

Vaccines

Live recombinant vaccines against tuberculosis that are safer and more potent than BCG

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Our approach to an improved replacement vaccine for BCG is the development of live recombinant BCG vaccines overexpressing key immunoprotective proteins of *Mycobacterium tuberculosis* (*Mtb*). As a vector, BCG advantages include well-established safety, a baseline level of protective efficacy, high acceptability worldwide, and an intracellular lifestyle and antigen presentation pathway similar to *Mtb*. We have previously described rBCG30, the first recombinant BCG vaccine against TB and the first vaccine more potent than BCG. rBCG30 overexpresses the *Mtb* 30-kDa major secretory protein (Antigen 85B; r30). In the demanding guinea pig model, rBCG30-immunized animals survived significantly longer than BCG-immunized animals after *Mtb* aerosol challenge, and, in 18 consecutive experiments, had significantly fewer *Mtb* in the lung (mean 0.8 ± 0.1 log CFU less) and spleen (mean 1.1 ± 0.1 log CFU less). In a Phase 1 human trial, rBCG30 was as well-tolerated as BCG and, in contrast to BCG, induced significantly increased Antigen 85B-specific immune responses.

To render rBCG30 safe for HIV-positive persons, who suffer disproportionately from tuberculosis but in whom BCG can disseminate, we engineered rBCG(*mbtB*)30, a recombinant BCG vaccine that overexpresses r30 and is mycobactin/exochelin-dependent, rendering it defective in iron acquisition and hence growth-limited *in vivo*. If preloaded with iron, rBCG(*mbtB*)30 can multiply several times *in vivo*, sufficient to induce strong immune responses but not to disseminate. In the guinea pig model, rBCG(*mbtB*)30 is significantly more potent than BCG; in SCID mice, rBCG(*mbtB*)30 is much safer than BCG.

These studies demonstrate the feasibility of vaccines that are concurrently more potent and safer than BCG.

Research and development of new generation of live vaccines against Tuberculosis

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Live vaccine candidates offer a high potential in the development of new vaccines against tuberculosis. The experience in production, distribution and the use of present vaccine in use, BCG, makes a new live vaccine a reliable candidate to replace BCG.

The transcription factor PhoP is involved in the regulation of complex networks of virulence in *Mycobacterium tuberculosis*. The live vaccine SO2, based on phoP mutation, is highly attenuated in severe combined immunodeficiency disease (SCID) mice. Balb/c mice vaccinated with SO2 are protected against challenge with *M. tuberculosis* H37Rv at levels comparable to mice vaccinated with BCG. A high dose aerosol challenge of SO2 vaccinated guinea pigs resulted in superior levels of protection when compared with BCG vaccination, as measured by Guinea Pig survival and reduction of bacteria and in disease severity in the lung.

One of the main concerns for live vaccine candidates is safety. Sensibility studies of SO2 showed full sensitivity to major antituberculosis drugs. Stability of phoP mutation was studied subcultivating SO2 strain for 6 months in the laboratory and after 3 months infection in SCID mice and not reversion of the phoP mutation was detected. Additional SO2 safety studies were performed in SCID mouse model in aerosol SCID mouse model and safety/toxicity in Guinea Pigs, showing full attenuation of SO2.

A new generation of live vaccines based on phoP deletion and containing a second additional deletion on fadD26 gene unmarked, following Geneva Consensus, has been constructed in order to be tested in clinical trials.

The dynamic hypothesis of latent tuberculosis infection offers a new rational to develop future therapeutic strategies

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Traditionally, it has been thought that individuals infected with *Mycobacterium tuberculosis* (MTB) retain for a lifetime latent bacilli, which remain dormant and reside within old lesions in the upper lobes of the lung. Reactivation of these dormant bacilli leads to active tuberculosis (TB), aided by resuscitation factors as well as the high oxygen pressure. These assumptions raise at least three relevant questions to answer: (1) how can the dormant MTB persist in the face of the continual cellular turnover and healing of injured tissues?; (2) How can the resuscitation factors get into the old lesions?; (3) How can isoniazid (INH) chemoprophylaxis be effective in 90 % of infected contacts, considering that it acts only against multiplying bacilli?

Characterization of latent tuberculosis infection (LTBI) in mice shows: (1) the drainage of non-replicating bacilli by foamy macrophages (FM) towards the alveolar spaces; (2) the constant formation of new granulomas; (3) the presence of local immunodepression; (4) the reduction of the immunological response and FM accumulation after a short-term chemotherapy, and bacillary reactivation once is finished; (5) immunotherapy with fragments of MTB (RUTI) allows the control of this reactivation by inducing a polyantigenic response against secreted and structural antigens.

The “dynamic hypothesis” suggests that LTBI would be caused by the constant endogenous reinfection of non-replicating bacilli. This hypothesis explains the efficacy of the 9-month isoniazid chemoprophylaxis, and supports a therapy based in the elimination of the local immunosuppression by a short-term chemotherapy, followed by a therapeutic vaccination against growing and non-replicating bacilli.

Tb subunit vaccines

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The current tuberculosis (TB) vaccine *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) does not prevent the establishment of latent TB or reactivation of pulmonary disease in adults. After a very active research effort in many different laboratories a number of highly active vaccine antigens have now been identified and the development of subunit vaccines has reached the point where single antigens as well as poly-protein fusion molecules have been evaluated in animal models and found to provide efficient protection against tuberculosis. The most advanced of these vaccines such as the fusion between ESAT6/TB10.4 and Ag85B are now in clinical trials and the first data on safety and immunogenicity are very promising. Currently the focus is on evaluating the influence of different adjuvants, routes and prime-boost regimes for optimal expression of immunity in the lung, boosting of BCG and maintenance of immunological memory. Subunit vaccines can be used to boost BCG immunity either administered together (Tandem administration), shortly after BCG (early boost) or in adolescence when BCG immunity starts to wane (Late boost). A late BCG boost would frequently be administered post-exposure to latently infected individuals and ongoing efforts are focused on understanding the impact this would have on existing vaccines and on the design of efficient booster vaccines.