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Editorial

ENVIRONMENTAL MYCOBACTERIA AND HUMAN DISEASE

in the latter part of the last century, the causative organisms of leprosy and tuberculosis were described, respectively, by Armauer Hansen in 1874 and Robert Koch in 1882. These bacteria were distinguished from all others known at that time by a characteristic acid-fast staining property by Paul Ehrlich in 1883. In 1898, when the generic name *Mycobacterium* was introduced, no other acid-fast bacillus had been formally described and named but from that time onwards many such organisms were isolated from environmental sources and from diseased mammals, birds and cold-blooded animals. A few were also isolated from patients, but their role as human pathogens was not seriously considered until the middle of this century when two new mycobacterial skin diseases, swimming pool granuloma[1] and *M. ulcerans* infection,[2] later termed Buruli ulcer, were described and when Runyon published his classical description of the four groups of mycobacteria causing pulmonary disease.[3]

Many unsatisfactory collective names including 'atypical', 'anonymous', 'tuberculoid' and 'MOTT (mycobacteria other than typical tubercle) bacilli' have been given to these mycobacteria but when it became apparent that these species were not primarily pathogenic but widely distributed in nature, the name 'environmental mycobacteria' (EM) was generally adopted. At present there are about 60 known mycobacterial species: 41 are included in the Approved Lists of Bacterial Names[4] published in 1980 and the others have been described subsequently.[5] Cultivable mycobacteria are divisible into two main groups: the slow and rapid growers, which, by antigenic analysis and DNA homology, appear to belong to two quite distinct subgenera.

The ecology of the environmental mycobacteria

It is not generally appreciated just how common the EM are in nature. Sphagnum marshes contain very large numbers[6] and they are also readily isolated from mud, compost, wet soil, surface water, rivers and estuaries.[7] Some species colonize piped water supplies and have been isolated from taps and showerheads. There is also evidence that mycobacteria are washed into estuaries, taken up into aerosols generated by breaking waves and wafted inland by sea breezes and thereby sensitize people who inhale them.[8] Some species or strains are more hydrophobic than others and thus more readily enter

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aerosols.[9] There is also some evidence of an association between hydrophobicity and virulence[10] so that strains entering aerosols may be more likely to cause disease than other environmental strains.

The population of EM varies quantitatively and qualitatively from region to region, being affected by temperature, dryness, pH and mineral content of the environment and possibly by the availability of nutrients derived from decomposing vegetable material. Thus, for example, slowly growing species are particularly prevalent in Burma while rapid growers predominate in Uganda. The EM present in a given region may be determined directly by culturing soil and water samples and indirectly by skin testing surveys based on use of sensitins prepared from a range of mycobacteria.[11]

Immunologically effective contact with mycobacteria

In view of the widespread distribution of EM in nature, exposure of man to them is a regular occurrence in most parts of the world. Thus EM may be consumed in drinking water, inhaled in aerosols or enter the skin through cuts and abrasions. Despite this repeated exposure, overt human disease due to them is very uncommon. On the other hand, there is increasing evidence that overt disease is just one of a complex range of interactions occurring between these commonly encountered micro-organisms and man. These interactions include transient or long term colonization of the intestinal and upper respiratory tracts and there is evidence that they may cross the pharyngeal or intestinal wall (translocation) and persist in the tonsils or gut-associated lymphatic tissue.[12] Exposure may also lead to 'immunologically effective contact' manifesting as reactions or cross-reactions on skin testing with tuberculin and other mycobacterial antigens.[11] More importantly, such contact early in life may affect the pattern of immune responses elicited by subsequent exposure to mycobacteria—a concept picturesquely termed 'Original Mycobacterial Sin'.[13]

In this context, there has, in recent years, been considerable interest in the possible effect of immunologically effective contact with EM on the way in which the host subsequently responds to challenge by BCG vaccination or by infection with *M. leprae* or *M. tuberculosis*. Much of this interest resulted from attempts to explain why an extensive BCG trial conducted in South India failed to reveal any protective efficacy of this vaccine.[14] This led to the restatement of the hypothesis, originally formulated in 1966, that contact with EM provided a form of natural vaccination.[15] If this is the case, a human population repeatedly exposed to EM might become so immune that administration of BCG could not add to this and would therefore appear ineffective in a clinical trial. The alternative hypothesis is that exposure to mycobacterial antigens can prime the host for a range of different immune responses—protective immunity, tissue destroying hypersensitivity and autoimmunity—and that the actual response is, in part at least, determined by the extent of the exposure to EM and the species to which the population is exposed.[16] This hypothesis has two important consequences. First, it may be important to give BCG vaccination before the 'wrong' antigens have been encountered, i.e. neonatally. Secondly, the judicious administration of mycobacterial antigens may lead to the enhancement of protective responses and the suppression of harmful ones. There have been some encouraging preliminary studies on this approach to immunotherapy.[17]

Species of EM causing human disease

Most of the species of slowly growing EM are able to cause opportunist disease in man but, with very rare exceptions, only two rapid growing species, *M. chelonae* and *M. fortuitum*, cause such disease. These two species were once called the 'cold blooded tubercle bacilli' as they were originally isolated from, respectively, turtles and frogs.

By far the most prevalent causes of human disease among the slowly growing EM are the closely related species *M. avium* (the avian tubercle bacillus) and *M. intracellulare*. Being closely related, they are not easily distinguished outside specialist reference centres and are therefore usually grouped together as the *M. avium-intracellulare* (MAI) complex.[18] Other commonly encountered slowly growing opportunist pathogens include *M. kansasii*, *M. xenopi* and *M. scrofulaceum*. Less common members of this group include *M. szulgai*, *M. simiae* and *M. malmoense*, with infections due to the latter showing a considerable, but unexplained, increase in frequency over the last few years. A few other species very rarely cause disease and others may eventually be added to their number.[19]

Epidemiology of human disease due to EM

The incidence and nature of human disease due to EM in a given region is dependant upon the number and species of mycobacteria in that region, the opportunities for contact with potential hosts and the susceptibility of those hosts to disease. As the source of the causative organisms is almost invariably the environment, control measures against tuberculosis, which are designed to break the chain of person to person infection, have no impact on the incidence of disease due to EM. Consequently, as tuberculosis declines in a given country, a relatively greater proportion of mycobacterial disease is due to EM. Also, as a result of an increase in the number of individuals with natural or iatrogenic immunosuppression, the absolute incidence of the latter is also increasing. As mentioned above, the two main opportunities for infection are inoculation through the skin and inhalation of aerosols. Thus, an important group of diseases due to EM, including the two named diseases Swimming pool granuloma and Buruli ulcer, are the result of inoculation.[20] Inhalation of aerosols as a cause of pulmonary disease is less easy to demonstrate but there is strong evidence that a cluster of cases of disease due to *M. kansasii* in pneumoconiotic coal miners in Czechoslovakia was related to the presence of this organism in the water supplying the shower houses used by the miners.[21]

Types of human disease due to EM

Overt disease is divisible into four main types: Inoculation mycobacteriosis, localized lymphadenopathy, pulmonary and disseminated disease.

INOCULATION MYCOBACTERIOSIS

This is usually caused by the rapid growers *M. chelonae* and *M. fortuitum*.[20] result from the use of contaminated needles or injectable materials—many so-called 'sterile' postinjection abscesses are caused by these mycobacteria. Such abscess may be

very chronic and reach very large sizes but they tend to remain localized and respond to surgical drainage, curettage or excision. There have been a number of 'mini-epidemics' of postinjection abscesses resulting from the use of contaminated vaccines and other injectable materials. Multiple abscesses or spreading cellulitis may occur in diabetics and these lesions usually require chemotherapy.

Other examples of inoculation mycobacterioses include posttraumatic corneal ulcers and lesions resulting from the accidental implantation of mycobacteria during operative surgery, particularly cardiac valve replacement.[22] These more serious forms of inoculation mycobacterioses require therapy as well as surgical debridement. Owing to the sporadic nature of these infections, there have been no formal comparisons of drug regimens but success has been reported with various combinations of erythromycin, trimethoprim, sulphonamides, gentamicin, amikacin, fluroquinolones and third generation cephalosporins. In the author's experience, erythromycin with trimethoprim, with the addition of amikacin in more serious cases, is often effective. Corneal infections are usually treated with topical amikacin and erythromycin but in many cases, especially in those caused by *M. chelonae*, relapses follow initial resolution or dense scarring develops. Hence, corneal grafting is often required.

The inoculation mycobacterioses include two named diseases: Swimming Pool Granuloma[1,23] (also known as Fish Tank Granuloma or Fish Fancier's Finger) and Buruli Ulcer.[2,24]

Swimming Pool Granuloma is caused by *M. marinum*, and manifests as warty lesions on trauma-prone parts of the skin, particularly the elbows and knees of swimming pool users and the hands of tropical fish enthusiasts. The lesions are usually localized, although 'sporotrichoid' secondary lesions may occur along the draining lymphatics. The disease is usually benign and self-limiting although excision and/or antibacterial chemotherapy is sometimes required.

Buruli ulcer is a much more serious disease which was first described in Australia[2] but mostly occurs in various limited localities throughout the tropics. The causative organism is *M. ulcerans* which is unique among the mycobacteria by having a very narrow temperature range of growth *in vitro*—32–34°C—and it is the only pathogenic mycobacterium known to produce a cytotoxin.[25] Although it has not yet been isolated from the environment, there is epidemiological evidence that *M. ulcerans* is a free-living mycobacterium that enters the skin by traumatic inoculation by, in most cases, spiky grasses and other vegetation.[26] Following such inoculation into the skin, the toxin released by the organism causes necrosis of the subcutaneous tissues and, eventually, the overlying skin, thereby forming deeply undermined skin ulcers which may reach enormous size. During the progressive phase of the disease, the lesion contains numerous acid-fast bacilli but there is no detectable immune response to them. At this stage, the patient fails to react to tuberculin and to Burulin[27]—a specific skin test reagent prepared from *M. ulcerans*.

A peculiar characteristic of Buruli ulcer is that a point is invariably reached when immune responsiveness develops, the patient reacts to tuberculin and Burulin, the organism disappears and the lesion heals, although often with severe residual scarring and deformity. Even more peculiarly, anergic and reactive lesions may be seen simultaneously in the same patient. The reason why there is a shift from an anergic, multibacillary state, analogous in some respects to lepromatous leprosy, to an abacillary healing stage is

unknown but an unravelling of this mystery could perhaps have profound significance for the development of immunotherapy for leprosy and other mycobacterial diseases.

Treatment of Buruli ulcer is not easy.[24] Although rifampicin and clofazimine are effective *in vitro* and in the mouse model, clinical experience has been rather disappointing. Treatment therefore usually involves excision, often requiring very major surgery with skin grafting. Radical excision is particularly important in the progressive stage in order to remove all infected tissue. For this reason, community education in the importance of presenting with early lesions is of great benefit. As *M. ulcerans* has a very restricted temperature range of growth—32–34°C, resolution of infection has been obtained by applying external heat to lesions for prolonged periods of time but the practical problems associated with this therapy greatly limit its usefulness.

LOCALIZED LYMPHADENOPATHY

This is caused by many species although the great majority of cases are caused by *M. avium-intracellulare*.[2] solitary lymph node in the neck is involved, although other nodes and age groups may also be affected. Although some cases are associated with congenital or acquired immunodeficiency, including AIDS, most such infections, particularly those affecting children, occur in otherwise healthy individuals. Treatment by excision of the affected node is usually curative and chemotherapy is not usually required. Drainage, rather than excision, should not be attempted as this may lead to chronic sinus formation.

PULMONARY DISEASE

This is usually due to the slowly growing species *M. avium-intracellulare*, *M. kansasii* and, in some countries, *M. xenopi*. Less common causes include *M. malmoense*, *M. scrofulaceum*, *M. simiae* and the rapid grower *M. chelonae*. Most cases occur in persons with local or systemic predisposing causes such as industrial dust-associated lung disease, old tuberculosis cavities, rheumatoid lung, cystic fibrosis, malignant disease and any form of congenital or acquired immunosuppression.[29] Some cases, however, occur in the absence of detectable predisposing conditions. Such disease is indistinguishable clinically and radiologically from tuberculosis and diagnosis depends on isolation and identification of the causative mycobacterium. The species of EM causing pulmonary disease may also occur as transient saprophytes in abnormal lung tissue. Great care must therefore be made to distinguish such colonization from actual disease. As a general rule, an EM should not be considered as the primary cause of lung disease unless it is repeatedly isolated from patients with compatible signs and symptoms and in whom all other possible causes of such signs and symptoms, including tuberculosis, have been rigorously excluded.

The causative organisms are usually resistant to most anti-tuberculosis drugs *in vitro*. Nevertheless, good results have been obtained in disease due to the slowly growing species mentioned above by use of first line antituberculosis drugs rifampicin, ethambutol and isoniazid.[30,31] Although infection due to *M. kansasii* has been shown to respond to 9 months therapy, disease due to other slowly growing species requires treatment of 1 year or 18 months duration. In contrast to tuberculosis, ethambutol appears to be a more

effective drug than isoniazid and should therefore be continued throughout the duration of therapy, with due attention to possible ocular side-effects. There are no well-tried regimens for pulmonary disease due to rapid growers but there are anecdotal reports of success with the various drug combinations used for the treatment of inoculation mycobacterioses due to these species (see above).

DISSEMINATED DISEASE

As in tuberculosis, disease due to EM may disseminate from the primary site of infection to other organs. Cases of solitary lesions due to EM in the genitourinary tract, bones and central nervous system have been described but they are very rare. Until the early 1980s, widely disseminated disease due to EM was very uncommon and the few described cases mostly occurred in children or young adults with congenital immune deficiencies, people with leukaemia and other malignancies and renal transplant recipients.

In recent years, however, there has been an increasing number of such infections associated with AIDS. For reasons that are not clear, such AIDS-related disease is mostly, though not exclusively, due to *M. avium-intracellulare* (MAI).[18] Furthermore, there is evidence that the population of strains of MAI involved in AIDS-related disease differs from those causing disease in HIV-negative patients. Thus, in one study, 73% of MAI from AIDS patients were of a particular genotype, compared with only 39% from non-AIDS patients and none of 8 strains from faeces of healthy persons.[32] The significance of this finding is unknown. The source of infection is uncertain: one possibility is that it is the result of activation of dormant MAI in the gut-associated lymphatic tissue (see above) rather than direct acquisition of the bacilli from the environment.[12]

Unlike tuberculosis, which often occurs early in the course of HIV infection, opportunist disease due to MAI usually occurs in patients with fully developed AIDS. Bacteria are often found throughout all internal organs and in the blood. Involvement of the intestinal wall leads to debilitating diarrhoea. The incidence of AIDS-related MAI disease varies from country to country, being uncommon in Africa but occurring in up to 50% of those dying of AIDS in the USA.[33]

There has been some controversy as to whether AIDS patients die of or with MAI infection and whether such infection reduces life expectancy. Although more information is required, therapy does appear to prolong life and enhance its quality. The most effective drug regimens currently available are based on rifabutin and clofazimine, sometimes with the addition of isoniazid and ethambutol but, even with this powerful combination, relapses are common.[34]

EM as possible causes of other diseases in man

There have been several claims during the last few decades that mycobacteria are responsible for a number of human diseases of unknown aetiology, notably sarcoidosis and Crohn's disease. In most studies, acid-fast bacilli have neither been seen in, nor cultured from, the lesions of patients with these diseases. Nevertheless, by analogy with tuberculoid leprosy, the failure to observe acid-fast bacilli in, or to culture them from, diseased tissue does not necessarily exclude them as the prime cause of that disease. There

have, however, been a few isolations of *M. paratuberculosis*, the causative agent of hypertrophic enteritis in cattle, from Crohn's disease tissue.[35] Clearly, these few isolations do not prove that this mycobacterium is a cause of Crohn's disease: the tissue changes may merely promote secondary saprophytic colonization by certain bacteria that are present in small numbers in the healthy bowel.

Several authors have postulated that mycobacteria associated with Crohn's disease and sarcoidosis exist in cell wall-free forms and, as such, are very difficult to detect and isolate.[36] For this reason, attention has turned to the detection of specific components of mycobacteria by sensitive chemical or molecular biological techniques but the limited data yielded so far have been somewhat conflicting and the aetiological significance of detected components remains unknown.

Tuberculostearic acid, a long-chain fatty acid specific to the genus *Mycobacterium*, has been detected in lymph nodes from patients with sarcoidosis.[37] By use of suitable DNA probes, DNA compatible with *M. paratuberculosis* has been detected in Crohn's disease tissue but it was also detected in tissue from cases of ulcerative colitis and non-inflammatory bowel disease.[38] More recently, use of a DNA–RNA hybridization technique showed that bowel tissue from Crohn's disease contained much more (almost five times as much) RNA binding to a non-species-specific mycobacterial DNA probe than normal bowel tissue. Likewise spleens from patients with sarcoidosis contained an equally increased amount of such RNA.[39] Not all attempts to detect mycobacteria in such tissues have, however, been successful. One group of workers failed to detect mycobacterial DNA in Crohn's disease tissue, even though they used the very sensitive polymerase chain reaction to amplify any such DNA.[40]

Thus, at present, the topic is a challenging yet controversial one but, with the increasing sophistication of the techniques available to the microbiologist of today, there can be little doubt that this subject will generate a large amount of work and, probably, even more controversy. In order to avoid drawing false conclusions, it is crucial that workers in this field should interpret their data with great caution, always bearing in mind that the well-established criteria for determining the causative role of micro-organisms in disease, namely Koch's postulates, are as relevant now as they were when introduced over a century ago.

Prevention of human disease due to EM

As EM are so common in nature, prevention of infection of man by them is virtually impossible. Any measures to control, treat or prevent predisposing lung damage, especially pneumoconiosis, and natural or iatrogenic causes of immunosuppression will limit the subsequent development of overt disease. Likewise, strict attention to the sterility of injectable materials, needles and syringes will have an impact on the incidence of inoculation mycobacterioses.

The effect of previous BCG vaccination on the incidence of disease due to EM is poorly understood. The effect on Buruli ulcer in Uganda was transient and limited. On the other hand, termination of neonatal BCG vaccination in Sweden was followed by an increased incidence of mycobacterial lymphadenitis in young children, providing indirect evidence that BCG afforded protection against that disease.[41] The effect of previous BCG vaccination on AIDS-associated disease due to EM, particularly *M. avium-intracellulare*,

remains to be established. If such disease is due to recent and direct infection from the environment, then any protective effect of BCG would probably be abrogated by the immunosuppression. If, on the other hand, disease is due to reactivation of dormant bacilli in lymphatic tissue, then BCG given early in life could well have a beneficial effect by preventing the establishment of such dormant foci of infection. It would be interesting to know whether the high incidence of AIDS-related disease due to EM in the USA is due to the fact that BCG vaccination was abandoned many years ago in that country.

Conclusions

Once regarded as a mere curiosity, overt disease due to EM is now recognized as being a serious cause of human illness. Once regarded as a rarity, the tragedy of the AIDS pandemic has assured the increasing prevalence of such disease. Future studies may well confirm the long-suggested causal link between EM and certain chronic diseases of at present unknown aetiology. Despite these actual and postulated associations with overt human disease, the main cause of interest in future may well relate to their ability to predetermine the nature of immune responses on subsequent encounters with the tubercle or leprosy bacilli. Detailed studies of the immunological consequences of the interactions between environmental mycobacteria and man, and the development of ways of manipulating these interactions, may enable 'Original Mycobacterial Sin' to be changed to Original Blessing!

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