanied by an increment in the number and activity of NK cells. The NK cells, particularly the CD4+NK1.1 T cells, produce high levels of IFNγ and are able to kill mycobacterium-loaded macrophages (53). By monitoring the CD4+ and CD8+ spleen cell populations in susceptible BALB/c mice infected with M. lepraemurium, a progressive increase in the CD8+ cell population, with no modification in the size of the CD4+ population, has been observed (O. Rojas-Espinosa, unpublished data). As infection leads to death in BALB/c mice, a CD8+ class-2 T cell population is probably the one that increases in the progressive disease. The CD8+ class-2 cells produce type 2 cytokines that exert anti-inflammatory (‘suppressive’) functions. Another study in favour of a central role for cell-mediated immunity in the control of (murine) leprosy, was reported by Rojas-Espinosa et al. (219); mice of a strain of intermediate susceptibility to leprosy (NIH strain) subjected to infection with M. lepraemurium, develop lesions in which macrophage activation ensues in the early stages of the disease. Activated macrophages exhibit a high degree of lysosomal activity, produce high levels of nitric oxide, and show strong mycobactericidal activity. However, this protective, activated state of the macrophages is not sustained, but vanishes as the infection continues, and completely disappears in the more advanced stages of the infection. Proliferation of bacilli is restrained when macrophages are activated, but proliferation occurs once these cells are deactivated. Links between macrophage activation and TH1 cell activity, and macrophage deactivation and TH2 cell activity have been proposed (216) and on this basis, a study of the role of TH1 and TH2 cells in murine leprosy is currently underway.

Activation of NK cells in murine leprosy has also been reported. Progressing murine leprosy in C57BL/6 (resistant) mice was associated with a sharp increase in splenic NK cell activity, which was abrogated by treatment with a monoclonal antibody against NK cells. Administration of this antibody enhanced C57BL/6 susceptibility to murine leprosy as observed by a decrease in survival time of mice infected with 10^7 M. lepraemurium (81 days versus 110 days) (53). In contrast, splenic NK cells were not detected by cytofluorometry in genetically susceptible BALB/c mice infected intravenously or intraperitoneally with 5 x 10^7 M. lepraemurium (O. Rojas-Espinosa, K. Wek-Rodriguez, L. Dominguez-Lopez, P. Arce-Paredes and J.A. Vargas-Hernandez, unpublished data). These results suggest that NK cells play an important role in resistance to murine leprosy, either by reducing M. lepraemurium growth in macrophages or by lysing heavily infected macrophages. Macrophage activation may also occur in the absence of T cells. Mice with the severe combined immunodeficiency mutation (SCID-mice) respond to infection by the production of high levels of NK cells, IFNγ secretion and macrophage activation (9, 11).

Immunotherapy in murine leprosy has been a neglected subject, although infusion of rIL-1 in BALB/c mice infected with M. lepraemurium leads to a reduction in the number of bacilli in liver and lymph nodes of the infused animals (54).

**Feline and canine leprosy**

**Feline leprosy**

Although granulomatous skin lesions in cats are not rare, the first report of cat leprosy appears to have been recorded in 1962 in New Zealand (27). Since then, cat leprosy has been described in several areas of the world (3, 138, 196, 212, 227, 228, 260, 277).

All authors described non-encapsulated, granulomatous lesions involving the skin, often with ulceration. In some cases, subepidermis and peripheral lymph nodes were also affected. Sections stained by the Ziehl-Neelsen method revealed large numbers of acid-fast bacilli within macrophages, and attempts to grow the organisms in conventional media for mycobacteria have so far failed. Cat leprosy bacilli passaged in mice have been isolated on 1% Ogawa yolk medium (172). The isolated bacilli, which were successively cultivated four times on Ogawa medium, produced lepromas in mice. Successful transmission of cat leprosy to laboratory animals has also been reported by Lawrence and Wickham (138), Poelma and Leiker (196), and Schiefer and Middleton (228). The characteristics of the isolated cat leprosy bacillus were identical to those of M. lepraemurium. The bacilli were slow growing, gave negative test results with heat-resistant catalase, heat-resistant phosphatase, arylsulphatase, niacin, hydrolysis of Tween 80, and urease, and produced light yellowish-white, rough colonies which were rich in coproporphyrin. Thus, on these grounds, cat leprosy bacilli appear to be identical to murine leprosy bacilli.

The similarity of the cat leprosy bacillus to M. lepraemurium was more recently demonstrated by Hughes et al. (102), using molecular analyses. These authors obtained PCR-amplified 16S ribosomal (r)RNA gene sequences from tissue specimens...
obtained from eight cats with suspected feline leprosy. Acid-fast bacilli were observed in all eight specimens, but culture of mycobacteria was positive in only one case, (identified as M. avium and M. chitae). The analysis of the V2 variable region of each 16S rRNA PCR product identified a sequence with 100% nucleotide similarity to the sequence of M. lepraemurium in the remaining cases. Molecular analysis therefore provided an accurate and rapid identification of M. lepraemurium as the causative agent of feline leprosy.

Canine leprosy

Although canine leprosy has never been reported, a rare case of indeterminate leprosy developing at the site of a dog bite was reported by Gupta et al. in India (90). As leprosy is endemic in India, this was very likely a case of infection with environmental M. leprae, which was facilitated by the dog bite, rather than a case of dog-transmitted leprosy.

Conclusion

While leprosy in wild nine-banded armadillos, chimpanzees, mangabey monkeys and macaques may constitute a zoonosis (273), murine leprosy appears to be restricted to mice, rats and cats, and is not a zoonosis. Naturally acquired leprosy in other species has not been documented.

Acknowledgements

This paper was written under the auspices of the Consejo Nacional de Ciencia y Tecnología (CONACyT) (Project 26427-M: ‘La lepra murina como modelo de la lepra humana: el papel de las células TH1 y TH2 en la inmunopatología de la enfermedad’) and the Dirección de Graduados e Investigación del Instituto Politécnico Nacional, Mexico.
habituelles aux mycobactéries mais il a pu être cultivé dans un milieu à base de jaune d’œuf. Des cas d’infection naturelle à *M. lepraemurium* ont également été observés chez les chats ; toutefois, la lèpre murine n’affecte ni l’homme, ni aucune autre espèce animale. Ainsi, contrairement à la lèpre humaine, la lèpre murine n’est pas une zoonose.

**Mots-clés**

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**Infecciones debidas a Mycobacterium leprae y a Mycobacterium lepraemurium en animales domésticos y silvestres**

O. Rojas-Espinosa & M. Levik

**Resumen**
*Mycobacterium leprae*, el agente etiológico de la lepra en el hombre, causa una enfermedad granulomatosa crónica que afecta primariamente la piel y los nervios periféricos, y secundariamente algunos órganos internos como los testículos y los ojos; las vísceras raramente son afectadas. Dependiendo de la resistencia del huésped, la lepra puede presentarse como una enfermedad benigna (lepra tuberculoiide) o como una enfermedad maligna (lepra lepromatosa), pero también puede presentar todo un espectro de formas intermedias. La inmunidad antileprosa depende de la inmunidad mediada por células del huésped y ésta se encuentra severamente deteriorada en la lepra lepromatosa. Aunque *M. leprae* todavía se mantiene como un microorganismo no cultivable, puede hacerse crecer en varios animales experimentales, incluyendo el armadillo, primates no humanos, y roedores, en cierto grado. Aparte del hombre, la enfermedad adquirida naturalmente se ha encontrado en armadillos silvestres de nueve bandas (*Dasypus novemcinctus*), en chimpancés (*Pan troglodytes*), en monos mangabey pardos (*Cercocebus atys*) y en macacos (*Macaca fascicularis*). Esto identifica a la lepra como una zoonosis.

La lepra murina es una enfermedad de las ratas y ratones causada por *Mycobacterium lepraemurium*. La enfermedad, crónica y granulomatosa, afecta primariamente las vísceras y la piel de estos roedores, y esporádicamente los nervios periféricos. Dependiendo de la cepa, la lepra murina puede adquirir las características de la lepra tuberculoide o aquellas de la lepra lepromatosa, y es probable que haya cepas de ratones que desarrollen las formas intermedias de la enfermedad. *M. lepraemurium* no crece en los medios convencionales para micobacterias pero se ha logrado cultivar en un medio sólido a base de yema de huevo. La lepra murina adquirida naturalmente se ha observado en ratas, ratones y gatos, pero no en el hombre ni en otras especies. Así, contrariamente a la lepra de los humanos, la lepra murina no es una zoonosis.

**Palabras clave**
References


