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Review article

Pathways of *Mycobacterium ulcerans* infection: A review

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Abstract

Buruli ulcer (BU), an often-deforming skin ulceration caused by *Mycobacterium ulcerans* (MU), is now considered by the World Health Organization to be the third most common mycobacterium infection. However, the reservoir of MU in the environment and the epidemiology of BU are poorly understood. The disease is prevalent in riverine, slow-flowing and swampy areas of several tropical and subtropical regions of the world, especially in West Africa where the disease is rapidly increasing in incidence. This paper presents a review of published literature concerning epidemiology of, transmission and susceptibility to MU infection. It considers several endemic natural environments and their influence on infection.

Keywords: *Mycobacterium ulcerans*; Toxin; Immunosuppression; Environments; Controls

Contents

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1. Introduction

Buruli ulcer (BU) is a skin disease caused by *Mycobacterium ulcerans* (MU) and is rated as the third most common mycobacterial infection after tuberculosis and leprosy (Josse et al., 1995). It usually begins with a painless nodule or papule in the skin and, without appropriate therapy, causes massive skin ulceration, which often results in grossly deforming sequelae (Connor et al., 1976). The disease has been reported in many countries, in particular the countries of West Africa, Australia, Papua New Guinea, Indonesia, Malaysia, Mexico, Bolivia, French Guiana, Surinam and Peru. In many of these countries, BU is known to afflict impoverished inhabitants living in remote areas where amenities of modern medical science are not available or are expensive (Guédénon et al., 1995). Of the BU-affected inhabitants in certain countries, many are children. For example in Ghana, about 76% of BU patients from Affram valley in the Eastern Region prior to 1989 were younger than 20 years (Van der Werf et al., 1989) and about 70% of BU patients from Ashanti Region prior to 1998 were younger than 15 years (Asiedu and Etuaful, 1998). The epidemiology of the disease is poorly understood, but the increasing incidence of BU in certain parts of the world, particularly in West Africa, led the World Health Organization (WHO) to recognize it as an emerging disease and called for urgent action to control it (WHO, 1998a).

The objective of this review is to present a general overview from published literature of MU, its infection, geographical distribution and environments of BU outbreaks with some emphasis on the possibility of a defect in the individual's immune system that could enhance BU development.

2. Microbiology of MU

MU is a slow-growing organism and is easily cultured on standard mycobacterial media at appropriate temperature. It grows optimally under microaerophilic conditions (Jenkins et al., 1982) at 25 and 28 °C than at higher temperatures (Tsukamura, 1983).

Molecular analysis indicates that MU is almost identical to another mycobacterium, *M. marinum* (Portaels et al., 1996) and the next closest being *M. tuberculosis* (Tonjum et al., 1998) but the diseases each of these mycobacteria causes are different.

3. Clinical presentation

BU is primarily a disease of subcutaneous fat and can be characterized as a non-ulcerative (papules, nodules, plaques and oedematous form) and secondary ulcerative disease. The disease usually begins as a firm, painless nodule, less often as diffuse, sometimes painful oedema. The focus of infection is the subcutaneous tissue (Van der Werf et al., 1999). When the skin ulcerates it shows an extensive zone of necrotic subcutaneous fat (necrotizing panniculitis), which extends laterally, undermining the dermal edges of the ulcer (Hayman and McQueen, 1985). There are some variations in the clinical presentation of BU. For example, in Australia the disease begins as a papule or pimple in the skin with no involvement of the subcutaneous fat (Van der Werf et al., 1999), whereas in Africa the subcutaneous fat is involved in the infection. Sometimes the entire trunk or limb may undergo serious necrosis of massive areas of the skin (Abalos et al., 2000). Some patients, especially in Africa, may develop osteomyelitis (Van der Werf et al., 1999) that may necessitate amputation (Connor et al., 1976).

4. Pathology and immune response

The progression of BU is characterized not only by damage to skin including nerves and blood vessels but also by absence of inflammatory response during the early and acute phases of the disease (Hayman, 1993; Pimsler et al., 1988; Hayman and McQueen, 1985; Connor and Lunn, 1966, 1965; Dodge, 1964). This suggests that the bacteria secrete a necrotizing and immunosuppressive toxin (Hockmeyer et al., 1978). This toxin was later found and named "mycolactone" (George et al., 1999). The main effect of this toxin is to produce fat necrosis, which may extend beyond the site of infection.

By suppressing the production of interleukin-2 (IL-2) and tumour necrosis factor (TNF), T-helper-1 (Th1) (the pro-inflammatory immune response) is down-regulated (Pahlevan et al., 1999); hence, the development of the disease. Development of MU infection therefore has much to do with the state of the individual's immune response (Van der Werf et al., 1999).

Studies by Gooding et al. (2002, 2001) indicate that those developing BU may already have (a) immune deviation resulting in down-regulation of Th1 and increased Th2 (humoral immune response) or (b) have inherent defect in the immune system that leads to failure to develop any strong response to mycobacterial antigens. He further found that the toxin of MU alone could not induce systemic immunosuppression. Johnson et al. (1999) support the fact that the toxin elaborated by MU results in the development of BU, but state that it may not be the only virulence factor in the pathogenesis of infection. Stienstra et al. (2001) suggest that host susceptibility factors, which could be genetic or environmental, need to be explored in order to understand the reason for the development of BU. A study by Barker and Ninkibigaya (1972), in which case control studies showed no differences in activity between BU patients and control, suggests that infection by MU could be due to the extent of an individual's exposure and response to MU infection, thus reinforcing the outcome of studies by Gooding et al. (2002, 2001).

5. Epidemiology

5.1. Geographic distribution

MacCallum et al. (1948) published the first clinical description of MU disease in a young child in Bairnsdale, Australia, in 1940. The authors then called it "Bairnsdale ulcer". Since that first report in Australia the disease still remains in the country with Douglas Shire having the highest incidence of five to six cases a year (Smith, 1997; Jenkin et al., 2002; Gooding et al., 2002). However, most patients now are from the Bellarine peninsular, near Melbourne where there have been more than 30

cases this year (2005). Cases have not been reported from South Australia but rather from south-eastern Australia (in the state of Victoria).

Many cases have since been reported in other countries of the world (Hayman and Asiedu, 2000) with most cases in the tropics, especially in rural Africa and catastrophically in West Africa (Nigeria, Benin, Togo, Ghana, Burkina Faso, Côte d'Ivoire and Liberia) (Monson et al., 1984; Cornet et al., 1992; Amofah et al., 1993; Amofah, 1995; Josse et al., 1995; Marston et al., 1995; Meyers et al., 1996; Aguiar and Stenou, 1997). Even before MacCallum's first publication, the disease was already known in Africa (Meyers, 1995). Several cases were reported from the Congo (Portaels, 1973) but it was in Uganda where the disease was named "Buruli ulcer" by Clancey et al. (1961) after the Buruli County, where there was a large number of cases during the late 1960s and early 1970s (Uganda Buruli Group, 1971; Clancey, 1964; Clancey et al., 1961; Dodge, 1964; Dodge and Lunn, 1962).

Other BU endemic areas of the tropical world include Papua New Guinea (Radford, 1974a,b), Malaysia (Pettit et al., 1966), Northern Sumatra (Shattock, 1965), Mexico (Aguilar et al., 1953), Bolivia (Lindo and Daniels, 1974), Peru (Radford, 1974a), Surinam (Portaels, 1989), French Guiana (Grosshans and Pradinaud, 1979) and Sri Lanka (Seevanayagam and Hayman, 1992). Few cases have been reported in the non-tropical world. Cases are reported from South Australia (Hayman, 1993), Japan (Tsukamura and Mikoshiba, 1982) and China (Faber et al., 2000).

The outbreaks, especially those in developing countries, have increased knowledge of MU infections. However, the precise mode of transmission of BU both in humans and animals remains elusive.

5.2. Transmission hypothesis

The mode of MU infection is described by two hypotheses. The first hypothesis is that trauma to the skin by a contaminated environment (e.g., soil, water, vegetation, insect vector) is one mode of transmission (Portaels et al., 2001). The second hypothesis is that, as MU has been shown to be aerosolized from suspensions of tap water (Hayman, 1991), it could be inhaled or ingested (Johnson et al., 1999; Hayman, 1991; Connor and Lunn, 1965) and then reactivated in low temperature areas of the body at the sites of trauma. This mode of transmission is said to be the cause of an extensive outbreak among residents of Philip Island in Australia (Ross et al., 1997; Stinear et al., 2000). Of the two hypotheses, however, the first seems to be more favoured (Radford, 1974a; Portaels, 1995; Hayman, 1991; Johnson et al., 1999).

Infection by MU occurs commonly in areas related to rivers, swampy terrain or lacustrine systems (Portaels, 1995). There have, however, been reports of endemic areas not associated with relatively large water masses (Christie, 1987). Animals (e.g., koalas) in Australia have been known to be infected (Mitchell et al., 1987) and it is thought that this could be from an environmental source. Observations also indicate that increased incidence of MU infections occur due to anthropogenic activities (Portaels, 1995). New endemic areas are associated with recent disturbances such as flooding (Barker, 1973; Hayman, 1991), mining (Aguiar et al., 1997), logging of rain forest (Hayman, 1991) and damming of rivers (Aujoulat et al., 1996; Van der Werf et al., 1999). However, it seems that the socio-economic environment of endemic communities is also an important factor to be considered (Calder and Jackson, 2000) with regards to MU infection.

6. Environments of BU outbreaks

6.1. Socio-economic environment

Malnutrition impairs several aspects of host defense (e.g., T-lymphocyte function) (Dai et al., 1998); and several aspects of cell-mediated immunity are sensitive to nutritional effects (McMurray et al., 1990; MacMurray, 1984; Suskind, 1977). Calder and Jackson (2000) indicate that undernutrition and infection are the main causes of morbidity and mortality in the developing world. Undernutrition, which includes protein-energy malnutrition and micronutrient deficiencies, helps to defect the host's immune defense mechanisms against pathogens; and once infected the malnourished status of the subject is aggravated. Increased infections may be attributed to specific nutritional deficiencies (Scrimshaw et al., 1968). Unfortunately, protein-energy malnutrition, for example, may consequently cause the deficiency of a micronutrient (i.e., zinc) and result in the susceptibility of the subject to toxin-producing bacteria (Wapnir, 2000). It has been suggested that the synergy of poverty, ignorance, poor hygiene, lack of good water supplies, poor housing, lack of modern health facilities, cultural practices and certain social setups create a poor nutritional environment as well as enhance exposure to pathogenic assaults (Calder and Jackson, 2000). Such an environment especially is more detrimental to children (WHO, 1998b) as infection with them becomes more severe.

Several studies (e.g., Aujoulat et al., 2003; WHO, 2003; Stienstra et al., 2002; WHO Fact Sheet, 2000; Bär et al., 1998; Asiedu and Etuaful, 1998; Portaels, 1995) have found that BU is prevalent among poor populations in remote rural areas where modern medical amenities are lacking. BU has often been referred to as the 'disease of the poor' (WHO Fact Sheet, 2000). In this environment of poverty the necessary proteins and micronutrients are lacking in staple food of endemic communities (Aujoulat et al., 2003) and due to lack of finances BU patients do not seek medical attention until it becomes the only option (Stienstra et al., 2002; Asiedu and Etuaful, 1998). There have been instances where BU patients were diagnosed with hypoproteinaemia (Bär et al., 1998), anaemia (Pszolla et al., 2003), some form of severe illness (e.g., sickle-cell anaemia) (Portaels unpublished observation.) or nutrition-related illness. Such severe illness coupled with MU toxin may enhance immunosuppression and subsequently dissemination and spread of MU (Johnson et al., 2002; Pimsler et al., 1988). Therefore poverty, its related undernutrition, its impact on immune function and susceptibility to pathogenic infection must be

considered a priority in the education of BU endemic communities as well as a challenge to the scientific community.

6.2. Riverine and volcanic environments

The earliest report (in 1957) of BU in Papua New Guinea occurred after the eruption of Mount Lamington in 1951. Following the eruption, floods devastated the area and most BU was found in settlements along the inundated portions of the rivers (Radford, 1974b). Infections occurred mainly along the Sepik and Kumusi Rivers. However, the majority of the disease was found in villages near the Kumusi River and hence the name 'Kumusi ulcer' (Radford, 1974b). The lack of scarring in the older people was an indicator that the disease might have begun after the eruption of Mt. Lamington; and that the eruption could be implicated in the disease (Hayman, 1998). Studies of Portaels (1989) in central Africa suggest that MU, like other mycobacterial species, is present in water but in very low concentrations. There has also been evidence of the disease associated with slow-flowing or stagnant water (Portaels, 1989). There are reported infections occurring near rivers in Uganda (Barker, 1973; Uganda Buruli Group, 1971), Côte d'Ivoire (Marston et al., 1995) and Ghana (Amofah et al., 1993; Mensah-Quainoo, 1998).

It has been suggested that insects (e.g., firefly larvae, *Naucoridae*, *Belostomatidae*) could be involved in the transmission of MU infection since they prey on water-filtering organisms, which might have concentrated MU (Portaels et al., 2001; Portaels et al., 1999). Marsolliers et al. (2002) subsequently carried out an experimental study to show that not only did this insect concentrate MU in its salivary gland but also that its bite transmitted infection to mice.

6.3. Agricultural environments

Farming activities in close proximity to a river has also been considered as a risk factor in MU infections (Marston et al., 1995). For example, a study by Barker and Carswell (1973), which relates to farming (i.e., crop irrigation), drinking water and frequency of MU infection showed that the disease (BU) was found in 6% of families using boreholes, 25% of families using seasonal swamps and 53% of families using permanent swamps at the edge of a section of the Nile River in Uganda. The construction of dams for agricultural purposes is also related to the extension of wetlands, which enhance MU infections (Ziefer et al., 1981; Monson et al., 1984). In Benin incidence of BU, especially around Zangnanado, was associated with recent construction of canals for irrigation purposes (e.g., for the cultivation of rice) (Portaels *pers. comm.*).

6.4. Lakes and reservoir environments

Aujoulat et al. (1996) indicate that, in Côte d'Ivoire, increased incidence of BU was very much related to areas around dammed rivers. The first report of MU infection in Côte d'Ivoire was a 7-year old boy living with his parents near an artificial lake (Lake Kossou) in the centre of the country (Peraudin et al., 1980). In Nigeria, BU incidence among Caucasians on the campus of Ibadan University (Oluwasanmi et al., 1976) was associated with a small stream near the university, which was dammed to make a 2.5-ha artificial lake. Similarly in Liberia, there were reports of BU cases after a dam construction following the introduction of swamp rice to replace upland rice (Ziefer et al., 1981; Monson et al., 1984). In Ghana BU is clustered along the Densu River (Mensah-Quainoo, 1998). An impoundment on the southern part of the river (Weija Dam) stores water for the western part of the capital city, Accra. BU occurred in settlements, both upstream and downstream of the impoundment. However, the upstream part and along the impoundment where wetlands have been created as a result, BU incidences were higher (with the highest occurring about 7 km north of the impoundment) than in the downstream part south of the impoundment where settlements were on higher elevations.

6.5. Swamps and related environments

Many of the MU infections occurred after flooding. Bainsdale, Australia, experienced its worst floods on record in 1935 (Hayman, 1998) and the first recorded case of BU in 1939 (MacCallum et al., 1948). Barker (1971) also postulated that the outbreak of BU incidences north of the Lake Victoria in the Busoga district in Uganda was related to unprecedented flooding from 1962 to 1964, which occurred as a result of heavy rains. Several references have been made to renewed outbreaks of BU after flood events (Meyers et al., 1996; Barker, 1974; Portaels, 1989, 1995; Radford, 1974b; Ravisse, 1977; Ravisse et al., 1975; Burchard and Bierther, 1986).

Outbreaks of BU on Philip Island were seemingly related to a road construction, which resulted in the formation of marshlands at the headwaters of an estuary (Johnson et al., 1995). Also on Philip Island, a golf course irrigated with recycled sewage and a nearby swamp was associated with an outbreak of BU between 1993 and 1995 (Ross et al., 1997; Stinear et al., 2000; Veitch et al., 1997). In this particular outbreak it was hypothesized that MU was transmitted via aerosols since it had been demonstrated that cells of MU could be aerosolized from suspensions of tap water (Hayman, 1991). Another evidence suggesting that water was not the only source of MU (but rather aerosols) was the occurrence of an outbreak in Kinyari (Uganda) refugee camp, located adjacent to swampy regions near the Nile River. The relocation of the refugees from the site drastically reduced MU infection (Bradley, 1971). MU has also been associated with slowly flowing or stagnant waters (Portaels, 1995; Meyers, 1994). Other places where BU outbreaks occurred in marshy environments include French Guiana (Pradinaud et al., 1974), Cameroun (Ravisse, 1977; Ravisse et al., 1975) and Uganda (Barker, 1971, 1973).

7. Seasonal variations and MU infections

Some authors (e.g., Revill and Barker, 1972; Meyers et al., 1996) have referred to the seasonal dimension of MU infections. For example, in Australia, it was noted that the disease appeared at the end of the autumn or winter (Hayman, 1985); and in Papua

New Guinea and Cameroun, it was observed that incidence of the disease increased during the dry season (Radford, 1974b; Ravisse, 1977). In Uganda, reports from two separate studies showed that the peak incidence of the disease occurred in low rainfall months between May and September (Uganda Buruli Group, 1971; Revill and Barker, 1972). In Ghana, the peak incidence of the onset of symptoms was in September and October (Amofah et al., 1993); and similarly in Côte d'Ivoire, Marston et al. (1995) found the peak incidence in the same months. Thus, there seems to be a temporal relationship between BU incidences and relatively dry periods.

8. Treatment and control

A wide range of drugs has been used against MU disease as well as surgical excision (Feldman and Karlson, 1957; Pattyn and Ermengen, 1968; Lunn and Rees, 1964; Portaels et al., 1998; Thangaraj et al., 1999). Complete surgical excision, however, of the affected area, with or without skin grafting remains the most effective treatment option. The disease generally does not respond to drug treatment alone (Johnson et al., 1999). It is therefore hoped that newer drugs may be more effective. Antibiotics, however, may be effective in preventing recurrence of the disease where surgical excision is inadequate. Besides, several individuals (especially of the rural population) in endemic areas consider hospital treatment expensive and sometimes ineffective and do not attend or report to the hospital when infected (Aujoulat et al., 2003; Amofah et al., 1993). In the light of this situation other control measures must be taken.

The difficulty in finding alternatives is the fact that the means of transmission is as yet unclear. One of the precautionary measures that has been recommended is limiting contact with the environmental source of MU (Portaels et al., 2001), which could be difficult for rural communities in the short run if it affects their source of water or farmlands. The wearing of trousers, boots and long sleeved shirts when on farms and swamps is said to have helped (Marston et al., 1995). However, this is only a partial solution since infection can affect every part of the body including the face (Amofah et al., 1993). Vaccination of Bacille Calmette-Guérin (BCG), which partially protect from BU for few months (Smith et al., 1977), could be implemented. Therefore, until knowledge of the mode of transmission is attained, there can be no clear preventive measures.

However, education, especially of the rural population where the disease is endemic, is important and the partial solutions or preventive measures can be used in the interim. The form of education must first focus on early detection of nodules by individuals so that they can report to the nearest clinic or hospital for removal. Another aspect of education is to stress the fact that BU is a result of a mycobacterial infection like any other disease and not as a result of sorcery (Aujoulat et al., 2003).

As BU mainly afflicts relatively poor communities and countries, the additional strain imposed on limited medical services is also an important consideration. When the disease reaches the ulcerative stage, confinement in a hospital bed is protracted and in itself an economic burden (Asiedu and Etuaful, 1998).

9. Summary

- MU is normally a slow-growing mycobacterium but grows luxuriantly at 25 and 28 °C under microaerophilic conditions.
- BU is a skin disease and is characterized as non-ulcerated and ulcerative disease. The focus of the disease is the subcutaneous tissue. MU secretes a toxin, which destroys the skin, nerves and blood vessels.
- The toxin elaborated by MU suppresses the immune inflammatory response and hence the disease development. The toxin alone, however, does not induce systemic immunosuppression and therefore any such situation in an individual suggests host susceptibility factors that must be explored.
- Geographically the disease is generally prevalent in the tropics with West Africa having a very high incidence. Few cases, however, have been reported in the non-tropical world.
- Because the exact mode of transmission is unknown, it has been hypothesized that infection could be via trauma to the skin by a contaminated environment or that MU is inhaled or ingested, later to be reactivated in sites of trauma.
- Commonly MU host environments are related to rivers, swampy terrain, lacustrine or reservoir systems or through environmental disturbances such as flooding, mining, logging of rain forest and damming of rivers.
- The disease has a seasonal dimension and incidences seem to have a temporal relationship with relatively dry periods.
- Since most endemic areas are rural populations who are poor and consider hospital treatment expensive, and since even the mode of transmission of the disease is unknown, alternative interim precautionary measures through education (of the rural population) are seen as a way to reduce human misery and economic burden imposed by the disease.

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