Mycobacterial disease—a challenge to biotechnology

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Received 16 May 1984; accepted 8 June 1984

Introduction

In 1896 Lehmann and Neumann introduced the generic name Mycobacterium to include the causative agents of two of the most feared of all afflictions of man, namely tuberculosis and leprosy. The former organism, Mycobacterium tuberculosis, had been observed and cultured 14 years previously by Robert Koch (1882). Observation of the leprosy bacillus, M. leprae, in infected tissue was reported by Hansen in 1874 but it remains one of the very few bacteria that has never convincingly been cultivated in vitro. Subsequently other mycobacteria were discovered and there are now about 40 recognized species (Skerman et al. 1980). Some of these cause disease in man but such infections are uncommon and, unlike those causing the two great scourges mentioned above, are hardly ever transmitted from person to person. These mycobacteria, and the diseases they cause, have been reviewed in detail elsewhere (Chapman 1977; Wolinsky 1979; Grange 1980). Many mycobacterial species, however, live freely in the environment and rarely if ever cause disease. There is increasing evidence that contact with such species has profound effects on the manner in which the immune system responds to later challenges by pathogenic mycobacteria.

The immense interest generated by Koch’s discovery focused on three major subjects: a remedy for tuberculosis, an effective vaccine, and a simple and reliable diagnostic test for the disease. If these lines of research had proved successful for tuberculosis and for leprosy then these afflictions might well have become extinct by now. The fact that there is at present an estimated 15-20 million sufferers from leprosy and 10 million new cases of tuberculosis (with 3 million deaths) each year is a clear indication that either our techniques for preventing, diagnosing and treating these diseases, or our endeavours to apply them in practice, are inadequate.

From the point of view of mycobacterial disease, the countries of the world are divisible into two main groups. There are countries with a high prevalence of these diseases but with limited financial and technical resources for overcoming them. In these countries the first priority is to locate the open or infectious cases and to treat them effectively. The second group of countries are those, usually wealthier and more technologically advanced, in which leprosy is virtually absent and the prevalence of tuberculosis is very low. This leads to an inevitable loss of interest in such diseases with the result that infectious cases of tuberculosis go undiagnosed and avoidable morbidity, and even mortality, is the result (Grange 1979; Horne 1984). Such a premature lack of concern is to be deprecated as, owing to the infectious...
nature of the disease, no one is safe until all are safe (Sodhy 1978). It is also regrettable that
nations that consider, albeit erroneously, that mycobacterial diseases no longer constitute a
‘problem’ are often unwilling to maintain a research interest for the sake of the less fortunate
nations.

As we now live in an age of ‘high technology’ it is pertinent to re-evaluate the methods
available for preventing, detecting and treating tuberculosis and leprosy and to look at the
ways in which recent advances in applied bacteriology, immunology and biotechnology
could possibly lead to significant advances in the battle against these two diseases.

Diagnosis

The leprosy bacillus cannot be cultured in vitro but, owing to its superficial nature, the disease
can usually be diagnosed by clinical or histological examination. Tuberculosis, on the other
hand, usually involves the lung or other internal organs and the only way to reach a firm
diagnosis is to isolate the causative organism, *M. tuberculosis*, in pure culture. This is a time
consuming procedure, both with respect to the time taken to prepare media, to treat speci-
mens so that organisms other than mycobacteria are killed (‘decontamination’), to inoculate
the media and to identify any cultured mycobacterium, and also with respect to the length of
time required for colonies of this slow-growing organism to appear on the medium. Furthermore
it is not always easy, particularly in some cases of non-pulmonary disease, to obtain
specimens containing tubercle bacilli. Even when viable bacilli are present in specimens the
harsh ‘decontamination’ procedures and the shock of being transferred from living tissue to
artificial media prevents their isolation in a substantial number of cases. It is indeed surprising
that so few technical innovations have been made in ‘routine’ tuberculosis bacteriology since
the introduction of egg-based media by Dorset in 1902 (c. Dubos 1954). Clearly much more
attention needs to be given to this prosaic, yet fundamentally important field of microbiology.

In view of the difficulties experienced in the diagnosis of mycobacterial infections by
bacteriological means, much effort has been put into the development of simple yet reliable
tests for these diseases. Two major classes of tests have emerged, skin tests and serological
examinations, yet neither has been very successful.

**Cross-reactivity**

Two great difficulties have emerged during the development of these tests: the occurrence of
immunological cross-reactivity between the pathogenic mycobacteria and those living freely
in the environment, and the difficulty in distinguishing between active disease, quiescent
infection and past sensitization.

The problem of cross-reactivity can be understood by looking at the results of immuno-
diffusion analysis of the mycobacterial antigens (Stanford & Grange 1974). This technique
reveals about 15 antigens in each species (more sensitive procedures reveal up to 90)
and these divide clearly into four groups as shown in Fig. 1. Group i antigens, about six in
number, are common to all mycobacteria and some of them also occur in related bacteria
especially the genera *Nocardia* and *Corynebacterium*. Group ii antigens are restricted to the
slowly growing species while Group iii antigens occur in rapidly growing mycobacteria and in
nocardias. The Group iv antigens are those that are unique to each individual species; these
antigens, depending on the species, number from two to eight and a small amount of intra-
specific variation is seen in some species. A small group of mycobacteria, including *M. vaccae*
and *M. leprae*, possess neither the Group ii nor the Group iii antigens and cannot be classified
serologically as either rapid or slow growers (Stanford & Rook 1983).