therapeutic vaccine has been contemplated as an adjunct to antibiotics in the therapy of TB.

BCG has an excellent safety record in immunocompetent individuals and a pretty good safety record even in HIV-infected persons (63); however, BCG can occasionally disseminate in immunocompromised persons, including AIDS patients, and cause serious and even fatal disease. Ideally, new vaccines would be as safe as BCG in immunocompetent persons and even safer in immunocompromised persons.

Vaccines Replacing BCG

BCG protects against childhood TB and disseminated forms of TB, such as meningitis and miliary TB (64,65). However, the efficacy of BCG against adult pulmonary TB, the most common form, has been highly variable (66). A large meta-analysis concluded that the efficacy of BCG is approximately 50% (64); however, this number obscures the fact that efficacy tended to be bimodal in epidemiologic studies, that is, the vaccine seemed to work well or not at all. In any case, there is need for a more potent and consistent vaccine to replace BCG.

All the leading candidate vaccines to replace BCG are live mycobacteria. While a large number of subunit vaccines have been tested for efficacy against MTB challenge in animal studies, including protein/adjuvant vaccines, lipid vaccines, DNA vaccines, and killed mycobacteria, none of these has ever been demonstrated to be more potent than BCG in animals, especially the stringent guinea pig model. In endemic areas of the world, health care providers are loath to allow any vaccine to replace BCG in newborns that is not at least as potent as BCG. For this reason, subunit vaccines are unlikely to supplant BCG as a first vaccine in newborns.

Several types of replacement live mycobacterial vaccines have been proposed, as described in the following sections.

Recombinant BCG Expressing MTB Proteins

These vaccines utilize BCG as a vector to deliver immunoprotective proteins of MTB. The fact that these vaccines comprise BCG enhances their acceptability as a replacement vaccine, since health care providers are reluctant to abandon BCG, except for something likely to be at least as efficacious. The acceptability of such vaccines is also enhanced by the fact that BCG has a very well-established safety profile, having been administered to approximately 4 billion persons.

The first rBCG vaccine expressing MTB proteins, and the first vaccine demonstrated more potent than BCG, was rBCG30, an rBCG vaccine overexpressing the MTB 30 kDa major secretory protein, a mycolyl transferase also known as antigen 85B (67–70). The enhanced efficacy of rBCG30 was demonstrated in the demanding guinea pig model of pulmonary TB. It significantly reduced the number of lung lesions and the extent of lung pathology, markedly and significantly reduced the burden of MTB in the lung and spleen, and significantly prolonged survival compared with the parental BCG Tice strain (67,68). The development of this vaccine followed from previous studies, showing that immunization with extracellular or secreted proteins of intracellular pathogens induces potent protective immunity against challenge with the relevant pathogen, first demonstrated for *Legionella pneumophila* (71,72) and subsequently for MTB (73).

Subsequently, a rBCG vaccine expressing two other MTB extracellular proteins, CFP10 and ESAT-6, which are in the RD1 region of BCG that was deleted from its genome during attenuation from *M. bovis*, was shown in one experiment to be more potent than BCG in the guinea pig model, reducing the burden of MTB in the spleen but not in the lung (74). This vaccine, however, was more virulent than BCG, and clinical development of the vaccine has not proceeded. A potentially safer alternative recombinant vaccine expressing the same extracellular proteins in an *M. microti* host has also been tested in preclinical studies; however, the potency of this vaccine in the guinea pig model was not significantly different from BCG (75). A similar rBCG vaccine expressing only the ESAT-6 protein was tested in mice, but it did not provide greater protection than BCG, either by itself or as part of a fusion protein linked to the hsp60 protein (76).

rBCG vaccines expressing other MTB extracellular proteins have also been reported. rBCG vaccine overexpressing a secreted MTB 38 kDa glycoprotein was tested in mice and found to prolong survival in one experiment (77). An rBCG expressing MTB 72f, a hybrid of two proteins, was tested in cynomolgus monkeys and appeared to induce marginally better protection than BCG, although differences between the two vaccines were not significant in this small study (78).

Recombinant BCG Escaping the Phagosome

A second strategy employs an rBCG vaccine that secretes listeriolysin, lyses the phagosomal membrane, and allows antigen translocation into the cytoplasm of the host cell (79). The rationale underlying this vaccine, as described below, is to enhance antigen presentation of BCG antigens and induce a more rigorous T-cell response against MTB. This vaccine induced efficacy superior to BCG in mice challenged with virulent MTB of the Beijing/W genotype family.

MTB Auxotrophs

Another strategy utilizes attenuated mutants of MTB as vaccines (80–88). The rationale for these vaccines is that they more closely resemble MTB than BCG. Differences between *M. bovis*, from which BCG is derived, and MTB are slight, as these strains are 99.9% similar at the DNA level (89). However, approximately 98 genes present in MTB are absent in *M. bovis*; moreover, during attenuation, BCG lost approximately 38 genes, some of which may contribute to immunoprotection against MTB (3). A number of attenuated strains of MTB have been tested (80–88). Most are no more potent than BCG, and some are less potent; however, one was found more potent than BCG in the mouse model (88) and another more potent in the demanding guinea pig model (83). Attenuated MTB present substantial safety concerns because of the possibility of reversion to virulence. Their clinical investigation will likely require multiple independent attenuating gene deletions to insure their safety (90); such additional attenuations are likely to reduce their immunogenicity. Given the safety concerns surrounding these vaccines and the failure of most to demonstrate superior efficacy to BCG, the future of these vaccines is problematic.

Recombinant BCG Secreting Cytokines

rBCG secreting various cytokines including IL-2, IL-18, GM-CSF, and IFN-γ have been shown to have enhanced immunogenicity in mice (91–95). However, such vaccines have not been demonstrated to induce enhanced protective immunity.

BCG Auxotrophs and Recombinant BCG Auxotrophs

The increased susceptibility of AIDS patients to disseminated infection with BCG has heightened interest in an even safer