EDITORIAL

***Mycobacterium ulcerans* – a mini-review**

In 1948, MacCallum *et al.* described six patients with unusual skin ulcers who lived in rural south-eastern Australia during the 1930s and 1940s [1]. The presence of acid-fast bacilli in the lesions led to an initial diagnosis of tuberculosis, but the organism failed to grow on appropriate media. Growth was eventually obtained on yolk agar after incubation at 30–33°C for several weeks. This was the first description of the third major mycobacterial pathogen of man: *Mycobacterium ulcerans*. In southern Australia, infection with this organism is still called ‘Bairnsdale ulcer’ after the main town in the original endemic region [2]. Elsewhere it is most often called ‘Buruli ulcer’, after a region in Uganda associated with a large number of cases during the late 1960s and early 1970s [3].

Fifty years on, the incidence of infection with *M. ulcerans* seems to be increasing sharply, particularly in West Africa [4–6]. In January 1998, the World Health Organisation launched the global Buruli ulcer initiative to respond to the emerging public health problem posed by the disease [7].

Epidemiology and transmission

Buruli ulcer affects otherwise healthy individuals irrespective of sex, age and race. Most disease occurs in rural equatorial Africa, where there are multiple endemic foci, but infections are also reported from Mexico, Malaysia, Papua New Guinea, Bolivia, French Guyana, Sri Lanka, Australia and several other countries. The global distribution of endemic foci suggests that *M. ulcerans* may have existed since Jurassic times [8]. In Africa, the disease was probably present long before the original description by Australian researchers. However, there appears to have been a marked increase in incidence centred in East Africa during the late 1960s and early 1970s [3], followed by the current epidemic in West Africa [6]. Although accurate incidence figures are not available, there is consensus that the incidence is rising. Field workers recognise regions with a high past prevalence of Buruli ulcer by the numbers of individuals with extensive old scars. Absence of these scars in adults in West Africa suggests that Buruli ulcer has arrived only recently in that region.

In Africa, most cases occur in rural villages. Children bear a disproportionate burden of disease [6], suggest-

ing that adults may be relatively protected by prior exposure to *M. ulcerans* or other mycobacteria. In Australia, where the incidence of infection is low, most cases occur in white adults [2].

M. ulcerans is an environmental mycobacterium that infects people who live in very specific regions. In Australia, these regions can be extremely small (1.5 sq km in one region [2, 9]). Other risk factors include minor skin trauma. It is possible that *M. ulcerans* is inhaled or ingested, is then widely disseminated in the body and reactivates in low temperature areas at sites of trauma, but direct inoculation seems most likely. The incubation period is usually *c.* 13 weeks, but much longer, and occasionally shorter, incubation periods occur. As with tuberculosis and leprosy it is likely that most exposed individuals do not develop disease. There is little evidence that concurrent HIV infection is responsible for the apparent increase of Buruli ulcer in rural West Africa, and HIV plays no role in the epidemiology of infection in Australia.

As most people with ulcers live near swamps, rivers or lakes, the organism may be transmitted by contact with contaminated water, soil or waterside vegetation, or it may be spread by aerosol. Person-to-person spread may occur, but is not thought to be of public health significance. Spread by biting insects is another possibility [10].

No one has yet isolated *M. ulcerans* by culture from any environmental site, and detection in water samples by PCR was first described only in 1997 [11]. The PCR method for environmental detection is based on insertion sequence IS2404, supported by detection of a second unrelated sequence, IS2606 [12]. IS2404 is repeated 50–100 times per *M. ulcerans* genome and appears to be organism-specific; IS2606 is also present in multiple copies. The identification of both sequences in environmental samples makes it almost certain that *M. ulcerans* is present. We have used PCR to demonstrate *M. ulcerans* in water samples and vegetation from two endemic areas in south-eastern Australia. Early findings suggest that the organism is present in only a few specific places, which may act as point sources of infection for human beings living nearby.

The changing epidemiology of Buruli ulcer has been attributed to flooding, population growth, mining, logging of rain forest and damming of rivers, but

direct evidence of a causal association is lacking. There is circumstantial evidence from Australia that nutrient enrichment of naturally occurring ground water may lead to a 'bloom' of *M. ulcerans* [2, 9, 11], but the organism has also been detected in a swamp with low nutrient levels. In Australia, infection can suddenly appear in regions with no previous experience of the disease [13], and this is probably true in Africa [4, 5]. A working hypothesis is that *M. ulcerans* is introduced into new regions by insects, human beings or other animals; once present in a village, hyperendemicity could develop as individuals with discharging lesions contaminate local bodies of water, which then act as a sump from which new human infections arise. Alternatively, the organism may already be widely distributed in the environment in low numbers, but is amplified to significant levels by events such as rain forest clearance or flooding. Available strain typing evidence suggests that there are relatively few clones of *M. ulcerans* [14, 15].

Pathogenesis

Histological examination of excised skin lesions characteristically shows skin ulceration with extensive necrosis of subcutaneous fat. Acid-fast bacilli are present in enormous numbers within the necrotic area and are typically arranged in spherules. These clusters of bacteria extend along thickened septa in the subcutaneous tissue. The granulomatous inflammatory response normally associated with mycobacterial infection may be seen in long-standing lesions, but is strikingly absent in early lesions. Necrosis frequently extends beyond areas where *M. ulcerans* is visible [16]. Many of these pathological features are probably mediated by a secreted toxin that is cytotoxic and immunosuppressive [17, 18]. A cell wall molecule, *M. ulcerans* lipid toxin, arrests cultured eukaryotic cells in G1, and reproduces many of the pathological changes caused by live bacteria when injected into guinea-pig skin [18]. The molecule is active at extremely low concentrations and is not present in spontaneously occurring non-toxic laboratory strains. This strongly supports a causal role for the toxin in the pathogenesis of infection, although it may not be the only virulence determinant.

Clinical features and diagnosis

In Africa, lesions often start as small mobile skin nodules that gradually enlarge over days to weeks. Most Australian patients first notice a small pustule or papule, often attributed to an insect bite, with no nodular stage. Lesions may resolve spontaneously, but most progress. There is usually no pain and no systemic inflammatory response, although patients may complain of itch. The surrounding tissue often becomes markedly indurated. Eventually the nodule or

papule breaks down at the centre and a relentlessly enlarging deeply undermined skin ulcer appears. Less commonly, patients present with an acutely swollen area of skin (typically an entire limb), but without a visible ulcer. Over the next few days to weeks the entire region sloughs, leaving a very extensive area of denuded subcutaneous tissue. A small but increasing proportion of African patients develop osteomyelitis, an ominous complication that usually necessitates amputation.

Death from *M. ulcerans* infection is rare, although overwhelming secondary bacterial infection can complicate areas of extensive ulceration. However, morbidity is very significant. Large skin ulcers frequently require grafting. Facial lesions can lead to major cosmetic deformity or the loss of an eye; on limbs there may be extensive scarring with permanently impaired function.

Diagnosis is not usually difficult for health workers in endemic areas, but in countries with a low incidence, *M. ulcerans* is often not considered and consequently the diagnosis is delayed. The presence of large numbers of acid-fast bacilli in a swab from a necrotic ulcer makes the diagnosis likely, especially in countries with a low incidence of other mycobacterial infection. *M. ulcerans* can be cultured from most cases provided the correct culture media and incubation temperature are used, and plates are kept for up to 12 weeks [1]. Punch biopsies of early lesions that show large numbers of mycobacteria, fat necrosis and minimal inflammatory response are highly specific; a more conventional granulomatous response may be seen in later lesions. Several PCR methods that can be performed on ulcer swabs are available [12, 19, 20].

Treatment and control

Surgery is widely regarded as the definitive treatment. It seems logical to remove necrotic tissue, but there is no consensus on how wide margins of excision should be. Several antituberculosis agents are active against *M. ulcerans in vitro*, but experienced clinicians favour surgery rather than drug therapy in initial management. *M. ulcerans* is usually sensitive to clarithromycin, rifampicin and ethambutol, and we have used these drugs in combination in a small number of cases in which the organism has been seen at the edges of resected lesions, or when disease has recurred after surgery. However, we have seen two recent cases in which disease has progressed despite these medications. Devices that can maintain skin temperature around 40°C, and hyperbaric oxygen may also have a role, but may not be practical in developing countries. Moreover, their efficacy has not been demonstrated conclusively.

There is no known way to prevent infection with *M. ulcerans* other than to avoid endemic areas – not a

practical solution for villagers in West Africa. However, an outbreak of infection in a refugee camp in Uganda halted when people were moved away from the Nile [3], and an outbreak associated with an irrigation system in Australia appeared to cease abruptly when the system was modified [2, 11]. Avoidance of skin trauma and the covering of exposed skin are also sensible preventive measures. Bacille Calmette-Guérin (BCG) is partially protective for a few months [21] and in highly endemic areas BCG vaccination programmes are likely to be cost-effective and relatively easy to implement. Another approach, at least in areas of high incidence, is to focus on early detection and intervention based on identification of *M. ulcerans* infection at the early nodular stage. Nodules can then be removed by simple surgery without grafting. Surgical outreach programmes are being established in several West African countries to try to reduce the financial burden and human misery caused by late diagnosis.

Very little is known about *M. ulcerans*, its transmission and its establishment in new endemic areas. Optimal therapy, including the role of drugs, heat and other non-surgical treatments, needs to be clarified. An effective vaccine is required urgently. As a recognised micro-organism, *M. ulcerans* was 50 years old in 1998; let us hope we will have learned how to control this emerging pathogen by the time we 'celebrate' its centenary.

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