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Tuberculosis Vaccines
Current Progress

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Abstract

Tuberculosis continues to be a major cause of disease and death throughout the developing world. Chemotherapy is the current method of control but with the continuing emergence of drug resistance, coupled with the reticence of major drug companies to invest in drug discovery, the identification of new vaccines to combat tuberculosis is a pressing need. Rational vaccine design requires knowledge of the protective immune response and, while this is not fully understood, it is clear that induction of a T-helper-1 type of immunity is critical to host resistance. A variety of animal models, but especially the mouse and guinea pig, can be used to determine the protective efficacy of new vaccines. These mostly consist of relatively short-term prophylactic models in which animals are vaccinated and then challenged by the aerosol infection route to determine their capacity to reduce the lung bacterial load. Several promising vaccine types have emerged, including subunit vaccines, DNA vaccines and vaccines based upon living vectors, such as recombinant bacillus Calmette-Guérin (BCG) vaccines and auxotrophic or gene disrupted mutants of *Mycobacterium tuberculosis*. A few of these have already entered early stage clinical trials.

The current tuberculosis global pandemic continues unabated, with close to 3 million deaths per year, over 8 million new cases, about 14–18 million active cases and several hundred million people harbouring bacilli in some form of latent state.[1] In sub-Saharan Africa over the past few decades the increasing incidence of tuberculosis has been driven by the HIV epidemic, and as this virus continues to spread in areas such as India and China we can anticipate further increases in tuberculosis in these regions as well.[2]

Chemotherapy of tuberculosis requires the lengthy administration of at least four drugs, which is expensive.[3] Patient compliance remains a big problem, hence the emergence of the 'directly observed therapy' approach where daily ingestion of the drugs is monitored along with a reward incentive of some kind. However, in the longer term, vaccination seems the answer, especially if the material needs to be given only once or twice and is relatively inexpensive to produce. At the moment, however, chemotherapy is the only viable option; the existing vaccine (bacillus Calmette-Guérin [BCG]) is ineffective in adults and there are no well tested alternatives.[4]

This situation is gradually changing and we can expect to see relatively large-scale clinical trials with a few new vaccine candidates in the near future. These may include fusion proteins (polyproteins), recombinant BCGs and virus-delivered boosting antigens. This is grounds for optimism, but we need to continue to test new candidates in rele-

vant animal models to feed these into the clinical pipeline. It may be several decades before we can see or measure truly significant effects on preventing tuberculosis, but we must start now or it could be too late.

1. Cell-Mediated Immunity to Tuberculosis

The basis of the infection process in establishing tuberculosis remains unknown.[5,6] Bacilli that penetrate the alveolar spaces are probably picked up in the surfactant by alveolar macrophages. The majority of the time this is the end of the story, as the bacilli are then killed by purely innate mechanisms. However, what happens next in the minority of exposed people is still unclear. Surviving bacteria begin to divide in their intracellular niche and, at the same time, this may be associated with adherence and extension of the macrophage across the alveolar wall. Somehow, at that point, bacteria erode across the basement membrane into the interstitium, thus establishing infection. The basis of this erosion is unknown, but could be due to production of certain mycobacterial proteins, such as culture filtrate protein (CFP)-10 and early secretory antigenic target (ESAT)-6, which have been implicated in cell cytolysis.[7]

Within the interstitium, bacteria are then engulfed again by macrophages, almost certainly including dendritic macrophages. Soluble inflammatory mediators such as histamine and prostaglandins are produced by local tissue cells, whereas macrophages produce an array of cytokines and chemokines that are proinflammatory in activity. As a result, the local site of infection swells with tissue fluid, facilitating the influx of cells crossing in from the blood.

These cellular mediators probably diffuse out across the lung tissues, and this may explain the local nonspecific activation of natural killer cells in the lungs. However, these cells do not appear to directly contribute to host resistance since depletion of this cell subset does not affect the bacterial load.[8]

Bacteria are carried from the primary lesion to lymph nodes or into the lymphoid tissues around the bronchi, and in the guinea pig model (perhaps the closest model to humans) rapid lymphadenopathy of the draining hilar lymph node cluster is a major aspect of the early disease process. It is not known what percentage is carried there by infected dendritic macrophages versus free bacteria somehow finding their way into lymphatic capillaries.

Dendritic macrophages infected with *Mycobacterium tuberculosis* undergo a process consistent with maturation, and both these and other macrophage types expand considerably in the lung tissues as the disease process gets underway.[9] Foamy macrophages, long known to be a component of the lung granuloma, start to express DEC-205, a marker of less mature dendritic macrophages, raising the intriguing possibility that these are of dendritic cell origin.[10]

Two to three weeks into this process CD4+ T cells, and to a lesser extent CD8+ cells, begin to accumulate in the lung lesions. These cells, in concert with monocytes coming in from the blood, join local foamy and epithelioid cells in starting the process that leads to formation of the granuloma. The T-cell populations secrete interferon-γ in response to interleukin (IL)-12 and IL-23 signals from infected macrophages, leading to macrophage activation and slowing of the bacterial load.[11-14] As a result, the progressive stage of the infection slowly transforms into one of chronic disease in which the bacterial load is relatively static or increases only very slowly.[15]

The chronic stage has erroneously been identified by some laboratories as a period of latent disease, whereas in fact this remains a dynamic stage. The granuloma continues to develop, eventually entering a stage of necrosis and breakdown, while the T-cell response in the lungs continues to consist of cells with an activated effector phenotype ($CD44^{hi}$ $CD62L^{lo}$ $CCR7^{neg}$).[16] Interestingly, at this time CD4+ and CD8+ T-cell populations occupy different spatial distributions in the infected tissues.[17]

Activated effector cells are usually short-lived, at least in studies using the dangerous pathogen

Table I. Overview of animal models for vaccine testing

Animal	Pros	Cons
Mouse	Highly cost effective Huge number of immunological reagents available	Poor DTH responses Differences in pathology to humans
Guinea pig	Highly sensitive to low-dose aerosol Pathology similar to humans Strong DTH reactions	Expensive Limited number of reagents available
Rabbit	Good model of lung liquefaction	Modest number of reagents available Animal husbandry issues (bacteria shed in urine) Expensive
Cow	Highly sensitive Huge mineralisation response	Limited reagents Animal husbandry (space!)
Primate	Final evaluation step Similar pathology	Very expensive; availability issues Animal husbandry (dangerous) Response varies from animal to animal

DTH = delayed-type hypersensitivity.

ovalbumin. In the chronic disease state caused by *M. tuberculosis*, it is as yet unknown if these cells are of a longer-lived variety. Even so, they must be being continuously replaced and, therefore, the question is from where? In our own studies it appears that BCG vaccination establishes this type of cell population in the lungs, but they seem to arise from a subset of memory CD4+ cells kept as a reservoir in major lymphoid tissues such as the spleen.[18]

Although the existence of memory T cells in *M. tuberculosis* infection has been known for some time, newer mainstream information suggests that more than one subset is involved and that this is the case for both CD4+ and CD8+ immune populations. Present ideas[19] suggest that naive cells become activated effector cells, whereas others become effector memory cells and then have the capacity to become central memory. How one turns into another, how this is controlled and how new vaccines might induce one type of population rather than another is fundamental information that is still needed to improve rational vaccine design.

2. Animal Models Used for Vaccine Testing

Many animal species have been used or at least evaluated over the years as a means to test new tuberculosis vaccines. And yet, historically, much of the time this involved either variation on a theme regarding BCG or, alternatively, testing various bacterial fractions in an attempt to replace the BCG vaccine with a simpler non-living vaccine. However, not only was the latter unsuccessful, but it was done at a time when elements such as the Toll receptor system, not to mention the nature of the T-cell response itself, was still unknown.

Now, a few decades later, the field is facing the same questions as tuberculosis global rates continue to worsen and a substantial research effort has been mobilised, both in the US and in Europe, to search for new vaccines.

As I have argued on many occasions, there is no perfect animal model, but each model can provide potentially useful information (see overview in table I). The real limitation, of course, is the expense of the animal, including the costs of keeping the animal under stringent level III biosafety conditions. For that reason alone, most initial new vaccine screening is done in the mouse model. This model is by far the most cost effective, and it generates a strong T-helper (T_h)-1 CD4 response. Unlike humans (and guinea pigs), however, necrosis is not a primary element of the granuloma until later stages of the disease process.

In the simplest form of the model, mice are vaccinated and then a few weeks later infected with virulent *M. tuberculosis*. Intravenous challenge models were historically the norm, but many laboratories now use the more realistic low-dose aerosol model. At various times after infection, usually 30 days in most screening regimens, the bacterial load

in the lungs and other organs such as the spleen and liver is determined and compared with that in the controls (saline, adjuvant only, empty vector, etc.). Materials giving rise to protection can then be further evaluated, including for the degree to which they generate T_h1-type CD4+ and CD8+ cells, the longevity of the vaccine effect and whether presensitisation of the animal with environmental mycobacteria has any deleterious effect.[20]

Under the National Institutes of Health testing program at Colorado State University (Fort Collins, CO, USA), the candidates performing well in these assays (maybe 10%) go to the more expensive guinea pig model. A 'day 30' type assay is performed first, as in the mouse. Advantages of the guinea pig model include the fact that the vaccine itself can be tested for induction of a delayed-type hypersensitivity response to tuberculin (i.e. to see if it could interfere with this diagnostic test), as well as whether protection induced by the vaccine can influence the lung pathology. The course of this process in saline controls has recently been well documented,[21,22] including by 3-dimensional imaging.[23] Key changes induced by vaccination that can be documented include exclusion of an early eosinophilia, prevention of the development of central necrosis in granulomas and induction of smaller, more lymphocytic lesions.

A further assay that can be used is the long-term survival assay in which the guinea pig is tracked for mortality. Because BCG by itself protects animals for 50–70 weeks or more, this assay can be used for 'BCG boosting' effects that may not be evident if one relies entirely on short-term 'day 30-style' assays alone.[20] Because of the lifespan of guinea pigs, this model is also appropriate for assays in which the vaccine to challenge interval is extended, although this is rarely performed because of the expense.

The primate model is even more expensive but seems to be a worthy final evaluation step.[24-26] In fact, several vaccines have undergone evaluations in this model, including two promising fusion proteins described in section 3.2.

3. Types of New Vaccines Under Development

3.1 Subunit Vaccines

Although some attempts were made several decades ago to vaccinate animals with crude fractions of dead bacilli, including cell walls or ribosomal fractions (the latter of course loaded with CpG motifs), the field only really began to emerge in the mid-1980s with the careful description of subcellular fractions, including those expelled into the culture filtrate by Abou-Zeid and colleagues.[27,28] At the same time, resulting from studies into the relative protective capacity of live and dead bacteria, the concept emerged that the filtrate proteins produced by live organisms were the primary set of antigens that induced protective immunity.[29]

Since then, many vaccines based upon subunit pools have been tried, with varying levels of success. Culture filtrate pools are protective but do not make good candidates because of the difficulty of standardising for manufacturing and patenting. Several individual proteins have been shown (in short-term assays at least) to give strong protective responses, such as Ag85 and ESAT-6, but at the current time this seems to be just the tip of the iceberg, with most mycobacterial proteins yet to be purified and tested.

A further issue is that protein subunit vaccines require strong adjuvants. Several materials have been described that have the ability to induce T_h1 responses, including monophosphoryl lipid and the saponin QS21.[30] The field is at an interesting point at the moment as a large pharmaceutical company has recently acquired control of the most potent materials, and it is as yet unknown how willing they will be to make these materials available to the research field.

3.2 Polyproteins

Recombinant polyproteins made from more than one immunogenic protein or peptide have recently attracted some attention. As new molecules they can be patented, and a vaccine consisting of a single

protein chain is easier to manufacture and less expensive to make. Two current examples of polyprotein vaccines are the fusion protein Mtb72F, which has shown good activity in mouse and guinea pig models, including demonstrating that it can boost BCG and double the survival time in the guinea pig model,[31,32] and vaccines consisting of a combination of Ag85A and ESAT-6. These proteins, produced in the two configurations by two separate laboratories, both look promising.[33,34]

3.3 DNA Vaccines

DNA vaccines have the advantage of avoiding adjuvants in general but, whereas several laboratories have reported excellent results in mice and guinea pigs, results using DNA vaccines in humans have tended to indicate only very weak immunogenicity. DNA vaccines have been constructed to a range of mycobacterial antigens, and work well by themselves, especially in the context of prime boost strategies.[35]

By themselves, DNA vaccines seem to be safe and there is no evidence of chromosomal integration, but safety issues arising after the animal has been exposed to *M. tuberculosis* have been reported, indicating that this class of vaccines should be used with caution.[36,37]

3.4 Recombinant Bacillus Calmette-Guérin (BCG) Vaccines

Because of the inherent safety and low cost of BCG it has certain attractions as the basis of recombinant vaccines. As an example, BCG over-expressing Ag85A has been generated, and this vaccine engenders improved protection in the guinea pig model.[38] Another approach has been to introduce the *RD1* gene region into BCG, in other words returning the genetic information that is believed to have been lost from *M. bovis* when BCG was first made nearly a century ago. Interestingly, however, BCG containing *RD1* did not appear to increase protection.[39]

BCG can also be used to carry genes for toxins. A BCG-expressing cholera toxin, a potent adjuvant, has recently been produced,[40] as has BCG that produces the lysin molecule of *Listeria* spp.[41] The idea here is to allow the bacillus, or at least its contents, to escape to the cytoplasm resulting in better, and earlier, CD8+ T-cell sensitisation.

3.5 Mutants of *Mycobacterium tuberculosis*

The idea behind the approach of using *M. tuberculosis* mutants is that most people's immune system destroys this organism, indicating it is highly immunogenic (a fairly obvious conclusion). Hence, why not use *M. tuberculosis* as the vaccine, but in a form in which it cannot survive? To do this, genes that encode for certain pathways have to be disrupted. These include genes at the *RD1* region, which seem to encode for proteins that cause local cell cytolysis (permitting dissemination?) and pathways such as the pantothenate pathway in vitamin metabolism. Using one pathway alone might be risky but the use of two unlinked pathways seems safe, and all of the available data obtained in various mouse models seems to support this.[42,43] The organism appears to be able to linger long enough to generate T cells but is then completely killed by the host.

This is an interesting approach, and probably only in its infancy since there are multiple genes/loci that could be targeted for this vaccine strategy. However, whether it would be accepted by those conducting clinical trials as a safe vaccine strategy has yet to be seen.

3.6 Prime-Boost Strategies

Several laboratories have pioneered the prime-boost approach. This approach has certain attractions, not the least of which being that the initial immunity engendered by the widespread practice of giving BCG to neonates can be built upon. Mice given BCG and then Ag85A in mid-life are clearly boosted,[44] and similar results have been seen with various DNA or protein subunit candidates. The immune response to the BCG vaccine can be augmented by over-expression of mammalian cytokine genes within the bacterial DNA, and this approach allows multiple delivery methods.[45] Currently, the most advanced method is an modified virus Ankara (MVA)-based viral delivery system targeting

Table II. Vaccines entering clinical trials

Candidate	Vaccine type	Data available	References
Mtb72F in AS02 adjuvant	Subunit (fusion)	Data in mice, guinea pig, primate models. Short- and long-term protection data	25,31,32
Ag85-ESAT fusion in IC31	Subunit (fusion)	Data in mice, guinea pigs, primates. Potency of adjuvant in sustaining T_h1 memory immunity still unknown	26,33
rMVA-Ag85	Prime (BCG) boost (viral)	Excellent data in humans. Under test in South Africa	46,47
rBCG-Ag85	Recombinant BCG	Data in guinea pigs	38
rBCGΔure:Hly	Recombinant BCG	Data in mice. Designed to boost CD8 responses	49

BCG = bacillus Calmette-Guérin; **ESAT** = early secretory antigenic target; **MVA** = modified virus Ankara; **T_h1** = T-helper-1 cells.

Ag85A,[46-48] which is already under evaluation in Africa (see table II).

4. Conclusions

There has been an explosion in knowledge over the past decade regarding new tuberculosis vaccines and it is therefore gratifying to see several candidates now in the early stages of clinical trials. However, having said that, there are still two primary areas in which far more work needs to be done.

The first is in the proteomics and bioinformatics area. There was some early enthusiasm for 'algorithm-predicted epitopes' allowing direct design of human vaccines but none have emerged. Proteomics has told us a lot about the proteins of *M. tuberculosis*[50] but vaccines, not to mention diagnostics, still seem to be based upon just a few of them. There are many proteins in the filtrate alone that need to purified and tested in stand-alone assays.

The second area is in animal models. Many people feel that such models provide useful information and even those who do not still realise (with a few exceptions) that most regulatory bodies want to see animal data before allowing human exposure. However, most models tend to look at prophylactic events in relatively short assays, thus missing important data such as vaccine longevity and degree of generation of memory. As for testing vaccines that could be used as post-exposure therapeutic vaccines or for the resolution of latent disease, the current models are completely inadequate.

Recommended Further Reading: An international conference was held in Montreal, Canada in 2003 entitled "TB Vaccines for the World". Papers presented at that meeting were subsequently published in the journal *Tuberculosis*[51] in 2005 and will provide the reader with a wealth of further information.

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People with promising vaccine candidates but not the level III facilities needed to test them should consider contacting Dr Angelo Izzo, Colorado State University, Fort Collins, CO 80523, USA (NIH programme) or Dr Ann Williams, Health Protection Agency, Porton Down, Salisbury SP4 0JG, UK (EEC program) for further information.

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