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Tuberculosis: Looking Beyond BCG Vaccines

Mustafa Abu S, Al-Attiyah R

Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait.

Abstract

Tuberculosis (TB) is an infectious disease of international importance and ranks among the top 10 causes of death in the World. About one-third of the world’s population is infected with *Mycobacterium tuberculosis*. Every year, approximately eight million people develop active disease and two million die of TB. The currently used BCG vaccines have shown variable protective efficacies against TB in different parts of the world. Moreover, being a live vaccine, BCG can be pathogenic in immunocompromised recipients. Therefore, there is an urgent need to develop new vaccines against TB. The comparative genome analysis has revealed the existence of several *M. tuberculosis*-specific regions that are deleted in BCG. The work carried out to determine the immunological reactivity of proteins encoded by genes located in these regions revealed several major antigens of *M. tuberculosis*, including the 6 kDa early secreted antigen target (ESAT6). Immunization with ESAT6 and its peptide (aa51-70) protects mice challenged with *M. tuberculosis*. The protective efficacy of immunization further improves when ESAT6 is recombinantly fused with *M. tuberculosis* antigen 85B. In addition, ESAT6 delivered as a DNA vaccine is also protective in mice. Whether these vaccines would be safe or not cannot be speculated. The answer regarding the safety and efficacy of these vaccines has to await human trials in different parts of the world. (J Postgrad Med 2003;49:134-140)

Key Words: Tuberculosis, BCG, *M. tuberculosis*-specific antigens, new vaccines

Tuberculosis is an infectious disease known to mankind since antiquity. The disease is prevalent throughout the world including the developing and industrialized countries. The global problem of TB is so grave that in 1993, the World Health Organization (WHO) declared tuberculosis (TB) “a global emergency”. In a relatively recent survey by WHO, it was estimated that one-third of the world population is infected with *Mycobacterium tuberculosis*, eight million people develop the disease and two million die of TB each year. Among the infectious diseases, TB is the topmost killer of adults and is among the overall top 10 causes of death in the world. If the present trend continues, about 60 million people will die from TB by 2020.

The global, regional and local problems of tuberculosis are worsening due to several factors including the increase in the incidence of multidrug-resistant (MDR) TB, the migration of people from high to low endemic areas and concomitant infections with HIV. All these factors have contributed to make TB a disease of both rich and poor people. In AIDS patients co-infected with *M. tuberculosis*, the immunodeficiency is associated with increased dissemination of TB, increased number and severity of symptoms, and rapid progression to death, unless prompt and specific treatment is provided. The combination of MDR TB and HIV infection has a very bad prognosis with a median survival of about two months only.

Global control of TB requires universally efficacious vaccines. The vaccine that is currently used to protect against TB is the vaccine was distributed around the world for the prevention of TB. Since then, BCG has been used extensively around the world to vaccinate humans against TB. Although BCG vaccine is the world’s most widely used vaccine it is one of the most controversial vaccines in current use. Estimates of protection imparted by BCG against pulmonary TB vary greatly. For example, BCG vaccination in British school children, in 1952, showed 77% to 84% efficacy, whereas in a relatively recent trial conducted in Chingleput, India, the protective efficacy was nil against pulmonary TB in adults. This variability has been attributed to variation of strains, de-
fects in the preparation of BCG, to environmental influences such as exposure to sunlight, poor maintenance of cold chain and to genetic or nutritional differences amongst populations, or exposure to environmental mycobacteria. The exposure to environmental mycobacteria may have the effect of reversing immunological benefits conferred by BCG vaccination, or equilibrating immunity between the control and the trial groups. Another explanation to account for the variable results in the trials of vaccine-efficacy could be a progressive over-attenuation of BCG during prolonged passage through the laboratory. Implementation of BCG vaccination faces two additional problems. Firstly, vaccination with BCG induces a delayed type hypersensitivity (DTH) skin response that cannot be distinguished from infection with M. tuberculosis and therefore it compromises the use of purified protein derivative (PPD) of M. tuberculosis (tuberculin) in skin tests for diagnostic or epidemiological applications. Secondly, BCG is a live vaccine with potential to cause disease by itself, particularly in immunocompromised subjects and therefore it cannot be used in all groups of people. In this regard, WHO recommends that children with symptoms of HIV or AIDS should receive all the vaccines except BCG. The shortcomings of BCG vaccine have provided the impetus for a lot of research to identify alternative vaccines for TB. One approach taken is the identification of M. tuberculosis-specific antigens with potentials for inducing protective immunity and further evaluation of such antigens in animal models of TB before conducting large-scale efficacy trials in humans.

Genomic hybridisation to identify M. tuberculosis-specific regions

Mahairas et al first described the existence of M. tuberculosis-specific genomic regions in 1996. By employing subtractive genomic hybridisation analysis to identify genetic differences between virulent M. bovis and M. tuberculosis and attenuated BCG, they identified three M. tuberculosis/M. bovis-specific genomic regions that were deleted in BCG. The regions deleted (RD) were designated as RD1, RD2 and RD3 (Table 1). RD3 was present in the virulent laboratory strains of M. bovis and M. tuberculosis and were deleted in all strains of BCG. However, RD3 was also deleted from the genomes of 84% clinical isolates of M. tuberculosis and therefore the antigens expressed from this region were not considered to be important for developing new vaccines against TB. RD2 was conserved in all virulent laboratory and clinical tubercle bacilli. However, this region was deleted in some but conserved in other sub-strains of BCG (Table 1). The analysis of studies conducted with different BCG strains failed to show the influence of RD2 encoded antigens on protection against TB, both in mice and in humans. Thus, antigens encoded by RD2 could also not be considered important for developing new vaccines against TB. In contrast to RD2 and RD3, the RD1 (a 9.5 kb DNA segment) was deleted from all BCG sub-strains but conserved in all the tested laboratory and clinical isolates of M. tuberculosis.

More recent studies indicate that the RD1 DNA segment is related to the virulence of M. tuberculosis as the pathogenic laboratory strain of M. tuberculosis H37Rv when artificially deleted of RD1, behaved very similar to BCG in human macrophages in vitro and C57BL/6 mice in vivo. Furthermore, knocking-in of RD1 in BCG (BCG::RD1) gave rise to bacilli which grew more vigorously than BCG in immunodeficient mice, inducing extensive splenomegaly and granuloma formation in the experimental animal. In immunocompetent mice, BCG::RD1 knock-in bacilli showed increased persistence and reversal of attenuation, whereas BCG controls were cleared. Knocking-in five other RD DNA segments did not affect the virulence of BCG. These studies suggested that the RD1 DNA segment contains genes that encode proteins involved in the virulence of M. tuberculosis, and therefore, an appropriate immune response against these proteins may lead to protective immunity against TB. In this context, RD1 was considered to be the most important deleted region to identify M. tuberculosis-specific antigens for the development of new vaccines against TB.

Bioinformatics of RD1

To initiate the work for identification of M. tuberculosis-specific antigens encoded by the RD1 DNA segment, it was necessary to identify the panel of deletion genes to be studied. In their original publication, Mahairas et al annotated eight potential open reading frames (ORFs) in the RD1 capable of encoding an equal number of proteins (Table 2). In the genome sequence of M. tuberculosis H37Rv, Cole et al. anno-

Table 1: Identification of M. tuberculosis genomic regions deleted in BCG by DNA hybridization

<table>
<thead>
<tr>
<th>Region Deleted</th>
<th>Deletion in</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD1</td>
<td>All BCG strains</td>
</tr>
<tr>
<td>RD2</td>
<td>Some BCG strains, i.e. Danish, Prague, Glaxo, Frappier, Tice, Pasteur, Connaught and Phipps</td>
</tr>
<tr>
<td>RD3</td>
<td>All strains of BCG, and some clinical isolates of M. tuberculosis</td>
</tr>
</tbody>
</table>

Studies indicate that the RD1 DNA segment is related to the virulence of M. tuberculosis as the pathogenic laboratory strain of M. tuberculosis H37Rv when artificially deleted of RD1, behaved very similar to BCG.
tated nine ORFs in this region, six of which matched those identified by Mahairas et al (Table 2). In another annotation attempt by Robertson and Thole, the RD1 sequence was analysed using GeneMark software. This software identifies ORFs based on initiation and stop codons, comparison of ORFs sequences to sequences in the database using probabilistic statistics, and analysis of the ribosomal sites sequence. A total of 16 potential ORFs were identified (Table 2).

Immune responses to RD1 ORF proteins

To immunologically characterise the putative proteins encoded by M. tuberculosis-specific RD1 ORFs, we first attempted to clone and express six of the ORFs (ORF10 to ORF15) as recombinant proteins in Escherichia coli. However, we were successful in expressing five and purifying only two (ORF11 and ORF14) of the six targeted proteins. The problems included low level of expression, degradation of the mycobacterial proteins and the presence of contaminating E. coli proteins in purified preparations. The problem of purification could be sorted out by designing modified plasmid constructs capable of providing new strategies for efficient purification of recombinant M. tuberculosis proteins expressed in E. coli.

When tested for serological reactivity, the purified ORF14 protein was frequently recognized by antibodies present in the sera of TB patients, but not healthy BCG vaccinated donors. However, when tested with peripheral blood mononuclear cells (PBMC) from TB patients and healthy donors for cell mediated immune (CMI) responses, ORF11 and ORF14 proteins showed weak reactivity in antigen-induced proliferation and IFN-γ assays (unpublished data). The weak CMI responses to these proteins were further confirmed in mice and guinea-pigs after active immunization with recombinant BCG and Mycobacterium vaccae expressing ORF11 and ORF 14 proteins (unpublished data).

To analyse the M. tuberculosis/M. bovis specific RD1 ORFs for immunological reactivity, a novel approach of overlapping synthetic peptides was subsequently used. An example of such overlapping peptides covering the sequence of ORF3 protein is shown in Figure 1.

Each peptide was 25 aa in length and overlapped with the neighbouring peptides by 10 aa. Since the length of Th1 cell epitopes is usually between 8 to 10 aa, the overlapping synthetic peptide strategy minimized the possibility of missing the potential Th1 cell epitopes. A total of 220 peptides were synthesised using fmoc chemistry and peptide pools corresponding to each ORF protein of RD1 were tested for reactivity with human PBMC in antigen-induced proliferation and IFN-γ assays. The tested proteins were designated as major and modulator stimulators when positive responses were observed in over 50% and between 30 and 50% donors, respectively. The analysis of the results showed that the RD1 region contained three major (ORF5, ORF6 and ORF7) and three moderate (ORF2, ORF3 and ORF9) antigens stimulatory for Th1 cells in IFN-γ assays (Table 3). However, ORF5 and ORF9 proteins were equally good stimulators of Th1 cells from TB patients and healthy donors, whereas ORF3, ORF6 and ORF7 proteins were better Th1 cell stimulators in TB patients and ORF2 protein in healthy donors (Table 3).

When tested with PBMC in a cattle model of TB, ORF2, ORF3, ORF5, ORF6 and ORF7 proteins were also found to be serologically reactive.

| ORF1 (Rv3870 in the Cole annotation) and ORF16 (Rv3880) represent flanking ORFs that are present in BCG. ORF2 (Rv3871) is a potential regulatory protein. ORF3 (Rv3872) and ORF5 (Rv3873) encode PE and PPE proteins, respectively, with ORF4 representing a potentially overlapping ORF on the opposite strand. ORF6 (Rv3874) and ORF7 (Rv3875) encode low molecular weight proteins- CFP10 and ESAT6 present in the short-term culture filtrate of M. tuberculosis. ORF8 is a potential ORF on the reverse strand, and ORF9 (Rv3876) encodes a member of a family of M. tuberculosis proline-rich proteins. The product of ORF10 (Rv3877) belongs to a conserved hypothetical family of M. tuberculosis membrane proteins. ORFs 11 and 13 correspond to the unknown ORFs annotated as Rv3878 and Rv3879 by Cole et al. ORF12 is the unknown ORF1G suggested by Mahairas et al. ORF14 overlaps ORF13 but on the positive strand. ORF15, also on the positive strand, is predicted to encode a low molecular weight protein (Table 2). |

| Table 2: Annotation of ORFs in the RD1 segment of M. tuberculosis genome |
|---------------------------------|------------------|-----------------|------------------|
| Annotation by Mahairas et al. | Cole et al. | Robertson and Thole | Protein length | Comments |
| ORF1A | Rv3870 | ORF1 | 261 | Present in BCG |
| ORF1B | Rv3873 | ORF5 | 371 | Hypothetical |
| ORF1C | Rv3875 | ORF7 | 95 | ESA T6 |
| ORF1D | Rv3876 | ORF9 | 666 | Proline rich |
| ORF1E | Rv3877 | ORF10 | 552 | Membrane protein |
| ORF1F | Rv3878 | ORF11 | 280 | Hypothetical |
| ORF1G | Rv3879 | ORF12 | 563 | Hypothetical |
| ORF1K | Rv3880 | ORF13 | 746 | Hypothetical |
| ORF1F | Rv3880 | ORF15 | 95 | Hypothetical |
| ORF1E | Rv3880 | ORF16 | 115 | Present in BCG |

When tested with PBMC in a cattle model of TB, ORF2, ORF3, ORF5, ORF6 and ORF7 proteins were also found to be serologically reactive.
be major stimulators of IFN-γ secreting cells present in the peripheral blood of *M. bovis* infected cattle (Table 3). However, PBMC from non-infected or BCG vaccinated animals did not respond to these proteins (Table 3). Thus, ORF5, ORF6 and ORF7 proteins represented major antigens recognised by Th1 cells in *M. bovis* infected cattle as well as in TB patients. Among these antigens, ORF6 (CFP10) and ORF7 (ESAT6) proteins when used as recombinant antigens or overlapping synthetic peptides have recently been reported as dominant Th1 cell antigens in TB patients with limited disease and patients recovered from TB after effective chemotherapy. In addition, both of these proteins have also been shown to induce IFN-γ responses in healthy donors exposed to *M. tuberculosis* or showing strong responses to complex *M. tuberculosis* antigens like whole cell preparations, cell walls and culture filtrate. Taken together, studies conducted by us and others show that RD1 gene products are strong stimulators of the protective type of Th1 cell responses and thus will be useful in designing new vaccines based on *M. tuberculosis* specific antigens. Furthermore, the studies also suggest that pools of synthetic peptides could be as effective and specific in monitoring T cell reactivity as the full-length recombinant proteins. Thus, overlapping synthetic peptides corresponding to *M. tuberculosis* specific genomic regions may help to identify antigens of protective importance.

### Immunodominant peptides of RD1 ORF proteins

To identify the immunodominant epitopes recognised by Th1 cells, the individual peptides of ORF3, ORF5, ORF6, ORF7 and ORF9 proteins were tested in antigen-induced proliferation and IFN-γ-assays with PBMC from TB patients and healthy donors. The results showed that a single peptide of ORF3 (P3.2, aa 16-40) was immunodominant for recognition by PBMC from TB patients, whereas none of the ORF3 peptides showed dominant recognition by PBMC from healthy donors (Table 4). Furthermore, peptide P5.9 (aa 121-145) of ORF5 was immunodominant in healthy donors as well as in TB patients (Table 4). In addition, both ORF6 and ORF7 proteins contained one peptide each, which were primarily recognized either by TB patients (P6.4, aa 46-70 and P7.1, aa 1-25) or by healthy donors (P6.2, aa 16-40 and P7.5, aa 61-85) (Table 4). In contrast, no single peptide could be labeled immunodominant in case of ORF9 protein as the responses with PBMC from healthy donors in antigen induced proliferation and IFN-γ assays were seen with peptides scattered throughout the sequence (unpublished data). A cocktail of the immunodominant peptides of ORF5, ORF6 and ORF7, which were primarily recognised by healthy donors in Th1 cell assays, could be useful as a peptide-based vaccine against TB.

### Promiscous Th1 cell antigens encoded by RD1 ORFs

Mycobacterial antigens are recognized by Th1 cells primarily in association with highly polymorphic HLA-DR molecules. Therefore, one of the requirements for any antigen to be effective in HLA-DR heterogeneous human population, to be considered as a candidate vaccine against TB is its recognition in association with a large number of HLA-DR molecules. The promiscuous nature of the major RD1 antigens was suggested when the sequences of ORF3, ORF5, ORF6, ORF7 and ORF9 proteins were analysed for binding to 51 HLA-DR alleles using the Proped graphical web tool for predicting MHC class II binding regions in antigenic protein sequences. All these antigens were predicted to bind more than 80% of the HLA-DR alleles studied (Table 5).

The recognition of RD1 ORFs, with ORF7 protein (ESAT6) as an example, was further confirmed in functional assays by

---

### Tables

| **Table 3: Major and moderate RD1-encoded Th1 cell antigens recognized by PBMC from humans and cattle** |
| **Tested group** | **Major Th1 cell antigens** | **Moderate Th1 cell antigens** |
| TB patients | ORF5, ORF6, ORF7 | ORF3, ORF9 |
| Healthy donors | ORF5 | ORF2, ORF6, ORF7, ORF9 |
| *M. bovis* infected cattle | ORF5, ORF6, ORF7 | ORF2, ORF3 |
| Non-infected cattle | None | None |
| BCG vaccinated cattle | None | None |

| **Donor group** | **Immunodominant peptides of RD1 ORFs** |
| TB patients | ORF3-P3.2 (aa 16-40), ORF5-P5.9 (aa 121-145), ORF6-P6.4 (aa 46-70), ORF7-P7.1 (aa 1-25) |
| Healthy donors | ORF5-P5.9 (aa 121-145), ORF6-P6.2 (aa 16-40), ORF7-P7.5 (aa 61-85) |

| **Table 4: Immunodominant peptides of RD1 ORF proteins recognized by PBMC obtained from TB patients and healthy humans in Th1 cell assays** |
| **Donor group** | **Immunodominant peptides of RD1 ORFs** |
| TB patients | ORF3-P3.2 (aa 16-40), ORF5-P5.9 (aa 121-145), ORF6-P6.4 (aa 46-70), ORF7-P7.1 (aa 1-25) |
| Healthy donors | ORF5-P5.9 (aa 121-145), ORF6-P6.2 (aa 16-40), ORF7-P7.5 (aa 61-85) |

| **Table 5: HLA-DR binding predictions for ORF3, ORF5, ORF6, ORF7 and ORF9 proteins of RD1 to 51 HLA-DR alleles** |
| **ORF protein** | **Protein length** | **% binding to HLA-DR alleles** |
| ORF3 (Rv3872) | 199 | 84.3 |
| ORF5 (Rv3873) | 368 | 80.4 |
| ORF6 (Rv3874, CF10) | 95 | 86.3 |
| ORF7 (Rv3875, ESAT6) | 100 | 80.4 |
| ORF9 (Rv3876) | 666 | 100 |

---

**A cocktail of the immunodominant peptides of ORF5, ORF6 and ORF7, which were primarily recognised by healthy donors in Th1 cell assays, could be useful as a peptide-based vaccine against TB.**
using HLA-typed antigen presenting cells and antigen-specific T cell lines. The results showed that multiple epitopes of ESAT6 were promiscuously presented to Th1 cells in association with HLA-DR and DQ molecules.35,36

**RD1 encoded antigens as candidate vaccines against TB**

After showing that a number of proteins encoded by *M. tuberculosis*-specific RD1 segment were strong Th1 cell antigens recognized promiscuously by PBMC in vitro, the next logical stage towards anti-TB vaccine development will be to demonstrate their protective efficacy in animal models of TB. The experiments performed with one of the proteins, i.e. ORF7 (ESAT6), strongly suggest the potential of the antigens encoded by the RD1 genes as a new vaccine. In these studies, mice immunised with ESAT6 in combination with appropriate adjuvants were protected against challenge with the pathogenic *M. tuberculosis*.37 Interestingly, the level of protection was comparable to that obtained after vaccination with live BCG.37 The protection studies were extended to determine the potential of peptide based vaccines by using single peptides from the ESAT-6 sequence as immunogens. These peptides were aa 1-20 and aa 51-70 and both of these peptides were previously shown to be recognized by Th1 cells secreting IFN-γ.38 When injected along with appropriate adjuvants, both the peptides induced IFN-γ secretion in mice. However, only peptide aa 51-70 induced protection that was equivalent to the level of protection achieved after vaccination with the complete ESAT6 molecule.38 The protective efficacy of ESAT6 was further enhanced after recombinantly fusing it with Ag85B,39 which is another major antigen of *M. tuberculosis* present in the culture filtrate and has been shown to provide protection in animal models of TB.40 In addition, DNA vaccine constructs expressing ESAT6 provided better protection in mice upon *M. tuberculosis* challenge than the protection provided by immunisation with DNA-MPT64;40,41 MPT64 is a major secreted T cell antigen encoded by a gene in the RD2 segment of *M. tuberculosis* genome.15,22 These results are highly encouraging with respect to the potential of subunit/peptide and DNA vaccines based on RD1 proteins as future vaccines to replace and/or supplement BCG.

**M. tuberculosis** genome sequence and identification of additional *M. tuberculosis*-specific regions

The phenomenal achievement of sequencing the complete *M. tuberculosis* H37Rv genome in 1998 by Cole et al.15 has opened up new frontiers of basic and applied research in tuberculosis, including the identification of new vaccine candidates. To identify additional *M. tuberculosis*-specific regions and genes, detailed comparative genome analyses of *M. tuberculosis* with BCG and other mycobacteria have already been performed.5,6,42 The results showed that in addition to RD1, RD2 and RD3, 13 other regions present in *M. tuberculosis* H37Rv (RD4 to RD16) were deleted in some or all strains of BCG. Among these deleted regions, 10 regions (RD4 to RD13) covering 108 ORFs of *M. tuberculosis* H37Rv are deleted from all BCG strains (Table 6). Three other regions (RD14 to RD 16) covering 27 ORFs are deleted only in some BCG strains (Table 6). By using the overlapping synthetic peptide approach, we are currently engaged in the identification of Th1 cell antigens and epitopes encoded by the genes present in these deleted regions. These studies may identify additional antigens as new vaccine candidates to protect against TB. In addition, the identification of major Th1 cells antigens present in some BCG strains, but lacking in others, may provide an explanation for variation in the protective efficacies of different strains of BCG.

**Delivery systems for new vaccines**

As described above, although, subunit vaccines in the form of recombinant antigens and synthetic peptides have been shown to induce protective immune responses in the animal models of TB, a long lasting immunity will most probably require appropriate delivery systems. In the recent years DNA plasmids carrying the genes for several mycobacterial proteins have been shown to induce protective immune responses to *M. tuberculosis* challenge in the animal models of TB.40,41,43,44 Other delivery systems include bacterial

<p>| Table 6: Identification of <em>M. tuberculosis</em> genomic regions deleted in BCG strains by comparative genome analysis |
|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>RD</th>
<th>Deletion in BCG</th>
<th>No. of ORFs</th>
<th>ORFs deleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>All strains</td>
<td>12</td>
<td>Rv1505c-Rv1516c</td>
</tr>
<tr>
<td>5</td>
<td>All strains</td>
<td>8</td>
<td>Rv2346c-Rv2353c</td>
</tr>
<tr>
<td>6</td>
<td>All strains</td>
<td>5</td>
<td>Rv3424-Rv3428</td>
</tr>
<tr>
<td>7</td>
<td>All strains</td>
<td>15</td>
<td>Rv1964-Rv1977</td>
</tr>
<tr>
<td>8</td>
<td>All strains</td>
<td>7</td>
<td>Rv3617-Rv3623</td>
</tr>
<tr>
<td>9</td>
<td>All strains</td>
<td>4</td>
<td>Rv2072c-Rv2075c</td>
</tr>
<tr>
<td>10</td>
<td>All strains</td>
<td>3</td>
<td>Rv0221-Rv0223</td>
</tr>
<tr>
<td>11</td>
<td>All strains</td>
<td>46</td>
<td>Rv2645-Rv2695c</td>
</tr>
<tr>
<td>12</td>
<td>All strains</td>
<td>5</td>
<td>Rv3117-Rv3121</td>
</tr>
<tr>
<td>13</td>
<td>All strains</td>
<td>3</td>
<td>Rv1255c-Rv1257c</td>
</tr>
<tr>
<td>14</td>
<td>Pasteur</td>
<td>9</td>
<td>Rv1765c-Rv1773c</td>
</tr>
<tr>
<td>15</td>
<td>Frappier, Connaught</td>
<td>12</td>
<td>Rv0309-Rv0312</td>
</tr>
<tr>
<td>16</td>
<td>Moreau</td>
<td>6</td>
<td>Rv3400-Rv3405</td>
</tr>
</tbody>
</table>
and viral vectors, e.g. attenuated Salmonella, Listeria, vaccinia virus, etc.\textsuperscript{45-47} In addition, work is also in progress to use highly attenuated BCG, which will be safe for use even in immunocompromised recipients and will not induce DTH reaction to tuberculin.\textsuperscript{48} Such a highly attenuated BCG strain could also be used to deliver M. tuberculosis-specific antigens to improve upon the protective efficacy of BCG.\textsuperscript{49} Moreover, efforts are also underway to attenuate the pathogenic M. bovis and M. tuberculosis by deleting the genes involved in pathogenesis and use the attenuated strains as candidate vaccines against TB.\textsuperscript{50-52}

**Conclusions**

The research conducted in the recent past has clearly shown that some of the M. tuberculosis-specific RD1 gene products are promising candidates as new vaccines against TB. Furthermore, additional M. tuberculosis-specific antigens of vaccine potential may be identified from the ongoing work with other RD DNA segments, e.g. RD4–RD13. In addition to M. tuberculosis-specific antigens, there are other candidate vaccines under investigation, including attenuated whole-cell live and whole cell inactivated mycobacteria, crossreactive antigens as subunit and DNA vaccines, and prime-boost vaccines etc.\textsuperscript{53-55} Some of these candidate vaccines have shown a level of protection in animal models of TB, which is equal or superior to that of BCG. However, the vaccines based on M. tuberculosis-specific antigens will have advantage over other formations because these will be free from the shortcomings of cross-reactive antigens that may not differentiate between vaccination and exposure to environmental mycobacteria. Furthermore, the vaccines based on M. tuberculosis-specific antigens can also be used to boost the immunity already provided by BCG vaccination and/or environmental mycobacterial exposure. However, the final clearance for an improved vaccine against TB will require long-term efficacy trials in humans conducted in different parts of the world.

**References**


