Advances in the development of more effective vaccines against bovine tuberculosis

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In developed countries, control of bovine tuberculosis in cattle herds has been based on the use of the intradermal test and slaughter of reactor animals. This regime has been very effective in decreasing the number of herds infected with *Mycobacterium bovis*. However, attempts to eradicate tuberculosis from cattle and farmed deer herds in New Zealand has been frustrated by the existence of wild-life reservoirs of *M. bovis* infection, particularly possums. Alternative control strategies including the use of tuberculosis vaccines for cattle, farmed deer and possums are being considered for the prevention of the spread of bovine tuberculosis. Research in the development of tuberculosis vaccines over the past 5-10 years has spawned some imaginative new approaches. While some of these approaches are showing substantial promise, the development of an effective vaccine for release in the field will not occur for at least 5-10 years.

Protective immune response to tuberculosis

Studies involving the transfer of serum or lymphocytes from immunised mice to recipient mice have provided evidence that protection against mycobacterial infections is primarily cell mediated. Antibody appears to have little effect in limiting the disease and antibody responses tend to indicate the progression of the disease. Several cell types are involved in protection. The macrophage plays a central role, acting as an effector cell for bacterial killing as well as acting as the host to the parasite. Mycobacteria are readily phagocytosed by macrophages, but have developed a range of mechanisms to evade being killed by these cells. These mechanisms include inhibition of lysosome fusion, resistance to oxidative and lysosomal attack and escape from the phagosome. Effective killing of mycobacteria within macrophages requires T-cell lymphocyte help. Macrophages process and present mycobacterial antigens to antigen-specific T-cells which become activated and produce soluble effectors or cytokines. Cytokines such as interferon-γ released from these T-cells activate macrophages resulting in the death of the intracellular bacteria. A second mechanism which has recently been shown to be important in protection against tuberculosis involves cytotoxic T-cell responses. Antigen specific cytotoxic T-cells can lyse macrophages which are expressing mycobacterial antigens. The mycobacteria which were surviving within these macrophages are now released, but are subsequently phagocytosed and killed by activated macrophages.

Immunisation with dead bacteria or bacterial components is less protective than immunisation with live mycobacteria. The reason may be that protection is induced by

antigens expressed *in vivo* or secreted by activated bacilli. Furthermore, live bacteria are more effective in inducing appropriate cellular immune responses. Immunisation results in the generation of antigen specific memory T-cells and upon subsequent exposure to the live bacteria, an accelerated secondary immune response is produced.

BCG Vaccine

BCG (bacille Calmette-Guerin), an attenuated *M. bovis* strain, has been the live vaccine widely used for immunisation against human tuberculosis and has also been used in cattle trials. Early BCG vaccination trials in cattle showed some protection against experimental challenge, although results from field trials were disappointing. In two cattle trials at Wallaceville, vaccination with BCG was effective in protecting 50-70% of cattle from developing tuberculous lesions following experimental challenge with virulent *M. bovis*. However, in a third trial where cattle had prior sensitisation to environmental mycobacteria, vaccination with BCG induced no protection. These results are similar to those in human tuberculosis trials, with vaccine efficacy ranging from 0 to 80 percent. The limited efficacy of BCG in developing countries particularly in the tropics, compared to developed countries suggested that prior exposure to environmental mycobacteria may affect vaccine efficacy.

Wallaceville studies have shown that BCG vaccination of possums by aerosol or intragastric routes could markedly reduce the severity of a subsequent experimental *M. bovis* challenge. Further studies are now necessary to demonstrate whether BCG-vaccinated possums can be protected from natural exposure of *M. bovis*.

Improved BCG vaccines

One approach to building new vaccines is to start with the existing BCG and try to improve it, thus retaining its useful properties including its ability to persist intracellularly and its native adjuvant qualities. Recent genetic studies have shown that there are genes present in virulent *M. bovis* and *M. tuberculosis* which are absent from BCG. Introduction of selected *M. bovis* genes into BCG may lead to a more immunogenic vaccine. A second approach could be the incorporation of cytokine genes into BCG. This is an approach currently being investigated by Glenn Buchan of Otago University. Incorporation of genes expressing interferon-γ, interleukin-2 or interleukin-12 into BCG could help promote the induction of the appropriate type of protective immune response in an animal. Studies would need to be undertaken to prove that these vaccines are effective and safe.

Rationally attenuated *M. bovis* vaccines

Techniques are currently being developed to allow the disruption of *M. bovis* genes. These techniques will allow the construction of defined mutants which are defective in

either genes required for virulence or in "housekeeping" genes which encode enzymes that are essential for growth (auxotrophs). A group from Wallaceville led by Des Collins and a number of groups outside of New Zealand have investigated these approaches to develop attenuated mutants. One challenging problem is to identify mycobacterial genes required for virulence. An ideal vaccine would be a double mutant to reduce the probability of reversion to wild type and must be capable of surviving long enough in the host to induce a protective response. Rationally attenuated *M. bovis* vaccines have the potential to be more effective than BCG, which despite all its bad press is still after 75 years the best tuberculosis vaccine available. A rational attenuated *M. bovis* vaccine would be cheap to produce and would be relatively easy to administer to wild life, but would need to be tested for safety.

Subunit vaccines

Subunit vaccines are produced by identification of mycobacterial antigens that induce protective responses and these antigens are then produced as recombinants, or by chemical means. These antigens would be combined with an adjuvant. The advantage of using a subunit vaccine would be in the safety of this type of vaccine since they are non-viable and these vaccines may not induce skin test reactivity to tuberculin. The disadvantage is that to date, none of these antigen combinations have been shown to be more effective than BCG and the major difficulty is in the induction of strong cellular immune responses.

Protective mycobacterial antigens delivered by heterologous hosts

Selected mycobacterial genes are incorporated into attenuated live vaccine vectors, such as vaccinia virus or *Salmonella spp.* in the hope that these recombinant vaccines will induce strong cellular immune responses. Unfortunately, none of these vaccines have been shown to be as effective as BCG.

DNA vaccines

A radical new approach in vaccine design has been the development of DNA vaccines. Plasmid DNA encoding an antigenic protein is injected intramuscularly into an animal and is taken up rapidly by animal cells such as muscle cells. Recombinant protein is expressed inside the cytoplasm of the antigen-presenting cells, which process the protein in a more effective way than when the protein is injected directly into the host.

A number of different tuberculosis DNA vaccines have recently been tested in mice and have been shown to induce protective immune responses. Conclusions from these trials are:

- DNA encoding a single mycobacterial protein antigen can be sufficient to generate protective immunity.
- the expression of protection involves cytotoxic antigen-specific T cells.
- the identity of the antigen may be less important than the mode of presentation.
- injection of DNA encoding the antigen (DNA vaccination) is a superior way of raising protective immunity compared to injection of the antigen itself.

Advantages of tuberculosis DNA vaccines include ease of production, high stability, induction of long-lasting immunity (>2 years in mice) and that their use may not compromise diagnostic test results. However, these vaccines have not been tested in cattle, deer or possums, the issue of safety needs to be addressed and these vaccines would be very difficult to administer to wild-life.

Assays to discriminate between vaccinated and infected animals

A perceived impediment to the use of live mycobacteria such as BCG as a vaccine for cattle is that immunised cattle can subsequently develop skin test responses to tuberculin. However, recent Wallaceville studies have shown that BCG-vaccinated cattle could be differentiated from *M. bovis* infected animals by using a diagnostic test incorporating an antigen which is present in *M. bovis,* but not in BCG. A mycobacterial antigen, ESAT-6 which was supplied by Peter Andersen, State Serum Institute, Denmark was used in a whole blood interferon-γ test in recent Wallaceville studies. Very weak interferon-γ responses were observed in the BCG-vaccinated animals, while strong responses were observed in all of the *M. bovis*-infected animals.

Conclusions

Advances in mycobacterial genetics and immunology in the past 10 years have lead to the development of some exciting new approaches in tuberculosis vaccine design. The goals of developing vaccines to completely protect cattle and deer from developing a *M. bovis* infection or to protect a highly susceptible animal such as a possum are indeed formidable. However, these new approaches in vaccine design offer some hope that vaccination can play an important role for control bovine tuberculosis in New Zealand in the near future.

Future reading

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