Current Tuberculosis Vaccine Development

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Information derived from the complete genome sequence of Mycobacterium tuberculosis makes it possible to develop a range of new vaccine candidates. Strategies currently under investigation include construction of whole cell live attenuated mycobacterial vaccines, as well as the use of individual antigens delivered by a variety of subunit vaccination procedures. Fundamental questions associated with the rational design, preclinical testing, and future application of new tuberculosis vaccines are reviewed.

Generating New Vaccine Candidates

Advances in mycobacterial molecular genetics, which have culminated in the establishment of the genome sequence of Mycobacterium tuberculosis H37 [1], make it possible to generate a vast new repertoire of potential tuberculosis vaccine candidates. Two broad strategies can be envisaged. Following the paradigm of BCG vaccine, live attenuated mycobacterial vaccines can be constructed with the aim of presenting the immune system with a challenge as close as possible to that of the natural infection but without the pathological sequelae associated with the virulent bacteria. Although BCG vaccine arose as the result of spontaneous deletion of fragments of the mycobacterial genome (including genes encoding important antigenic determinants) [2, 3], it is now possible to use the tools of gene replacement and transposon mutagenesis to allow precise inactivation of individual genetic loci [4, 5]. This strategy has been applied successfully in the design of live vaccines for the prevention of other bacterial infections. Mutations that affect the ability of a pathogen to cause disease can be introduced in combination with mutations that impose particular nutritional requirements in order to “fine-tune” the efficacy and to ensure safety of new vaccine candidates. For example, a growth requirement for the amino acid leucine generates mycobacterial mutants unable to survive in immunodeficient mice, which provides a possible route to develop live vaccines that are safer to use in immunocompromised human populations [6]. An alternative approach to improved live vaccines is to manipulate BCG vaccine itself, restoring expression of antigens lost during its original derivation or enhancing its immunogenicity by engineering expression of recombinant mammalian cytokines [7].

A second general approach to vaccination is to boost the immune response to selected antigenic determinants that are delivered in the form of subunit vaccines. Such vaccines have advantages over live mycobacteria with respect to safety and quality control. Several strategies are available for delivery of subunit vaccines. For example, promising results have been obtained in experimental models of tuberculosis by immunization with purified mycobacterial proteins mixed with appropriate adjuvants [8–10]. An exciting alternative involves direct injection of DNA encoding the relevant antigens. DNA taken up by host cells directs expression of the relevant antigen, which results in induction of a range of immune responses. Although the potency and safety of DNA vaccination in humans are subjects of ongoing clinical trials, results of animal models demonstrate a degree of efficacy against challenge with M. tuberculosis [11–13]. Establishing the complete set of 3924 genes of M. tuberculosis provides the starting point for design of a very extensive panel of attenuated mutants and subunit vaccines.

Selecting New Vaccines

The next challenge is to select from the vast repertoire of possible vaccines those candidates that have sufficient promise to merit testing in clinical trials. Ideally, this challenge would be met rationally, by learning the nature of the immune response required for protection and then selecting candidates capable of inducing that response. Significant progress has been made in uncovering the immunologic mechanisms that are essential for protection. There is compelling evidence that a “type I immune” response—involving IL-12 modulation of CD4+ T cells and consequent IFN-γ-mediated activation of macrophages—is required for immunity in both mice and humans [14–16]. Additional mechanisms are also required, however. In particular, activation of CD8+ T cells has been shown to be essential in murine models [17], and there is evidence for involvement of an array of additional T cell subsets and cytokines [18, 19]. It seems likely that protection results from multiple immune functions appropriately coordinated with respect to both timing and precise localization.

Because we have only an incomplete understanding of this complex phenomenon, how should we best proceed with a “rational” selection of new vaccine candidates? Should we attempt to prime the whole gamut of immune responses by using a live
attenuated vaccine, or should we prime key initial events with a subunit vaccine and rely on the natural immune response to supply additional functions? In view of our current rather rudimentary understanding of the regulatory mechanisms that govern the cell-mediated immune system, it is prudent to include both possible approaches in the search for better vaccines.

Wanted: Dead or Alive?

Since the early part of the century, it has been recognized that immunization with living mycobacteria imparts protection in animal models superior to the protection provided by the same bacteria delivered in killed formulations [20]. Why should this be the case? It may be that the repertoire of antigens expressed by the live organism within the infected host differs from that provided by the in vitro-grown killed mycobacteria, or that the live vaccine may deliver the same set of antigens as the dead mycobacteria but in a way that results in induction of a qualitatively different type of immune response. Finally, persistence in infected tissues may endow the live vaccine with an ability to imprint the immune system with a more robust “memory” response. Antigens that are secreted by live mycobacteria and are generally absent from killed preparations could play an important role as targets for immune recognition during the early stage of an infection. This hypothesis has been productively applied to the selection of candidates for subunit vaccines from among the repertoire of M. tuberculosis proteins secreted into the culture filtrate in vitro [8–11]. The presence of secreted antigens may provide a partial explanation for the relative efficacy of live as compared with killed mycobacterial vaccines, but differences in the way that the bacteria are handled within the body and within cells are also likely to be important. For example, the ability of live mycobacteria to interfere with intracellular trafficking pathways might influence the presentation of antigens to CD4+ and CD8+ cells [21, 22].

Natural or Supernatural Immunity?

A classical approach to vaccine design relies on the observation that recovery from initial infection confers protection against subsequent exposure, the goal of the vaccine then being to mimic immunologic aspects of the natural infection. Is this a valid model for tuberculosis? On the one hand, it can be reasoned that the natural immune response to tuberculosis results in a very high level of protection (<10% of infected individuals develop disease), but the association of tuberculosis with reactivation or reinfection in previously “immune” individuals suggests that there is room to improve on natural immunity. Perhaps in selecting vaccine candidates we should also consider approaches that deliberately avoid reproducing the natural infection, which would be particularly true if M. tuberculosis has in fact evolved an ability to induce immune responses that augment rather than prevent infection. An example of this type of response might be the 19-kDa lipoprotein antigen, which is itself a potent immunogen, but has the effect of reducing low level protection conferred by rapidly growing mycobacteria in a mouse model [23]. The mechanism of this effect remains unclear but seems to be associated with an ability of the 19-kDa antigen to distract the attention of the immune system and so inhibit development of a response to other mycobacterial antigens. We are currently testing the hypothesis that removal of this antigen might potentiate the protective efficacy of BCG vaccine. Identification of 2 large gene families encoding proteins resembling those involved in antigenic variation in other pathogens (the PE and PPE proteins) provides another hint that M. tuberculosis might have evolved ways of positively misleading the immune response [1].

High Throughput Screening for Vaccines

In light of uncertainty surrounding the rational basis for vaccine design, it is sensible to maximize the range of potential candidates included in preliminary screening for new vaccines. Currently, the only test available for assessment of protective immunity is evaluation of the response to virulent challenge in experimental animal models, most commonly in mice and guinea pigs. Two drawbacks to this approach are evident. First, testing requires prolonged incubation in specialized and expensive containment facilities, severely limiting the number of candidates that can be screened. Second, it is not clear how the efficacy of vaccine candidates in animal models will relate to their activity in humans. Strategies to address some of these technical limitations include the use of luciferase reporter strains to facilitate rapid assessment of mycobacterial viability in infected tissues [24, 25] and simultaneous evaluation of multiple DNA vaccine candidates that are injected as a single pooled sample [26]. However, the leap from experimental models to humans is a formidable barrier. There is a need to identify some means of testing immune status that would provide a reliable correlation with the ability to resist infection. Such a test would facilitate high throughput screening of vaccine candidates in experimental models and—more crucially—would provide some basis for initial assessment in clinical trials.

Vaccine Efficacy in Humans

In designing vaccine screening in laboratory models, it is important to consider how the final product might actually be used in humans. There are two obvious paradigms. A “better BCG vaccine” could be given at birth and would confer lifelong protection against disease. Although this would seem to be the ideal long-term solution (and is the option addressed by most current preclinical screening programs), difficulties can be anticipated in clinical trials of such a vaccine. First, before replacement of BCG vaccine, it will be necessary to demonstrate that the new vaccine can at least match the efficacy of BCG
vaccine in protection against childhood tuberculosis; second, a 20-year follow-up will be required to assess protection against adult disease after neonatal vaccination. An alternative strategy would be to target the young adult population at highest risk of tuberculosis in countries where tuberculosis is endemic. This strategy, called “postexposure” vaccination, would involve developing a vaccine that would build on previous immunity resulting from BCG vaccination or natural infection with *M. tuberculosis*.

The postexposure strategy has a significant advantage in terms of the feasibility of vaccine efficacy trials, in that clinical cases would arise in the trial population within a relatively short time frame. Perhaps a realistic scenario would be to perform an initial efficacy trial of a postexposure vaccine in order to validate surrogate measures of protective immunity, which could subsequently be used in optimization of neonatal vaccination. A caveat is that mechanisms of protection—and hence immunologic correlates—may differ in different forms of the disease.

**Conclusion**

The exciting technical opportunities for generation of new tuberculosis vaccine candidates are matched by frustrating conceptual problems associated with understanding protective immunity. A pragmatic approach to break this stalemate combines empirical testing of candidates in experimental models with study of fundamental mechanisms of protection and persistence. The absence of a test that provides a suitable correlate of protective immunity is an important limitation to initiating clinical trials of new vaccines; paradoxically, validation of such a test may well be contingent on its application in the context of a vaccine trial.

**References**

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