New concepts for the control of tuberculosis in the twenty first century

ABSTRACT—As the end of the twentieth century approaches new methods are needed for the treatment and control of tuberculosis. Vaccination needs to be rethought, and BCG either improved or replaced. Chemotherapy is no longer enough to meet the needs of impoverished countries, non-compliant patients, and increasingly drug-resistant organisms. The next major step forward should logically come from immunology. Following the clear differentiation of two pathways of cellular immune response to mycobacterial challenge, and the recent description of two functional types of helper T cells, ideas of what controls them have allowed the logical development of a potential new vaccine and a new immunotherapy. These are based on a killed environmental organism, *Mycobacterium vaccae* NCTC 11659. With this simple preparation together with chemotherapy we may be armed as never before to face the inevitable challenge that tuberculosis will present to the twenty first century.

Parallels recognised between cell death in tuberculosis and infection with the human immunodeficiency virus open the possibility that the progress made in immunotherapy in tuberculosis might be applicable to HIV. If this proves the case then we may also have control over the latest, and worst, risk factor for tuberculosis at the time we need it most.

A mere decade ago we thought we understood the pathogenesis and bacteriology of tuberculosis, we had confidence in preventive measures, and treatment by short course chemotherapy appeared quite straightforward. The disease was almost forgotten in many developed nations and it seemed only a matter of time, and limited financial investment, before tuberculosis would be conquered in the developing world. Yet, in 1993, we can be confident of one thing only—that the struggle against tuberculosis will continue well into the next millennium. Why is this disease of antiquity, apparently so readily preventable and curable, proving so difficult to eradicate?

There is no simple answer. Without doubt, the problem has been exacerbated by the unexpected advent of the HIV pandemic [1], but this is by no means the only reason that tuberculosis is flourishing today. Another reason is the nature of the host-pathogen relationship, particularly the ability of the bacillus to persist in the host tissues for years or decades [2].

To a great extent, however, we ourselves are responsible for failing to eradicate tuberculosis. We have allowed pharmacies to dispense powerful antituberculosis drugs over the counter in many countries and have permitted inexperienced physicians to prescribe inadequate drug regimens [3], thereby facilitating the emergence of multidrug resistance. We have failed to take into account the natural propensity of many people not to comply with their prescribed treatment. We have failed to put global campaigns against tuberculosis on a firm financial basis.

Only an appreciation of the problems and a concerted and practically directed programme of research will lead to the conquest of tuberculosis in the twenty first century [4]. The principal needs are threefold. First, a prophylactic vaccine to prevent primary disease and the persistence of tubercle bacilli in the tissues of the primarily infected person. Second, preventive therapy to stop infected persons (who make up about one third of the world's population) from developing overt disease. Third, shorter and more effective therapeutic regimens for those with clinical disease, including that caused by multidrug resistant bacilli.

Vaccination

Most people do not consider the possibility that there could be an alternative to BCG as a vaccine against tuberculosis, perhaps operating through a quite different immunological mechanism. The notion, stemming from the statements of Koch and Trudeau a century ago, that a vaccine strain effective against tuberculosis must be a close relative of its virulent counterpart and must be living and capable of inducing a 'limited tuberculous process' has rarely been challenged [5]. Yet, if the recent advances in the understanding of the bacteriology and immunology of tuberculosis are to have a practical impact, such a challenge must be made.

In particular, the concepts of 'determinants of virulence' and 'protective epitopes' require careful reappraisal. Most of the surface of a mycobacterium is glycolipid, many epitopes of which are common to the whole genus. There are surprisingly few secreted protein antigens [6] and these too share most of their epitopes with all other mycobacteria. The highly

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conserved heat shock proteins, some of which are secreted, not only have a common structure among mycobacteria but have about 50% homology with analogous human proteins. Owing to this homology, heat shock proteins are amongst the antigens that most readily induce antibody formation [7] and, through epitope spreading, such antibodies may contribute to the pathogenesis of disease by facilitating autoimmune reactions [8]. Although the non-secreted cytoplasmic proteins show great antigenic complexity, they are irrelevant to protective immunity as they are never exposed by an intact, living bacterial cell and there is no benefit in eliciting protective immune responses to dead ones.

Despite extensive investigations, no clear-cut differences between avirulent environmental mycobacteria and virulent tubercle bacilli have been found. The differences are likely to be very small, perhaps in just a few surface and secreted antigens. Yet these small differences are extremely important to the induction of the immunopathological features of active tuberculosis.

There is now evidence from a number of different sources, including the demonstration of cross-protection by BCG against leprosy [9,10], that the 'protective' antigens of mycobacteria are to be found amongst those common to all mycobacteria [11]. This being the case, it is both unnecessary and illogical to develop vaccines from tubercle bacilli. Likewise, there is no need for a second generation vaccine derived from BCG by genetic manipulation [12]. One of the problems of BCG vaccine is that, although unable to cause overt disease (unless the recipient is immunocompromised), it is clearly able to induce allergic reactions. These reactions are directed towards species-specific epitopes and are part of the mechanism of the T-cell mediated tissue destruction, known as the Koch phenomenon [13–15], which is the basis of the pathological process of tuberculosis. This is the reason why BCG should not be given to persons who are already tuberculin positive and why it is ineffective in countries such as Burma and India where individuals are usually highly sensitised by environmental mycobacteria before vaccination [16,17]. Some common environmental species such as *M. scrofulaceum* and *M. intracellulare,* which are known to induce Koch type immune reactivity, predispose against the efficacy of BCG. Thus a new vaccine based on a mycobacterial species totally devoid of the species-specific epitopes of the tubercle bacillus must be sought.

The dogma that the vaccine must be live and able to induce a limited tuberculous process is also questionable. Although the mode of action of BCG is unknown, we do not need a replacement that has the same effect but a vaccine enabling rapid recognition of tubercle bacilli by their surface antigens and neutralisation of the pathogenic effects of their secreted antigens. Such a potential vaccine strain might be found among those species of environmental mycobacteria that never cause human disease and rapidly disappear from mammalian tissues.

For these various reasons, the ideal vaccine against tuberculosis could well be a suspension of dead bacilli of a harmless environmental species. Indeed, the immunising potential of casual exposure to certain species of environmental mycobacteria has been known for a quarter of a century [18]. Such an organism would possess the antigenic determinants that elicit protective cellular immune reactions against the mycobacterial cell surface and secreted antigens, this, together with the powerful adjuvant effect common to all mycobacteria, producing antibodies that bind to shared epitopes on the secreted proteins of the tubercle bacillus.

The amount of BCG that can be given by injection is limited because it can provoke severe allergic reactions It is, therefore, not possible to inject enough killed BCG to elicit lasting immunity. By giving it live, a slow rate of replication in the tissues generates sufficient antigen to induce lasting immunity while avoiding the allergic manifestations. A vaccine containing large numbers of bacilli of a species that does not induce the tissue-necrotising Koch phenomenon could, however, be given with impunity, whatever the immunological status of the recipient [5]. It would not need to persist in the tissues as the immune responses elicited by it would be boosted by day to day contact with many species of environmental mycobacteria. A number of harmless species might be candidates for such a vaccine, but the conquest of tuberculosis will also require a protective immunotherapeutic agent for those who already harbour tubercle bacilli in a persistor state [2].

Although the evidence is not substantial, the best time for giving a vaccine for eliciting cell-mediated immune mechanisms appears to be from 4 to 12 weeks after birth [10]. At that time, the adjuvant effects of a mycobacterial vaccine might be used to improve the efficacy of dip-tet-pertussis (triple) vaccine which induces a series of protective antibodies. Such vaccine combinations would make enormous economic savings that could be directed towards further vaccine-oriented research. Preliminary unpublished evidence based on combinations of mycobacteria with rabies vaccine supports this basic concept (Bui Van Thiu, personal communication).

Preventive immunotherapy

Primary infection by tubercle bacilli only causes overt disease in a small minority of people, but the bacilli often persist in the tissues and years later may give rise to overt tuberculosis in a proportion of those infected [2]. Studies in the USA indicate that courses of antituberculosis drugs, including isoniazid monotherapy for one year, reduce the chance of developing overt tuberculosis. It is not known whether this preventive therapy actually kills mycobacteria or prolongs the persistor state in some other way. Whatever the mechanism, the

cost effectiveness, problems of compliance, possible emergence of drug resistance and the occurrence of toxic side effects, as well as doubts as to its efficacy, render this approach impractical in all but a few special circumstances [19]. There is an urgent need for a simple means of killing the persisting bacilli and priming the immune response to kill further infecting organisms rather than holding them in the persistor state.

Evidence that the persistor state depends on host factors as well as bacterial ones has come from HIV infection [1,2,5]. One of the earliest infections complicating HIV is tuberculosis. Most such cases are due to reactivation of persistors as a result of the very earliest effects of the HIV infection on CD4+ T helper cells. The underlying mechanism may be the progressive switch of human T cell function from T_H1 to T_H2 activity which is known to occur in asymptomatic HIV-infected persons [20]. Thus a vaccine-like agent promoting T_H1 activity may be what is required. Exactly the same activity is likely to be required for successful immunotherapy of overt tuberculosis. It is even possible that an agent promoting T_H1 activity could not only have a beneficial effect on HIV-related tuberculosis but also on the immunopathology of the HIV infection itself. In this respect, it is noteworthy that tuberculosis and HIV infection appear to have a synergistic effect in hastening the patient's progress to full-blown AIDS [1,21].

A stimulus towards protective immunity, by which invading bacilli would be eradicated, could be combined with a T_H1 adjuvant in one preparation. The immunising potential of harmless environmental mycobacteria [18] has been mentioned above and the possibility that some of these species might also regulate T cell activity away from tissue-damaging reactivity has been suspected [22]. Such regulation has been demonstrated in man by use of mixtures of skin testing reagents (new tuberculins) prepared from a range of mycobacteria. Extracts of some, but not all, fast-growing species were found to convert incipiently necrotising tuberculin reactions, which are the skin test equivalent of the tissue destroying Koch phenomenon, to non-necrotising responses [23]. From among these strains, one, with the characteristics of *M. vaccae* subspecies *aurum*, was selected for investigation as a potential vaccine and immunotherapeutic agent.

Immunotherapy for tuberculosis

Modern short course chemotherapy for tuberculosis has been hailed as one of the most clinically and cost effective treatments available for any chronic infectious disease. If the patient has drug-susceptible disease, and in most regions 90% do, if the prescribed regimen is a good one and the patient rigorously complies with the therapy, then about 98% of patients can be cured. In practice, the cure rate usually lies between 75% and 90%. Non-compliance, however, is the greatest bar to effective tuberculosis control worldwide. Virtually all patients will take their drugs for the first two months but thereafter a progressive number default. Non-compliance is greatest, often in excess of 60%, in the very parts of the developing world where tuberculosis is most prevalent. It is, however, a universal problem not necessarily related to socio-economic status and education.

If the duration of treatment could be reduced to those two months of full compliance, a dramatic reduction in the morbidity and mortality of tuberculosis, as well as in the dissemination of disease, could be attained. Only an immunotherapeutic approach is likely to achieve this aim as all the available antituberculosis drugs depend for their efficacy on their ability to disrupt one of the metabolic processes of the bacillus. Resting bacilli have very slow metabolic rates and are thus protected from the bactericidal activity of the drugs. The use of prednisolone to reduce the immune restriction on persistors is a logical but dangerous approach to overcoming this problem.

Tuberculosis control programmes are increasingly faced with the additional problem of disease caused by bacilli resistant to many of the antituberculosis drugs. Although some new drugs (including newer quinolones and macrolides) are available, there is as yet no evidence that they will permit the problem to be overcome. In the USA, multidrug resistant tuberculosis can be cured in about 50% of cases but often at a cost of over $200,000 for each patient. Again, an immunotherapeutic approach is urgently needed.

Three properties are required of a successful immunotherapeutic agent for tuberculosis. First, it must stimulate the host's immune responses to destroy the bacilli and prevent even small numbers from persisting in the tissues. Second, it must divert T cell function away from tissue destruction. Third, it must suppress the harmful activities of circulating cytokines, which include disruption of the proper control of adrenal corticosteroid release. In other words, the requirements for an immunological approach to vaccinating uninfected persons, preventing infection progressing to active disease and to curing active disease are the same [24].

Evidence is steadily accumulating that a killed suspension of the selected strain of *M. vaccae* can fulfil all three requirements.

The evidence

The first use of killed *M. vaccae* was as an additive to BCG in an attempt to improve its efficacy as a vaccine against leprosy for children living in close contact with leprosy patients in Iran [25]. It was also used on its own as a vaccine booster for such children who had scars of past BCG vaccination or who, being tuberculin positive, were unsuitable for BCG vaccination. Three to ten years later, when many of the children were re-examined, none had developed leprosy but a high

Table 1. Conversion to dermal reactivity to leprosin A three to 10 years after vaccination of Iranian children in close contact with leprosy patients. The vaccines used were BCG alone, BCG + 10^7 killed *M. vaccae*, or 10^8 *M. vaccae* alone. The last of these was only used in children already tuberculin positive or with a scar of past BCG vaccination. All children were negative to leprosin A on entering the study.

Vaccine	Close contact		Casual contact
BCG alone	62% (447/721)	$p < 0.00001$	50% (217/438)
	$p < 0.00001$		n.s.
BCG + *M. vaccae*	88% (267/303)	$p < 0.00001$	55% (245/444)
M. vaccae alone	77% (24/31)*		

* This is the result achieved in children selected for their failure to develop leprosin A reactivity despite a past BCG vaccination, or development of tuberculin reactivity.
Note that closeness of contact with leprosy patients has a highly significant effect on reactivity to leprosin A, whichever vaccine was used.

proportion had developed reactivity to leprosin A (Table 1).

A study performed in India has shown that dermal reactivity to leprosin A is a marker of protection against developing leprosy. A group of 517 people living in an area highly endemic for leprosy were skin tested with leprosin A. Nine years later, eight of them had developed leprosy and all were from among those originally negative to the skin test. No cases of leprosy occurred among the 193 people originally responding to leprosin A ($p < 0.03$). Thus it may be inferred that a vaccine that is able to induce a high level of leprosin A reactivity is also likely to confer protective immunity against leprosy.

Subsequent studies showed that killed *M. vaccae* alone, or with BCG, achieved this desired effect by enhancing recognition of common mycobacterial antigens (Fig 1). Thus persons so vaccinated could rapidly recognise leprosy bacilli [26], or any other individual mycobacterial species in their environment by their common antigens and subsequently develop positive reactions to species-specific antigens in skin test reagents prepared from them. This has been shown to be the case in Iran, India, Kuwait and Vietnam.

The potential of *M. vaccae* as a vaccine against tuberculosis has been less well investigated but it should facilitate responsiveness to tuberculin, just as it does for leprosin [24,25], and no cases of tuberculosis are known to have occurred among those vaccinated. More formal studies to establish the vaccinating potential of *M. vaccae* in close contacts of tuberculosis patients are in progress.

The ability to down-regulate T cell mediated tissue destruction in tuberculosis, the Koch phenomenon [15], was first demonstrated in patients undergoing chemotherapy for tuberculosis at the Middlesex Hospital [27]. Two small groups of these patients were skin tested with tuberculin and then given either an injection of killed *M. vaccae* or saline. The patients were skin tested a second time one month later. All eight of those who had received *M. vaccae* had softer, less painful and less inflamed responses to their second tuberculin test—quite different from the response to their first test. The two patients given saline responded to tuberculin with angry, red, hard and tender responses on both occasions.

It has been established in studies on leprosy patients in Spain [28] and on tuberculosis patients in Kuwait

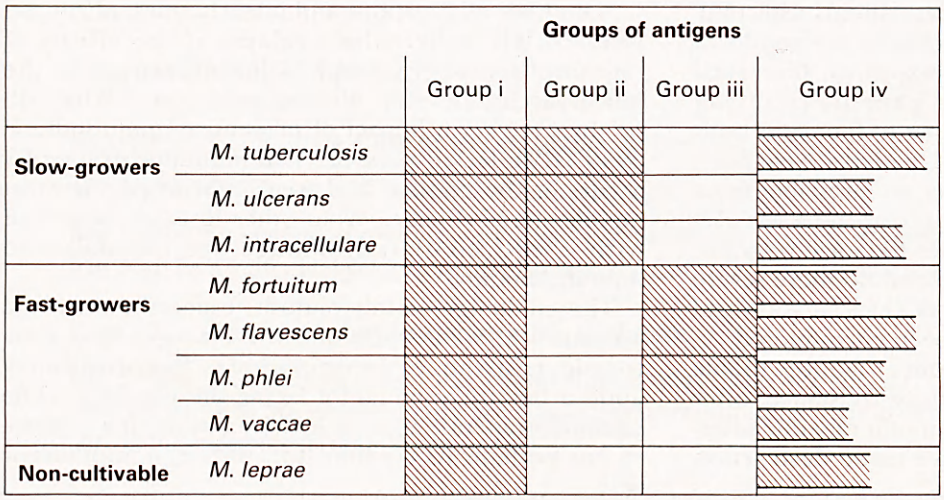

Fig 1. *The antigenic structure of the genus* Mycobacterium. Antigens common to all species (group i); antigens restricted to the slowly growing species (group ii); antigens restricted to the rapidly growing species (group iii) and species-specific antigens (group iv)

[29] that the optimal dose of *M. vaccae* for immunotherapy is about 1mg wet 10^9 weight, equivalent to 10% bacilli, preferably killed by autoclaving. In the case of leprosy, there is some evidence that the addition of a tenth of the routine strength of tuberculin to the *M. vaccae* improves its efficacy. Subsequent studies on the use of heat-killed *M. vaccae* in the treatment of tuberculosis have been carried out, or are in progress, in Gambia, Nigeria, South Africa, Romania, Argentina, Mexico, India, Iran and Vietnam. It has been highly effective in those countries from which we have received data so far.

A randomised and blinded study in the Gambia, with a non-optimal preparation of *M. vaccae*, showed a reduction in mortality ($p < 0.06$) and in treatment failure ($p < 0.03$) occurring during the course of standard or short-course chemotherapy for pulmonary tuberculosis in patients without scars of previous BCG vaccination. Initial data from a similar study in progress in Vietnam, based on an optimised preparation of *M. vaccae*, show a significant improvement ($p < 0.02$) in cure rates in patients receiving short course (eight months) chemotherapy.

In Nigeria a randomised study of patients receiving very inadequate courses of chemotherapy showed that a single injection of an optimal preparation of *M. vaccae* given between 7 and 21 days of starting chemotherapy reduced mortality over the next 10 to 12 months from 40% to 2% ($p < 0.00001$) [30]. This study illustrates the tremendous impact of immunotherapy even under very difficult operational conditions where chemotherapy is woefully inadequate.

In Romania, where immunotherapy has been used in the re-treatment of chemotherapy failures, sputum culture positivity was reduced from 47% in the control group to 8% in the immunotherapy group six months after injection ($p < 0.005$). In Vietnam, a study of immunotherapy for patients relapsing at the end of a first full course of chemotherapy achieved a 91% cure rate, compared with an expected cure rate of 74% for chemotherapy alone in such patients.

In a study performed in Iran on patients with multidrug resistant pulmonary tuberculosis, the results of immunotherapy were compared with an historical cure rate of less than 1 in 100 patients receiving chemotherapy alone [31]. After one to four injections of *M. vaccae* at intervals, 11 of 41 patients have been cured ($p < 0.00001$). Similar data are available from Vietnam and early reports of a study in India are also encouraging. In all of these studies the local response to injection of the *M. vaccae* has been no more severe than those elicited in children by BCG vaccination and generalised side effects have not been encountered.

It should be stressed that treatment failures (Table 2), including patients with multidrug resistance, offer the most rigorous test of an immunotherapeutic intervention as there is usually massive tissue destruction and dense scarring in the lungs.

Table 2. Pooled data from Iran and Vietnam on the response to immunotherapy of patients with drug resistant bacilli. The resistance patterns of patients responding or failing to respond to immunotherapy with *M. vaccae*. In most cases, the chemotherapy given was isoniazid (H), streptomycin (S), rifampicin (R) and ethambutol (E). A few patients received kanamycin (K) or pyrazinamide (Z).

Resistance patterns	Treatment outcome failures		successes
HSREK	11		5
HSRE	9		1
HSRK	2		–
HREK	1		–
HSR	13		6
HRE	5		2
HSE	2		1
HRK	1		–
HR	6		1
HS	3		1
HZ	1		–
HE	–		1
SR	2		–
SE	1		–
H	1		1
S	1		–
	23 of 59 (39%) resistant to 4 or more drugs	n.s	6 of 19 (32%) resistant to 4 or more drugs

Prospects for the future

With the combination of ultra-short chemotherapy and immunotherapy, we may be re-armed against the tubercle bacillus in a way that we have not been since the discovery of streptomycin almost half a century ago. A killed vaccine based on *Mycobacterium vaccae*, which would be effective in all environments and in those already harbouring tubercle bacilli as well as in uninfected persons, should prove a worthy successor to BCG.

A number of questions and uncertainties, of course, remain. Will tuberculosis relapse if the effects of immunotherapy wear off? Will children given the killed vaccine develop lifelong protection? What will be the long–term impact of preventive immunotherapy in HIV-infected persons? Will immunotherapy play a part in the control and treatment of opportunist mycobacterial disease? What other diseases associated with T cell mediated damage are amenable to immunotherapy?

The challenge of tuberculosis, especially since the advent of HIV infection and the emergence of multidrug resistance, is enormous. Revolutionary approaches are essential for facing this challenge. The chemotherapeutic approach alone has not led to victory but perhaps its combination with immunotherapy will.

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Errata

Volume 27 No. 2, April 1993

Pages 111–5. West: **A look at the statistical overview**

The legends to Figs 1 and 3 should be interchanged.

Fig 1 shows a statistical overview of seven cohort studies in acute myeloblastic leukaemia.
Fig 3 gives a statistical overview of 17 trials of rehabilitation after myocardial infarction.

Page 172. Moxon: **Microbes, molecules and man**

Fig 5 – the second sentence of the legend should read: 'The *bex* genes (A,B,C,D, indicated by light grey rectangles) are provisionally considered to be required for export of the polysaccharide from across the cytoplasm membrane.'