vaccine than BCG. This has prompted the investigation of auxotrophs of BCG and rBCG as these have been found to be safer than BCG in severe combined immunodeficiency (SCID) mouse models. Both a leucine and methionine auxotroph of BCG have been reported to induce protection against MTB challenge in guinea pigs, although protection is inferior to that induced by BCG; interestingly, the leucine auxotroph does not induce a cutaneous response to tuberculin (96).

An rBCG auxotroph engineered to have curtailed growth in macrophages and the immunized host and overexpressing the MTB 30 kDa major secretory protein (antigen 85B), induced protection greater than BCG in the guinea pig model (97). This rBCG has a defect in iron acquisition, but if preincubated with iron and mycobactin before immunization, it can undergo several cycles of replication in the host. This rBCG is much safer than BCG in the SCID mouse model.

Booster Vaccines

About 7 to 8 million of BCG-vaccinated individuals nevertheless develop active TB each year. A booster vaccine might augment the immunity of BCG-vaccinated people and improve their capacity to ward off active TB.

Few vaccines have successfully boosted the level of protection conferred by BCG vaccination in animal studies. The MTB 30 kDa major secretory protein (antigen 85B), administered once intradermally, has enhanced significantly the protection conferred by intradermally administered BCG in the guinea pig model. This is the only booster vaccine that has proven itself capable of enhancing the protection conferred by BCG in the guinea pig model (98).

Modified vaccinia virus Ankara (MVA) expressing the MTB 32 kDa major secretory protein (Antigen 85A) (MVA85A) has been demonstrated to boost the level of protective immunity in mice conferred by BCG, but only when the prime is delivered intranasally (99); intranasal delivery of BCG differs from the intradermal route by which humans are routinely vaccinated with BCG. Boosting BCG with MVA85A failed to enhance the protection conferred by BCG in the guinea pig model (100,101). However, boosting BCG sequentially with MVA85A and a recombinant fowlpox virus expressing Antigen 85A enhanced survival in guinea pigs in a single small experiment (101). The MVA85A vaccine has been evaluated in humans and is discussed further below (102).

Mtb72f, a hybrid of two MTB proteins, was shown to enhance survival in the guinea pig model when coadministered with BCG (103); however, it has not been reported to enhance the protective efficacy of BCG in mice or guinea pigs when administered in a prime-boost vaccination protocol. Mtb72f failed to enhance protection conferred by BCG in a rabbit model of tuberculous meningitis (104). However, in a preliminary report, boosting BCG with Mtb72f was said to enhance survival in the cynomolgous monkey model (105).

An antigen 85B-ESAT-6 hybrid vaccine (Hybrid 1) has also been tested in a prime-boost regimen. It has been shown to enhance the level of protection conferred by BCG when delivered intranasally in the mouse model (106,107). The H1 did not induce greater protection than BCG in a small study in a high dose challenge exploratory guinea pig model that was tested in the EU TB Vaccine Cluster (100). The very similar vaccine based on antigen 85B-TB10.4 (H4) administered in IC31 was recently tested as a BCG booster in a large guinea pig experiment with 30 animals in each group and was found to prolong guinea pig survival after MTB aerosol challenge (Skeiky and Sadoff, personal communication).

Finally, a DNA vaccine encoding MTB Antigen Rv3407 was tested in a mouse model in which BCG was administered intravenously and the DNA vaccine subsequently administered twice; boosting with this vaccine slightly enhanced the level of protection conferred by BCG (108).

Postexposure Vaccines

Most people exposed to MTB contain the infection and never develop active TB. However, in about 10% of exposed individuals, active disease ensues, either soon after exposure (primary TB) or after a period of latency (reactivation TB) that may last for years or even decades. Hence, people exposed to MTB might benefit from a postexposure vaccine that would help keep the latent MTB bacteria within them in check and diminish the likelihood of reactivation TB. In essence, a postexposure vaccine is a booster vaccine for those individuals whose immunity has been primed by exposure to MTB.

Whether booster vaccines akin to those discussed in the previous section also would serve as efficacious postexposure vaccines or whether specially designed vaccines are needed to combat latent MTB infection is a matter of conjecture. One strategy for a vaccine especially designed to suppress latent MTB is a vaccine comprised of proteins expressed by MTB during latency, for example, α-crystalline (HspX) (109,110). Whether postexposure vaccines comprised of such latency-expressed proteins would be more efficacious than vaccines comprised of proteins expressed during active disease is unknown.

The evaluation of vaccines for efficacy in preventing reactivation TB is cumbersome. Such studies generally utilize the Cornell model or variations thereof, in which animals are sequentially (*i*) infected with MTB; (*ii*) treated with antibiotics to reduce the infection to a low level, a state thought to mimic latency; (*iii*) vaccinated with the test vaccine; and (*iv*) immunosuppressed, typically with dexamethazone, to reactivate TB. To what extent this model recapitulates latency and reactivation in humans is unclear.

A cocktail of 10 MTB antigens encoded by DNA constructs gave very modest protection in a modified Cornell model, not significantly different from the vector control (111). A second DNA vaccine encoding the *M. leprae* hsp60 protein initially appeared highly efficacious in a mouse model (112). However, in a subsequent report, vaccination of mice with DNA encoding the same *M. leprae* hsp60 protein was ineffective both in a prophylactic mode and in a Cornell model of latent TB. Additionally, when given in an immunotherapeutic mode, the vaccine induced a severe Koch-like reaction, characterized by cellular necrosis throughout the lung granulomas (113). A similar reaction was observed when a DNA vaccine encoding Antigen 85A was administered in an immunotherapeutic mode (113). In a separate report, prophylactic immunization with DNA vaccines encoding the MTB hsp60 or hsp70 heat shock proteins were not protective in mice or in guinea pigs, and guinea pigs vaccinated with the vaccines exhibited a moderate to severe necrotizing granulomatous bronchointerstitial pneumonia with bronchiolitis (114). These reports have cast doubt on both the safety and efficacy of DNA vaccines encoding heat shock proteins.