Therapeutic Vaccines

Therapeutic vaccines have a potential role as adjunctive therapy against TB. The emergence of MDR strains of MTB, including strains resistant to nearly all conventional antibiotics used to treat TB, may strengthen the rationale for such a vaccine.

Preclinical trials of therapeutic vaccines typically entail challenging animals with MTB, and after a period of time, vaccinating the animals one or more times with the vaccine being tested. Therapeutic vaccines have generally not fared well in preclinical studies. As noted in the previous section, a DNA vaccine encoding the *M. leprae* hsp60 protein was found highly efficacious in an initial study but ineffective and potentially dangerous in subsequent studies. A DNA vaccine encoding the MTB Antigen 85A and a vaccine comprising a crude extract of MTB extracellular proteins were ineffective at reducing the burden of MTB in the lungs of mice; however, these vaccines reduce the burden of MTB in the spleens of the mice (115). In the same study, BCG had no immunotherapeutic benefit (115).

In human studies, heat-killed *M. vaccae* have been extensively studied as an immunotherapeutic vaccine, yielding variable results (116). Recently, particularly promising results were reported in a randomized partly blinded study conducted in Argentina in which newly diagnosed HIV-negative patients were treated with placebo or heat-killed *M. vaccae* administered in a three-dose regimen; all patients also received chemotherapy. Patients treated with the *M. vaccae* regimen showed faster and more complete clinical improvement than patients administered the placebo (117).

SELECTED VACCINES IN OR APPROACHING CLINICAL DEVELOPMENT

Vaccines to Replace BCG

rBCG30

Rationale. rBCG30 is a recombinant BCG vaccine expressing the MTB 30 kDa major secretory protein, a mycolyl transferase known as Antigen 85B (67). Since unmodified BCG expresses a homolog of the MTB Antigen 85B that has an identical amino acid sequence, rBCG30 in essence overexpresses this protein. It expresses five to six times as much 30 kDa protein as the parental BCG Tice strain. rBCG30 stably expresses the 30 kDa protein after repeated subculture for more than one year in broth in the absence of selective pressure and after passage through guinea pigs (67).

The rationale for the selection of the MTB 30 kDa protein for expression by BCG derives from the extracellular protein hypothesis for vaccines against intracellular parasites, which holds that extracellular proteins of intracellular parasites are key immunoprotective antigens because their release inside the infected host cell makes them available for proteolytic processing and subsequent presentation on the surface of host cells as major histocompatibility complex (MHC)-peptide complexes (16,20). Such MHC-peptide complexes alert the immune system to the presence of a live bacterium within the host cell and allow T-cells capable of recognizing the complexes to exert an anti-microbial effect against the host cell, either by activating the host cell such that it inhibits the multiplication of the intracellular pathogen, or by lysing the host cell, thereby denying the intracellular pathogen its preferred intracellular niche. Three types of observations support this hypothesis. First, immunization of animals with live but not killed *L. pneumophila* and MTB induces strong protective immunity (118–121). Second, guinea pigs infected with *L. pneumophila* and mice and guinea pigs infected with MTB develop strong T-cell responses to secreted proteins (73,122,123). Third, immunization of guinea pigs with major extracellular proteins of *L. pneumophila* and MTB induces potent protective immunity against aerosol challenge with these pathogens (71–73,124–127). Importantly, the major secretory protein of *L. pneumophila* induces potent protective immunity despite the fact that it is not a virulence determinant in the guinea pig model, indicating that it is the processing and presentation of this molecule to the immune system rather than the neutralization of a virulence determinant that results in protective immunity (125).

Of the major extracellular proteins of MTB, the 30 kDa mycolyl transferase is the most abundant protein released by MTB in broth culture, making up one-quarter of the total protein released (126). Moreover, it is among the major MTB proteins of all types expressed by MTB within human macrophages (128). Hence, macrophages infected with MTB should present a rich display of MHC-peptide complexes derived from the 30 kDa protein on their surface for T-cell targeting.

The MTB 30 kDa major secretory protein is highly immunogenic (126). Immunization of guinea pigs with purified protein in adjuvant induces a strong cell-mediated immune response and protective immunity (126). Peptide mapping of the protein in humans and guinea pigs has revealed abundant immunodominant epitopes (129).

The rationale for selecting BCG as a vector for the delivery of the MTB 30 kDa protein was fourfold. First, like MTB, BCG is an intracellular organism and it follows a similar intracellular pathway in host cells, residing and multiplying within a phagosome; hence, antigens released by BCG should be processed and presented similarly to antigens released by MTB and result in the generation of T cells subsequently capable of recognizing and targeting MHC-peptide complexes derived from the antigen on host cells infected with MTB. Second, BCG has an excellent safety profile. Third, the BCG vector, which is highly homologous with MTB at the DNA and protein level, provides a baseline level of protection against TB. Hence, any improvement should result in a vaccine more potent than BCG. Finally, rBCG are essentially ''BCG+'' and thus should have high acceptability in TB endemic areas.

Immunogenicity and efficacy in animal models. rBCG30 has been extensively evaluated in the guinea pig model of pulmonary TB (67–69). Guinea pigs were sham-immunized or immunized with BCG or rBCG30, challenged 10 weeks later with highly virulent (Erdman strain) MTB by aerosol, and euthanized 10 weeks after challenge for enumeration of pathology and organ burden. Compared with guinea pigs vaccinated with BCG, guinea pigs vaccinated with rBCG30 had significantly fewer lung lesions, significantly less lung pathology, and significantly fewer MTB in the lung and spleen. On average, rBCG30–immunized guinea pigs had 0.8 ± 0.1 log fewer colony-forming units (CFU) in the lung and 1.1 ± 0.1 log fewer CFU in the spleen than BCG-immunized animals in 15 consecutive experiments ($n = 280$ animals total for BCG Tice and $n = 281$ animals total for rBCG30 Tice), differences that were highly significant in each of the fifteen experiments. rBCG30 is effective in guinea pigs over a broad dose range (10^1–10^6 CFU) (70). rBCG30 was evaluated for capacity to enhance the survival of guinea pigs after challenge; rBCG30-immunized animals survived significantly longer than BCG-immunized animals (68).