Development of cattle TB vaccines based on Heterologuos prime-boosting strategies

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Abstract

Development of a TB vaccine for cattle is a research priority in Great Britain. Two challenges need to be addressed. Firstly, vaccine strategies enhancing the efficacy of *M. bovis* bacille Calmette Guérin (BCG), currently the only potentially available TB vaccine, and secondly the development of a diagnostic test to be used alongside vaccination to differentiate vaccinated and infected animals (DIVA test). Significant progress in developing TB vaccines for cattle has been made over the last 7 years. Specifically: (i) DNA, protein, or viral subunit subunit vaccines used in combination with BCG have been shown to give superior protection against experimental challenge in cattle than BCG (heterologous prime-boost), (ii) neonatal BCG vaccination provides protection, (iii) prototype reagents that allow discrimination between vaccinated and infected animals have been developed; and (iv) and correlates of disease severity have been identified that can predict the success or failure of vaccination. The present overview provides details of some of these advances. [*Ethiop.J.Health Dev.* 2008;22(Special Issue):100-104]

Introduction

In 1996, an independent scientific committee reviewed the problem of bovine TB in GB. One of the recommendations put forward was that vaccination of cattle offered the best long-term solution for controlling TB in the National Herd. Cattle TB vaccination could also be an attractive and cost-effective control strategy in developing countries where other control strategies are difficult and expensive to implement. The development of novel vaccines against bovine TB has to some degree closely followed that of the human TB vaccine effort and there is significant alignment between the human and bovine TB vaccine programmes (1) and vaccines like recombinant viruses aimed at the development of human TB vaccines have been already tested in cattle (see below).

Mycobacterium bovis Bacille Calmette Guerin (BCG)

BCG is the most widely used human vaccine in the world. It was derived from a strain of *M. bovis*, which was isolated from a cow with tuberculous mastitis. Challenge experiments and field trials in cattle since 1919 have resulted in data showing a high degree of variability in the ability of BCG to protect cattle against infection with *Mycobacterium bovis*, the causative agent of bovine TB, almost some degree of protection was imparted in most of these studies (see (1-4) for reviews) . Importantly, BCG vaccination sensitises animals to the tuberculin skin test, and vaccinated animals will therefore, at least for a significant period post-vaccination, test positive in the classical skin test. For this reason, test and slaughter-based control strategies based on tuberculin skin testing were favoured above BCG vaccination. More recent experimental studies with BCG have confirmed its potential to protect cattle to some degree against bovine TB by reducing disease severity and pathology (5,9) . In addition, BCG vaccination was more effective when delivered to neonatal calves than to older animals (10,11). BCG has some of the qualities required for a veterinary vaccine (low costs, excellent safety profile), but it does not confer complete protection and therefore the aim of TB vaccine programmes is to improve its efficacy. However, the most promising vaccination strategies identified to date have mostly involved improving upon BCG vaccination rather than replacing it (see below). BCG remains the prototype, gold standard vaccine with which to judge the efficacy of any novel vaccine.

Cattle models to test TB vaccines

The fact that cattle TB vaccines can be experimentally tested for efficacy directly in the target species is a big advantage over human vaccine development. However, to be able to compare vaccines tested in different laboratories it is important to use standardised infection models. The most commonly used experimental infection model infects calves via the intratracheal route (Table 1) (7,8). This is a robust model resulting in pathology mainly in the lower respiratory tract thereby closely reflecting the pathology seen in the majority of infected cattle. Its advantages are that almost 100% of infected animals produce productive disease with reproducible location and severity thus requiring relatively small group to detect significant protection. Its short duration (3-4 months post-infection) also make it attractive. Potential disadvantages are that, due to the relatively high infection doses required to achieve infection and disease in most animals, the immune system can be overwhelmed and potentially effective vaccines could be classified as non-effective. To overcome some of these limitations, we have developed a vaccination model where transmission of disease is facilitated by in-contact with naturally infected cattle (Table 1). The advantages are that a natural route and infective dose is used, which is unlikely to overwhelm the immune system, and that data generated in this model will be highly relevant to the actual field situation to guide the design of field trials. The disadvantages are larger group sizes necessary to achieve the required statistical power, due to the lower infection rates compared to intratracheal infection. Encouragingly, our preliminary experiments conducted in GB have demonstrated a relatively high transmission rate (>50% based on immunological conversion of in-contact animals to interferon-gamma (IFN-□) test positiviity and mycobacterial culture,

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Vordermeier et al., unpublished data) potentially allowing smaller group sizes. As part of the Wellcome Trust project *'Bovine tuberculosis in the developing world'* (Animal Health in the developing world initiative), a similar in-contact transmission experiment is at the moment being conducted in Ethiopia to test vaccine efficacy after neonatal BCG vaccination. Vaccines giving promising results may then be tested in larger field trials, which would likely require large numbers of cows that would run for a considerable time.

Table 1: **Examples of cattle models to test vaccines**

Model	Advantages	Disadvantages
Experimental challenge: intratracheal model	'Few' animals required (n ≤10-12/group): 100 % infection rates of control animals	Immune system may be overwhelmed without giving vaccine a chance (high challenge dose: 1-5000 CFU)
	Short duration (3-4 months): highly standardised, synchronised infection, defined infection strain, defined disease kinetics and pathology)	
'Field experiment' In contact transmission	Natural route and infective dose: data highly relevant for trial designs	More animals required (>20/group): low infection rates of controls
		Long in-contact period (12 months): no synchronised infection, disease kinetics not defined, pathology less defined
Field trial	Real-life situation: routes, doses, management	Very large numbers required (n = 100-1000s)
		Long and expensive (years)

Recent progress in developing cattle TB vaccines that are better than BCG vaccines

Several strategies have been implemented to improve the efficacy of BCG, namely the use of subunit vaccines in the form of DNA vaccines, protein subunit vaccines administered with a suitable adjuvant, live recombinant vaccines like attenuated recombinant viruses expressing mycobacterial antigens, or recombinant BCG expressing additional antigens not, or under-expressed in BCG. Another possible strategy involves the development of rationally attenuated *M.bovis* strains (see (12-14) for reviews. The practicality of these strategies have been greatly facilitated by the elucidation of the genome sequences of *M. bovis*, *M. tuberculosis*, and *M. bovis* BCG (Pasteur) (15-17).

Recent results in cattle have also shown that the most effective vaccination strategies against bovine TB have been based on priming the immune system with BCG followed by boosting with subunit vaccines (*heterologous prime-boost strategy*) containing protective antigens that are present in BCG. Heterologous prime-boost immunisation strategies involve using two different vaccines, each expressing the same antigen.

Heterologous prime-boost strategies based on DNA vaccines. DNA vaccines can be useful as part of heterologous prime-boost protocols. We tested heterologous prime-boost protocols in cattle based on priming the immune response with a cocktail of 3 DNA vaccines encoding the mycobacterial proteins, HSP65, HSP70 and APA (which were not protective by themselves), followed by boosting with BCG [6] (Table 2). This induced significant enhancement of protection in six parameters used to determine vaccine efficacy, compared to BCG which induced significant protection in only 2/6 of these parameters (6). Subsequent experiments showed that superior protection to BCG could be achieved with this combination of vaccines irrespective of whether the DNA vaccines or BCG were used for the priming immunisation (18) (Table 2).

Heterologous prime-boost strategies based on protein subunits. Conceptually, protein subunits are very attractive. However, in contrast to DNA vaccines, protein subunits are unlikely to induce cellular immune responses in the absence of an adjuvant. Therefore, a high priority for the development of protein subunit vaccines is the identification of adjuvants that enhancing the development of cellular immune responses in cattle. A recent important development has been the definition of CpG motifs as adjuvant units within DNA vaccines (see (19) for review). Synthetic oligonucleotides containing such CpG motifs can be synthesised to produce short immuno-stimulatory sequences (CpG ODN), which can be added to vaccine formulations to enhance immunogenicity. Therefore, *M. bovis* culture filtrate proteins (CFP) were used in conjunction with such CpG ODN as cattle TB vaccines and they significantly enhanced the cellular immune responses of CFP Importantly, significant protection was also seen in

Table 2: **Vaccine strategies improving BCG efficacy in experimental challenge experiments**

Study N	Vaccine	Antigen	Adjuvant/ live vector	Comment	Reference
1	**DNA/BCG**	DNA vaccine cocktail: HSP65,HSP70, Apa	None ('in-built' adjuvant activity of DNA vaccines)	BCG Pasteur, 6 months old calves	[6]
2	**DNA/BCG or BCG/DNA**	As above	None ('in-built' adjuvant activity of DNA vaccines)	BCG Pasteur, neonatal calves	[21]
3	**BCG/protein**	*M. bovis* CFP	Emulsigen/bovine specific CpG ODN (ODN2007)	BCG Pasteur, 6 months old calves	[21]
4	**BCG/MVA85A**	Ag85A	Attenuated vaccinia virus (modified vaccinia Ankara strain, MVA)	6 months old calves. BCG SSI (freeze-dried)	[23], and unpublished data
5	**BCG/Ad85A**	Ag85A	Attenuated, replication-deficient human adenovirus type 5	6 months old calves. BCG SSI (freeze-dried)	[24], and published data

animals vaccinated with CFP plus CpG ODN, although the protective efficacy was inferior to that observed after BCG vaccination (20).

Based on these findings, further prime-boost experiments were performed in cattle using culture filtrate proteins delivered in the presence of CpG containing ODN to boost primary immune responses induced by BCG. Groups of cattle were vaccinated with either BCG, with BCG and CFP plus CpG at the same time followed by two CFP/CpG boosts. The results indicated that boosting BCG with CFP in CpG gave superior protection than vaccination with BCG alone. (Table 2) (21).

Heterologous prime-boost strategies based on recombinant viruses. Some to the advantages of live attenuated viruses over protein subunit vaccines are better induction of strong cellular immunity, and potentially lower production costs and simplified batch release test protocols. The first Phase I human trial of a new TB vaccine was based on a heterologous prime-boost strategy involving boosting BCG-mediated immunity with an attenuated vaccinia virus expressing Ag85A of *M. tuberculosis* (MVA85A) (22).

So far, in a collaborative study with the group of Professor Adrian Hill at Oxford University, we have performed immunogenicity studies of the BCG/MVA85A heterologous prime-boost regimen in cattle. Prime-boost protocols using recombinant MVA85A and BCG in either combination resulted in significantly higher frequencies of Ag85-specific IFN-□ secreting cells than the viral vectors or BCG used alone. The most promising combination was BCG priming followed by one MVA85A boost (23). Similarly, we have shown that a prime boost protocol applied to cattle that consisted of BCG priming followed by heterologous boosting with a recombinant adenovirus expressing the same antigen, Ag85A, (Ad85A) developed by Professor Xing's group at McMaster University, Toronto, Canda (24) resulted in superior antigen-specific IFN-□ responses as well as improved central T cell memory compared to BCG vaccination alone (24). Furthermore, in a recent challenge experiment using the intratracheal infection route, we could demonstrate that both MVA85A and Ad85A when used to boost BCG-induced immunity conferred significant protection, superior to BCG vaccination alone (Table 2).

Thus, significant advances have been made to develop prototype vaccine strategies that can enhance BCG vaccination efficacy based on DNA, protein and viral subunit vaccination. Further work is required to determine which of these approaches is the most effective, and how efficacy determine in experimental challenge experiments will translate into field efficacy. Thus, these candidates will be tested in the model involving in contact challenge as described above. In addition, it will be necessary to define, in addition to Ag85A, further protective antigens that can then be used as subunit vaccine candidates. This work is on-going, but further discussion is beyond the scope of this review.

Differential diagnosis of infected from vaccinated individuals, and correlates of protection.

In order to use a vaccine as part of a control strategy for bovine TB, discrimination between infected and uninfected vaccinated animals (so-called DIVA test) is a pre-requisite so that test and slaughter control strategies can be carried out alongside vaccination regimens. Over the last decade, encouraging progress has been made to make the implementation of a DIVA strategy alongside effective cattle TB vaccination likely. Conceptually, antigens whose genes are expressed in *M. bovis* yet absent from BCG constitute candidates for DIVA reagents. The antigens CFP-10 and ESAT-6 have been shown to be useful as diagnostic reagents to discriminate between BCG vaccinated and *M. bovis*-infected cattle (5,25-28) and constitute a prototype DIVA reagent. Both proteins are encoded by genes located on the RD1 region of the *M. bovis* genome that is deleted from the genomes of all

strains of BCG (29-31). The genomes of *M. tuberculosis*, *M. bovis* and BCG Pasteur have now been sequenced (15-17) and systematic comparative genome comparison have been performed to identify further cattle DIVA antigens (25,32-33).

TB vaccine development would be greatly facilitated by the definition of immunological correlates/surrogates of protection. Although some progress has been made, for example by defining ESAT-6 and CFP-10-induced IFN-□ responses as inverse correlate of protection (5), further surrogates still await closer definition. It is beyond the scope of this manuscript to present these advances in detail and I refer to recent reviews for more detailed discussion (13, 14, 34, 35).

Conclusion

Significant progress has been made in the development of TB vaccines for cattle: Subunit vaccines based on DNA, proteins or viral subunits used in combination with BCG have resulted in better protection against experimental challenge with *M. bovis* than BCG vaccination on its own. BCG vaccination of neonates has also proved to be highly protective. DIVA reagents that allow discrimination between vaccinated and infected animals have been developed. Finally, correlates of disease severity are being actively sought that can predict the success or failure of vaccination hopefully in future shortening experimental protocols.

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