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Mycobacterium tuberculosis: approach to development of improved strategies for disease control through vaccination and immunodiagnosis

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Tuberculosis is a major health problem throughout the world causing large number of deaths, more than that from any other single infectious disease. Estimates till date ascertain the fact that Tuberculosis (TB) is continuing to be the leading cause of death worldwide. The infection from single infectious agent *Mycobacterium tuberculosis* is killing about 3 million individuals every year and accounts for around 18.5% of all deaths in adults between the age group of 15 and 65. An average of 1.79 billion people, which constitutes roughly one-third of the world's population, is infected with the causative agent *M. tuberculosis and* is at risk of developing the disease. This situation highlights the relative shortcomings of the current treatment and diagnosis strategies for TB and the limited effectiveness of public health systems, particularly in resource-poor countries where the main TB burden lies. The timely identification of persons infected with *Mycobacterium tuberculosis* and rapid laboratory confirmation of tuberculosis are two key factors for the treatment and prevention of the disease. Novel molecular assays for diagnosis and drug susceptibility testing offer several potential advantages over the above methods including faster turnaround times, very sensitive and specific detection of nucleic acids, and minimal, or possibly no, prior culture. The need for new technologies for rapid diagnosis of tuberculosis is clear. Most studies of mycobacterial immunity attributes focus on proliferation of T cells, production of cytokines and cytolytic activity. A proper vaccine for tuberculosis can be developed by using a combination of antigens and adjuvants capable of inducing appropriate and long-lasting T cell immunity. Development of new vaccines against TB should include some important aspects learned from BCG use such as mucosal routes of immunization; revaccination of BCG immunized subjects, booster immunization and prime-boost strategy with wild-type BCG, and other vaccine candidates. Here, we review current and future strategies toward the rational design of novel vaccines against TB, as well as the progress made thus far, and the hurdles that need to be overcome in the near and distant future.

Key words: Immunodiagnosis, *Mycobacterium tuberculosis,* tuberculosis, vaccines

Introduction

Tuberculosis (TB), a disease which is both curable and preventable, still kills 2-3 million people every year (North et al 2004). After decades of neglect, the immense public health impact of TB is now widely recognized, and the development of new

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tools to combat and control the epidemic has become an international priority (Pai et al 2008). The current strategy for TB control is based on reducing the spread of infection through effective treatment of individuals with active disease and vaccination of children (Whittaker et al 2008). The WHO has initiated the Directly Observed Therapy (DOTS) campaign in many regions, but so far this programme has not been able to control the global TB epidemic or prevent the increase in Multi-Drug Resistant (MDR) strains of *Mycobacterium tuberculosis* (Starke et al 2003). The goals have been to develop reliable procedures that can detect and identify mycobacteria directly in clinical specimens, methods for testing anti mycobacterial drug susceptibility, and methods for assessing bacillary loads in tuberculosis patients to determine the efficacy of chemotherapy. The significant advances that have been made in the last decade towards these goals are described in this review. The current TB vaccine *Mycobacterium bovis* bacille Calmette-Guérin (BCG) is the most widely used vaccine worldwide (Horwitz et al 2000). BCG provides efficient protection against TB in newborns, but does not prevent the establishment of latent TB or reactivation of pulmonary disease in adults (Roche et al 1995). Being a viable organism, the activity of BCG depends on its initial replication, and it therefore cannot be used as a booster in an adult population that is already sensitized by prior BCG vaccination, exposure to environmental mycobacteria or latent TB (Trunz et al 2006). A novel, effective vaccination strategy against adult pulmonary TB is therefore a crucial goal and an active field of research, development and clinical evaluation.

Immunodiagnosis of Tuberculosis

Identification of Mycobacterial Species from Culture

Mycobacterial isolates have traditionally been identified to the species level based on their reactions in a series of phenotypic and biochemical tests. However, the biochemical reactions of isolates of the same species may vary from each other and from time to time, and in many cases no definitive identification is obtained (Abebe et al 2007). Because biochemical testing is slow, cumbersome, and may yield ambiguous results, laboratories are increasingly using molecular methods for species identification (Lalvani and Pareek 2010).

Amplification techniques for direct detection

The polymerase chain reaction

The advent of nucleic acid probe technology offered promise of rapid, specific and direct microbial detection in clinical samples. However, laboratory experience demonstrated that if the number of target molecules in a clinical sample is low, the sensitivity of nucleic add probes was unacceptably low (Haldar et al 2009). With the description of the polymerase chain reaction (PCR) for amplification of nucleic acids in 1987, researchers in the field quickly recognized the technology's potential to provide more sensitive tuberculosis diagnostics and possibly obviate the need for mycobacterial culture. By 1990, several PCR assays designed to amplify mycobacterial nucleotide sequences had been described (Takahashi et al 2008). Subsequently other amplification technologies were developed and applied to the detection of mycobacteria. Kox et al (1997) designed a multiplex PCR assay for co-amplification of the *M. tuberculosis* complex specific *IS6110* and a highly conserved stretch of the 16S rRNA. PCR products of this multiplex assay were analyzed in reverse cross blot hybridization with species-specific probes and a *Mycobacterium* specific probe (Brodie and Schnwger 2005). This multiplex PCR enabled identification of *M. tuberculosis* and the most important opportunistic mycobacteria in clinical

specimens (Takahashi et al 2008). An added advantage was the ability to detect simultaneous infections by more than one mycobacterial species.

Targets for detection

A suitable target for amplification may be a single copy gene in the mycobacterial genome or one that is present as a repeated sequence (Andersen et al 2007). The choice of target and design of primers within the gene target are equally important in terms of assay sensitivity and specificity. Both genus-specific and species-specific gene targets have been utilized. Some of the targets include the genes for insertion elements (IS6JJO, IS1081), immunodominant antigens (38-kDa antigen (Pab), 65-kDa protein, MPB70 (18kDa) (Barry et al 2009). 85 protein complex (30/32 kDa), (MPB64) and ribosomal sequences (16S rRNA 23S rRNA) (Covert et al 2001). Predominant among these is the insertion sequence IS6JJO/IS986 which is typically present in multiple copies in *M. tuberculosis* (Chen et al 2009).

Commercial amplification tests

The commercial PCR test for the detection of *M. tuberculosis* complex is marketed by Roche Diagnostic Systems. The Roche Amplicor MTB amplifies a region of the 16S rRNA sequence that is genus specific and detects PCR products by hybridization with a *M. tuberculosis* complex-specific probe (Tascon et al 1996). Another version of this test available and employs additional species-specific probes that allow detection and identification of *M. avium* and *M. intracellulare*. The Gen-Probe Amplified *Mycobacterium tuberculosis* Direct (MTD) Test is a transcription-mediated amplification (TMA) system (Andersen 2001). The basis of TMA is conversion of the target (in this case 16S rRNA) into eDNA by reverse transcriptase, using a primer containing an RNA polymerase promoter. The product can therefore be transcribed by RNA polymerase to produce large numbers of RNA transcripts, which become templates for reverse transcription and further transcription in a cyclic geometric amplification. RNA products are detected by a hybridization protection assay that uses an acridinium ester labeled DNA probe complementary to the rRNA target (Chambers et al 2002). The MTD test can detect <103 copies of rRNA, equivalent to one bacillus, and <5 bacilli even in the presence of a high number of unrelated organisms, thus being *M. tuberculosis* complex specific (Cole et al 1998). Increased sensitivity of mycobacterial detection can also be achieved with probe amplification technologies, one such method being the ligase chain reaction (LCR).

Diagnosing extrapulmonary tuberculosis with amplification methods

The real value of PCR diagnosis is in situations where the clinical picture is less clear or where smear and culture are less reliable. It is extra-pulmonary tuberculosis (meningitis, pleuritis, peritonitis, pericarditis, lymph node tuberculosis, skin tuberculosis, etc.) for which a rapid and accurate laboratory diagnosis would be most beneficial. Limitations of smear and culture are due to the lower number of organisms normally present in these types of specimens (Anderson et al 2000). There have been many investigations of PCR amplification of cerebrospinal fluid for the diagnosis of tuberculosis meningitis (Pai 2010). These were limited by the small numbers of patients studied, lack of corresponding culture results, and inadequate clinical diagnoses (Dosanjh et al 2008). More clinical studies are needed to evaluate the diagnostic yield of PCR in extrapulmonary tuberculosis.

Species identification of cultured mycobacteria

Nucleic acid probe identification

Nucleic acid probes for the identification of

M. tuberculosis complex and *M. avium* complex were introduced by Gen-Probe in 1987. Use of these radio isotopic DNA probes for rapid identification of cultures at the species level was the first application of molecular biology techniques in the clinical mycobacteriology laboratory. By 1990, chemiluminescent probes (AccuProbes) were available, and probes for speculating *M. kansasii* and *M. gordonae* had been developed. The AccuProbes are single stranded oligomers complementary to the rRNA of these particular species of mycobacteria.

Polymerase chain reaction combined with restriction enzyme analysis

Amplification of a highly conserved gene combined with restriction enzyme analysis of PCR products has been applied to the identification of several commonly encountered mycobacterial species. Plikaytis et al (1992) were the first to describe such an assay in which a portion of the highly conserved heat-shock protein 65 *(hsp65)* gene was amplified using primers common to all mycobacteria and the PCR product digested separately with two restriction enzymes. Telenti et al. (1993) developed a similar method which differed in the 65-kDa primers and restriction enzymes used. A third method described by Vaneechoutte et al. (1993) was based on the 16S rDNA target. With these methods the restriction fragment patterns were distinctive for *M. tuberculosis, M. bovis, M. avium, M. intracellulare, M. kansasii* and *M. gordonae;* however, the patterns occasionally varied within a species. Members of the *M. tuberculosis* complex consistently displayed the same patterns and could not be differentiated on a species level (Hernandez-Pando et al 2000). Strains of *M. avium* were tightly clustered, whereas *M. kansasii, M. intracellulare* and *M. gordonae* each showed greater variability within their clusters (Mahairas et al 1996).

Nucleic acid sequence determination

Direct sequencing of mycobacterial genes has become an increasingly important method for identifying mycobacterial species (Muttucumaru et al 2006). This approach is also useful for the detection of growth-deficient mycobacterial species directly in clinical specimens and in taxonomic characterization of mycobacterial strains (Behr et al 1999). Genes that have been examined include those for the 165 rRNA, *dnaJ,* superoxide dismutase, *hsp65,* and 32-kDa protein (Muttucumaru et al 2006). In general, the gene chosen for PCR and sequencing based identification should be found in all relevant mycobacterial species and not in other bacteria, it should contain enough sequence diversity between different species to allow for easy identification, and there should be very little variation among the strains belonging to one species (Bellamy 2005). The 165 rRNA gene, with its conserved and variable regions, has become the preferred target.

LINE-PROBE assay

The Line Probe assay (LiPA; Inno-Genetics N.V., Zwijndrecht, Belgium) has been developed for rapid detection of RIF resistance (de Beenhouwer et al 1995). The test is based on the reverse hybridization method, and it consists of PCR amplification of a segment of the *rpoB* gene and denaturation and hybridization of the biotinylated PCR amplicons to capture probes bound to a nitrocellulose strip. The bound amplicons are then detected with alkaline phosphatase-conjugated streptavidin and BCIP/NBT chromogen, producing a color reaction. The LiPA test strip contains five probes for wild-type *rpoB* sequences and four probes for specific *rpoB* mutations, in addition to a conjugate control and *M. tuberculosis* control probes. The interpretation of the banding pattern on the strip allows for identification of *M. tuberculosis* complex and

detection of *rpoB* mutations. The test can be performed on *M. tuberculosis* cultures or directly from clinical specimens (Hirano et al 1999). The turnaround time for the test is, 48 h. Overall concordance of the LiPA test with phenotypic susceptibility testing and direct sequencing, when performed from cultures, has been reported to be good, varying from 92.2% to 99.0%. Although the LiPA test only detects 4 of the 35 distinct *rpoB* mutations, 75% of the RIF-resistant clinical isolates carry 1 of the 4 mutations, making the LiPA test a useful method for rapid detection of RIF resistance (Traore et al 2000). However, the test cannot be used for detection of rare mutations.

DNA microarrays

DNA microarray technology described for mycobacterial species identification can also be used for rapid detection of mutations that are associated with resistance to TB drugs. This approach has the potential of becoming the most effective and rapid method for detection of drug resistance mutations in *M. tuberculosis*.

The future of molecular diagnostics

Recent technological advances have enabled the clinical mycobacteriology laboratory to detect *M. tuberculosis* in clinical specimens and to screen for resistance to the commonly used anti tuberculosis drugs within 24-48 h. Many clinical laboratories are routinely using either a commercial amplification system or an in-house PCR assay to test acid-fast, smear-positive respiratory specimens for primary diagnosis. Unfortunately, these rapid diagnostic tests have not replaced acid-fast smears or mycobacterial cultures. Smear microscopy provides an index of the degree of contagiousness, facilitating informed decisions regarding public health measures.

Mycobacterial cultures allow determination of complete drug susceptibility profiles, which are recommended for all patients to ensure optimal treatment. High sensitivity and specificity must be achieved with all sample types before amplification techniques can replace classic diagnostic methods (Cardona 2007). High specificity can be achieved if the laboratory staff is properly trained and complies with the stringent quality control requirements. Lack of sensitivity most likely results from the use of small sample volumes and irregular dispersion of the organisms in the paucibacillary samples (Anderson et al 2000). These shortcomings suggest the need for improved sample preparation methods and/or the performance of more than one test on each sample. Among the most promising recent developments is the microarray technology (DNA chips) which, combined with DNA or RNA amplification, could provide rapid identification of a wide range of mycobacterial species and drug susceptibility results.

Selection of candidate vaccines

The ideal TB vaccine would prevent the primary acquisition of infection in naive individuals (pre-exposure), post-primary or reactivation of latent *M. tuberculosis* infection in adults already infected (post-exposure), and would be equally active against all drug-sensitive and resistant strains. A perfect vaccine would also be effective in active disease, allowing shortening and simplification of treatment regimens. It would be safe in all age groups, the effects would be long-lasting (without the need for repeat boosting) and would also be safe in HIV infection and active TB disease. Desirable characteristics for new vaccines against tuberculosis are summarized below. It is not clear that a single new vaccine will be able to meet all criteria. For example, it is possible that certain antigens and immune responses (e.g., antibody) may be more effective in preventing bacteremic or disseminated disease and that other antigens or antigen delivery systems and immune responses (e.g., cytotoxi-

city) will be more effective in preventing reactivation of latent infection (Connolly et al 2007). Immunogenicity in persons with previous BCG immunization would be desirable in vaccines targeted for imminent testing (Brandt et al 2002). Candidate vaccines should be economical, and single-dose vaccines would be preferable to those that require multiple doses or boosters. It would be advantageous to have a vaccine that does not require parenteral immunization, although this goal seems unlikely to be achieved in the near future.

Desirable characteristics of a vaccine for the prevention of tuberculosis:

- Induction of both humoral and cellular immune response.
- Immunogenicity in both newborns and mycobacteria-experienced adults.
- Immunogenicity in HIV infection.
- Efficacy against primary disease, including pulmonary and bacteremic disease (meningeal, disseminated).
- Efficacy against reactivation disease, including pulmonary, disseminated, and other extrapulmonary disease.
- Efficacy in persons with previous BCG immunization (an interim advantage but not an ultimate requirement).
- Prolonged duration of immunity or effective booster regimen.
- Safety in newborns, adults, and immune suppressed persons, including those with HIV infection.
- Low cost.

Environmental mycobacteria and bacille Calmette-Guerin failure

The immunological cross-reactions between the various mycobacteria that cause disease and that with BCG and the ubiquitous non-pathogenic mycobacteria in the environment, raise paradoxes that we have not resolved and that threaten to undermine new vaccines. BCG vaccination at birth, prior to sensitization to any other mycobacteria, confers substantial protection against the relatively noninfectious childhood forms of tuberculosis but protection probably declines with time after vaccination (Cruz and Starke 2010). Vaccination given later gives protection that ranges from high (e.g. almost 80% in the UK) (van Altena et al 2011) to zero (e.g. in South India) against the predominantly pulmonary and infectious adult forms. Exposure and sensitization to environmental mycobacteria (and *Mycobacteria tuberculosis*) is relatively light in the UK so that declining protection probably reflects declining immunological memory, but it is not known if repeating the BCG vaccination would make a difference (Bishai and Mercer 2001). In contrast in South India, and in other regions where BCG efficacy against tuberculosis is low, exposure and sensitization to environmental mycobacteria is intensive (Udwadia et al 2012). Indeed, it was the prediction that environmental mycobacteria might interfere with BCG that led to the sighting of the major controlled clinical trial in South India that duly failed to show any protective effect. However, it is still not clear whether sensitization by the environment can confer full protection (equal to the maximum effect of BCG), (Brooks et al 2001) thereby leaving no room for BCG to add anything, or if the immune system becomes subverted so that protective responses can no longer be evoked by BCG, perhaps even enhancing harmful responses (Behr 2001). The distinction is important because it could profoundly affect the whole approach to finding something better than BCG.

Features required of a replacement vaccine

Whatever the mechanism whereby environmental sensitization reduces BCG efficacy against

tuberculosis, the primary requirement of a replacement for BCG is that it should be significantly more efficacious in those same environments. The best bet for achieving this would be to aim to have a vaccine that produces a protective response in infancy that is both (i) intrinsically stronger and (ii) long lasting than that normally induced by BCG or tuberculosis infection. If it is to be used beyond infancy, or as a therapeutic vaccine, it should probably also be capable of redirecting a pre-existing non-protective response. Since disease is usually a consequence of regrowth of the bacteria at secondary sites of infection after dissemination, often after long periods of quiescence, a vaccine that minimizes persistence and reactivation of dormant bacteria is also desirable.

Vaccine approaches

Recombinant mycobacteria

The recombinant mycobacterium is the most difficult approach and is primarily motivated by the desire to use BCG as a vehicle to express antigens cloned from unrelated pathogens. Such a multivalent vaccine might protect against a range of diseases (Bappaditya et al 2011). Although doubts about the stability of long-term expression of foreign genes remain, the technical feasibility is demonstrated by the integration and expression of foreign proteins in BCG and by the immunological activity of foreign proteins in BCG (Macedo et al 2011). Most interestingly, BCG has been engineered to secrete murine cytokines that can change the nature of the immune response, although it is not clear how acceptable these would be for use in a prophylactic vaccine (McShane et al 2004, Bappaditya et al 2011). Auxotrophic mutants of BCG have been generated with a view to having a vaccine vehicle that is safer to deploy than the original strains now that we have entered the AIDS era with its attendant increased risk of live vaccines multiplying to cause disease in severely immunodeficient people (Horwitz et al 2000).

Other recombinant vehicles

Efforts to use recombinant vaccinia and other pox viruses as vectors of mycobacterial genes to protect against mycobacterial disease have so far had only limited success, perhaps because of residual activity of viral proteins that interfere with immune responses (Macedo et al 2011). In the most recent study, although other antigens did not give protection, vaccinia vectors expressing the 38-kDa antigen, now known as PstS-1 (formerly PhoS, pab. Ag78 or Ag5) (Grode et al 2005), or indeed expressing the 19-kDa antigen that abrogated protection when expressed in *M. vaccae,* protected mice from *M. tuberculosis* challenge given 2 weeks later (Skeiky et al 2004). *Streptomyces lividans* and the more distantly related bacteria, *Escherichia coli* and *Salmonella typhimurium*, have also been briefly tested as potential vaccine vehicles for mycobacterial antigen (the 65-kDa heat-shock protein (hsp65)) but failed to generate protective responses (Grode et al 2005).

Advantages

- Those vectors that are not only safe but also easy to grow and store can be chosen.
- Antigens which do not elicit protective immunity or which elicit damaging responses can be eliminated from the vaccine.

Disadvantages

- Since the genes for the desired antigens must be located, cloned, and expressed efficiently in the new vector, the cost of production is high.
- When engineered vaccinia virus is used to vaccinate, care must be taken to spare immunodeficient individuals.

Protein subunit vaccines

Secreted proteins

The subunit approach, originally aimed at using purified proteins or other chemically homogeneous fractions directly as a vaccine, has evolved since the initial focus on antigens that are present in whole bacterial extracts and give good antibody responses. It was realized that antigens released by multiplying bacteria may be among the most important for protection and these would not necessarily be either present in extracts or be good antibody inducers. Culture filtrates that are collected during the early phase of mycobacterial growth and before bacterial lysis has occurred contain a large number of proteins (Seibert 1934). Novel antigens are present, to which T cells from purified protein derivative (PPD)-sensitive people and BCG-vaccinated mice give strong specific cellular responses (Olsen et al 2001). Responses to culture filtrate antigens appeared early during infection in mice and in humans, suggesting that some of these antigens at least were related to, and probably identical to, those released by live mycobacteria during the early stages of infection.

Advantages

- If the peptide that induces protective immunity is identified; it can be synthesized easily on a large scale.
- It is safe and can be administered to immunodeficient and pregnant individuals.

Disadvantage

- Poor antigenicity. Peptide fragments do not stimulate the immune system as well as a whole organism vaccine.
- Since peptides are closely associated with HLA alleles, some peptides may not be universally effective at inducing protective immunity.

DNA vaccination

Perhaps the most remarkable recent development has been the realization that individual mycobacterial protein antigens can generate protection to a degree similar to that obtained with live BCG vaccine. Findings with purified proteins and adjuvants were summarized above, but the strongest evidence for this has come from studies in which mycobacterial DNA is taken out of the bacteria and expressed directly within mouse cells (Fonseca et al 2001). Initially this was shown with the gene encoding hsp65 of *M. leprae* expressed from a retroviral vector in a mouse macrophage cell line that was used as the vaccine (Turner et al 2000). This was followed by the finding that protection could also be generated by direct DNA vaccination and that a range of different mycobacterial antigens can also be protective when they are expressed from plasmid DNA (Tascon et al 1996), including 36-kDa proline-rich antigen, Ag85, hsp70 and ESAT-6, PstS-1 and MPT83 (Macedo et al 2011, Aagaard et al 2009, Jain et al 2008**)**. The essence of this approach is that plasmid DNA encoding the antigen of interest is introduced directly into the normal tissues and cells of the body which then synthesize the antigen under the control of appropriate viral or eukaryotic promoters (Olsen et al 2001). This approach offers many attractions. For example, it avoids the problems of purifying proteins without denaturing them and offers a way of rapidly testing different combinations and mixtures, selecting the best epitopes, omitting or modifying unwanted epitopes, targeting different antigen-presenting pathways, incorporating DNA encoding cytokines, etc (Bappaditya et al 2011, Aagaard et al 2011).

Furthermore, tests in mice suggest that DNA vaccination of newborn infants could raise good immune responses. DNA vaccines have an inherent adjuvanticity that can play a strong part

Table 1 : Types of immunization strategies against tuberculosis and examples of candidate vaccines

Vaccine type	Description	Comments
Whole-cell live	Single dose replicates, induces mild infection and immune response	Durable immunity; safety in HIV infected persons a potential issue; may not replicate in persons with prior BCG or NTM infection (Guleria et al 1996)
Attenuated BCG or *Mycobacterium tuberculosis*	Mutant strains with nutritional deficiency (auxotrophs) or with other gene deletions	Some attenuated strains safe inimmunodeficient animals (Jackson et al 1999)
Proapoptotic BCG	BCG construct with diminished superoxide dismutase activity	Safe in animal model (Kernodle et al 2001)
Enhanced BCG (Ag85)	Recombinant BCG enhanced by over expression of Ag85	Safety in animals equal to parent BCG (Horwipz et al 2001)
BCG/IL-12 or ODN	BCG administered together with IL-12 or ODN to enhance Th1 response	Potential for enhanced virulence
Whole-cell inactivated	Multiple doses induce immune response, effective against tuberculosis in humans	Boosters required, expected to besafe in HIV infection (Freidag et al 2000)
Mycobacterium vaccae	Infection with NTM protects against tuberculosis	Requires multiple doses; safe in HIV Infection (Von-Reyn et al 2001)
ESAT/Ag85B	Combination of immunodominant secreted antigens	Multiple doses with adjuvant (Olsen et al 2001)
DNA	Candidate antigens in DNA, intracellular antigen expression to enhance cellular immune response	Safe in HIV infection (Mc Shane et al 2004)
Hsp65	Highly conserved antigen from *Mycobacterium leprae*	Safety of Hsp60 vaccine a potential Issue (Tascom et al 1996)
BCGrMVA-Ag85	BCG, followed by MVA expressing Ag85	BCG vehicles may not replicate in those with prior BCG immunization (Huygen et al 1996)
DNA ESAT + MPT63-MVA	DNA vaccine, then MVA expressing same 2 Ags	- (McShane et al 2004)

in biasing the immune response towards type 1 cytokine production and this is exactly the kind that is needed for tuberculosis (Bappaditya et al 2011, Cruz et al 2010).

Advantages

- DNA is very stable, it resists extreme temperature and hence storage and transport are easy.
- DNA sequence can be changed easily in the laboratory.
- The inserted DNA does not replicate and encodes only the proteins of interest.

- There is no protein component and so there will be no immune response against the vector itself.
- Because of the way the antigen is presented; there is a cell-mediated response that may be directed
- Against any antigen in the pathogen.

Disadvantages

- Potential integration of DNA into host genome leading to insertional mutagenesis.
- Induction of autoimmune responses: anti-DNA antibodies may be produced against introduced DNA.
- Induction of immunologic tolerance: The expression of the antigen in the host may lead to specific non responsiveness to that antigen.

Vaccines and strategies under development include whole-cell live, whole-cell inactivated, subunit, DNA, and prime-boost vaccines. Characteristics of these approaches and comments are summarized in table 1.

Future Vaccines for tuberculosis

Tuberculosis will be eliminated only if new, more effective vaccines are developed. Modern molecular genetics and biotechnology techniques should be applied rapidly to this to this problem. Because almost two billion people in the world already are infected with *M. tuberculosis,* vaccine research should address both preventing infection and halting progression of established infection to tuberculosis disease (Dorhoi et al 2011). The varying effect of BCG vaccines may be, in part, due to our imperfect understanding of the determinants of protective immunity against tuberculosis (Derrick et al 2011). The significant antigens of *M. tuberculosis* are poorly defined and difficult to produce owing to the complex structure and chemistry of the mycobacterial cell wall (Orme et al 2001). Because genetic factors may influence the response to BCG, environmental mycobacteria, and infection with *M. tuberculosis,* molecular genetics study in animal and humans should be broadened. Animal studies have revealed that genetically determined susceptibility to mycobacteria infection in mice is regulated by a gene called *Bcg, Ity*, or *Lsh. Bcg* gene (Muttucumaru et al 2006, Jain et al 2008), which has been indentified and characterized, codes for a membrane transport protein called Nramp. The actual function of Nramp is not yet fully understood, but it appears to influence susceptibility to tuberculosis disease. Mapping of the genome sequence of *M. tuberculosis* has been completed and should bring new light to potential vaccine development.

Innovative new approaches in tuberculosis vaccine development have been emerged, including

1. Plasmid DNA vector based vaccine, which delivers genes encoding antigens (Ag85 or hsp60) by use of vectors.
2. Recombinant and mutant BCG vaccines, which use BCG as the delivery vehicle with improvement.
3. Subunit vaccines, which use individual mycobacterial protein antigens to produce an immune response.
4. Attenuated *M. tuberculosis* vaccines, which lack the genes essential for virulence and contain the genes needed for protection.

All of these new strategies appear promising and perhaps one of these techniques or combination thereof and yield an effective new vaccines against tuberculosis.

Future approach

Major Field trials for BCG or any new tuberculosis vaccine will be tremendous challenge given the necessary scope, duration, and expense of adequate trials. There is a pressing need for

identification of some correlate of natural and vaccine-derived protective immunity based either an animal model or a measure of immune response in humans. Current animal models are inadequate, and human markers of protective immunity have not been determined. Tuberculin sensitivity after vaccination is not an adequate assay. Some advances have been made in diagnostic tests for tuberculosis, but there methods are still being developed and tested. A reliable serological test for latent tuberculosis infection and tuberculosis disease would make vaccine trials more feasible by requiring a shorter duration. Continued research on operational variables such as the age of optional vaccination and the role of booster vaccinations is needed. Finally, the research on the safety and efficacy of mycobacterial vaccines in HIV infected children and adults should be sought vigorously.

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