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Development of vaccines against bovine tuberculosis

P. J. Hogarth, R. G. Hewinson and H. M. Vordermeier

Abstract

Bovine tuberculosis caused by *Mycobacterium bovis* remains an economically important problem in Great Britain with potential zoonotic consequences, and the incidence is rising exponentially. In 1997 an independent scientific review recommended that the best option for disease control in Great Britain was the development of a cattle vaccine. Bovine tuberculosis remains a significant problem in countries of the developing world. Indeed, more than 94% of the world's population live in countries in which the control of bovine tuberculosis in buffalos or cattle is limited or absent. Effective vaccination strategies would have a major impact in countries that cannot afford expensive test and slaughter-based control strategies. Here, we present a review of progress toward that goal, and discuss how this progress has shaped our research strategy for the development of a vaccine.

General introduction

Bovine tuberculosis caused by *Mycobacterium bovis* remains an economically important problem in Great Britain (GB) with potential zoonotic consequences. GB employs a test and slaughter policy which relies on the intradermal tuberculin skin test to identify infected animals, with test positive animals being removed. This strategy however has failed to eradicate disease and the incidence of bovine tuberculosis has been rising exponentially since 1988. By June 2005, approximately 4% of the national herd was under restriction and in the west of the GB this figure was approximately 11% (Defra 2005). An independent scientific review in 1996 of the situation in GB concluded that the best option for controlling disease in the national herd was the development of a cattle vaccine and associated diagnostic test (Krebs 1997) allowing the discrimination of *M. bovis* infected from vaccinated animals.

Bovine tuberculosis remains an economic problem in other countries with endemic wildlife reservoirs, like New Zealand and the Republic of Ireland. However, it has been eradicated in countries such as Australia, and most countries of Western Europe (Cousins 2001).

Importantly, bovine tuberculosis remains a significant problem in countries of the developing world. Indeed, more than 94% of the world's population live in countries in which the control of bovine tuberculosis in buffalos or cattle is limited or absent (Cousins 2001). Effective vaccination strategies would have a major impact in countries that cannot afford expensive test and slaughter-based control strategies.

BCG

BCG (Bacillus Calmette-Guerin) 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 and during serial passage lost its ability to cause disease (reviewed in Daniel et al (1994)). In the laboratory, BCG protects a wide range of mammals against experimental infection with tuberculosis. 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 tuberculosis (Francis 1947, 1958; Skinner et al 2001; Hewinson et al 2003) in a way similar to results of human BCG vaccination trials (Bloom & Fine 1994).

TB Research Group, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, Surrey, UK

P. J. Hogarth, R. G. Hewinson, H. M. Vordermeier

Correspondence: P. J. Hogarth, TB Research Group, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, Surrey, UK. E-mail: p.j.hogarth@vla.defra.gsi.gov.uk

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Following experimental BCG vaccination in cattle, intratracheal *M. bovis* challenge primarily resulted in reduced pathology rather than complete clearance of infection. However, BCG vaccination has failed to induce protection in the majority of field studies against natural infection (reviewed in Francis 1947, 1958; Skinner et al 2001; Hewinson et al 2003). Importantly, BCG vaccination sensitizes animals to the tuberculin skin test, and vaccinated animals will therefore, at least for a significant period post-vaccination, test positive in the classical intradermal tuberculin skin test (Francis 1947).

More recent experimental studies with BCG, conducted to optimize protocols for BCG vaccination, have confirmed its potential to protect cattle to some degree against experimental bovine tuberculosis by reducing disease severity and pathology (Buddle et al 1995a, b; Wedlock et al 2000, 2003; Skinner et al 2003). In addition BCG vaccination was more effective when delivered to neonatal calves than to older animals (Buddle et al 2003; Hope et al 2005). This could be due to a difference in the neonatal immune response, or to a detrimental effect on vaccination in older animals caused by environmental mycobacteria. However, full protection (i.e. the absence of pathology and failure to culture *M. bovis* from tissues) was not obtained.

Nevertheless, BCG exhibits some of the qualities required for a veterinary vaccine (low costs, excellent safety profile). Moreover, the most promising vaccination strategies identified to date have mostly involved improving upon BCG vaccination rather than replacing it, for example by applying heterologous prime-boost strategies (see below). BCG therefore remains the prototype, gold standard vaccine against which to judge the efficacy of novel vaccine strategies. It is a useful model to aid the development of differential diagnostic strategies and to study protective immunity.

Subunit vaccines

Subunit vaccines consisting of either protein or DNA have shown considerable promise in protecting small laboratory animals against *M. tuberculosis* (reviewed in Wang & Xing (2002) and McMurray (2003)). This potential has also been demonstrated for proteins (Bosio & Orme 1998; Hogarth et al 2005) and DNA (Chambers et al 2000, 2002) in *M. bovis* challenge models.

Despite these promising reports in small animal models, both protein and DNA subunit vaccination in cattle have been shown to induce no (Skinner et al 2003; Wedlock et al 2003) or significantly less (Wedlock et al 2005a) protection compared with BCG. In one case it was observed to actually exacerbate disease (Wedlock et al 2003), although this was a combination of both DNA and protein subunits.

One possible reason for the failure for at least some of the protein subunit vaccines to induce protection in cattle may well be the choice of adjuvant. Most adjuvants used were chosen on the basis of inducing strong cellular immunity in mice or guinea-pigs (Hogarth et al 2003, 2005), but studies have shown that adjuvants that have proved effective in small animals do not necessarily act in the same way in cattle (Wedlock et al 2000, 2002; Hogarth & Vordermeier unpublished data). Thus, in formulating effective vaccines for cattle immunization, it may be necessary to empirically test prospective formulations directly in the host species.

Heterologous prime-boost strategies

Over the past few years there has been a growing realization within the human tuberculosis vaccine community that new tuberculosis vaccines will need to be effective in the face of prior BCG vaccination. This is because although BCG does not appear to protect against pulmonary tuberculosis in adults, it is recommended for use in children as it is effective at protecting them against severe extrapulmonary forms of the disease. Thus, in the short term at least, vaccination strategies are now being targeted at boosting the efficacy of neonatally administered BCG.

Moreover, results from our bovine tuberculosis vaccine programme have shown that the most effective vaccine strategy has been based on priming the immune system with BCG followed by boosting with subunit vaccines consisting of DNA vaccines or protein vaccines in suitable adjuvants (Skinner et al 2005; Wedlock et al 2005a). Thus, there is an increasing alignment between the human and bovine tuberculosis vaccine programmes.

Heterologous prime-boost immunization strategies involve using two different vaccines, each expressing the same antigen, several weeks apart. Over the last decade heterologous prime-boost vaccination strategies have been considered for a number of diseases, for example, HIV, malaria and human tuberculosis (McShane et al 2001, 2004; Dunachie & Hill 2003; Moore & Hill 2004; McShane & Hill 2005). In most cases, superior vaccine efficacy was demonstrated using this approach compared with conventional vaccination strategies. The first heterologous prime-boost vaccination strategies applied to tuberculosis consisted of either combinations of DNA vaccines and recombinant viruses expressing the same antigen, a combination of two different recombinant viruses expressing identical vaccine antigens, or a combination of BCG and recombinant viruses expressing BCG antigens. The best results were obtained using a combination of recombinant viruses expressing the mycobacterial antigen Ag85A with BCG (McShane et al 2001, 2004; McShane & Hill 2005).

DNA–BCG prime-boost strategies

Despite the discouraging results obtained in cattle using DNA as stand-alone vaccines, the proven ability to induce T helper 1 (Th1) responses in a number of tuberculosis models (reviewed in Chambers et al (2003)), and the encouraging results from studies in

mouse and man, led to the hypothesis that DNA subunits could be useful if combined with BCG in a prime-boost strategy in cattle.

A heterologous prime-boost protocol in cattle was employed, based on priming the immune response with a cocktail of three DNA vaccines encoding the mycobacterial proteins, heat-shock protein (HSP) 65, HSP70 and APA (which were not protective by themselves), followed by boosting with BCG (Skinner et al 2003). This prime-boost regimen induced significant enhancement of protection in six parameters used to determine vaccine efficacy, compared with BCG which induced significant protection in only two of these six parameters (Skinner et al 2003) (Table 1). These results demonstrated the principle that prime-boost strategies using BCG can result in better protection in cattle than BCG alone. 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 immunization (Skinner et al 2005).

When a similar experiment was undertaken in mice, where it was logistically and financially possible to test a larger number of different groups, it was found that the noncoding DNA vaccine plasmid backbone was able to enhance protection when combined with BCG (Hogarth et al 2006), and the presence of the genes encoding specific mycobacterial antigens was therefore not required. With hindsight this was perhaps not surprising, as it has been reported that the plasmid backbone contains unmethylated CpG motifs (McCluskie et al 2000), which are potent immuno-stimulators. Indeed it has been demonstrated recently that CpG motifs were able to confer a degree of protection against tuberculosis challenge to mice, either alone or in combination with BCG (Freidag et al 2000). As described below under 'Protein–BCG prime-boost strategies', we and our collaborators are utilizing the adjuvant properties of short synthetic oligonucleotide sequences (CpG-ODN) to enhance adjuvant preparations for cattle.

Protein–BCG prime-boost strategies

As with DNA subunits, protein subunits have performed poorly as stand-alone vaccines in cattle and as discussed previously this may be due in part to use of inappropriate adjuvants. But, in common with DNA subunits, protein subunits delivered in suitable adjuvants have the potential for use as priming or boosting vaccines in conjunction with BCG. Therefore a priority for protein–BCG prime-boost strategies remains the identification of suitable adjuvants for use in cattle.

A recent important contribution to vaccinology has been the definition of adjuvant units within DNA vaccines that stimulate the innate immune response, i.e. CpG motifs. Synthetic oligonucleotides containing unmethylated CpG motifs can be synthesized to produce short immuno-stimulatory sequences (CpG-ODN), which can be added to vaccine formulations to enhance immunogenicity. Interestingly, these motifs exhibit some degree of species-specificity, and cattle-optimized sequences are now available (Pontarollo et al 2002; Rankin et al 2002).

To investigate the potential of CpG-ODN, cattle-specific CpG-ODNs were added to culture filtrate protein (CFP)-containing vaccine formulations that did not induce strong interferon-γ (IFN-γ) responses on their own. Addition of the CpG-ODNs significantly enhanced the cellular immune responses of CFP formulated in commercially available veterinary adjuvants Emulsigen or Polygen, although these levels did not reach those observed after BCG vaccination. More significantly, whilst BCG gave the best protection overall, significant protection was seen in animals vaccinated with CFP-Emulsigen plus CpG-ODN (Wedlock et al 2005a).

Further experiments used prime-boost protocols in cattle using CFP delivered in the presence of CpG-ODN to boost primary immune responses induced by BCG. Groups of cattle were vaccinated with either BCG, with BCG and CFP in CpG/Emulsigen simultaneously followed by two CFP/CpG/Emulsigen boosts, or with CFP in CpG and Emulsigen administered three times. The results indicated that boosting BCG with CFP in CpG and Emulsigen gave superior protection than vaccination with BCG alone (Wedlock et al 2005b) (Table 2). This study not only re-emphasized the potency of heterologous-prime boosting to supplement BCG, but it also demonstrated that, in addition to DNA vaccines, protein/adjuvant formulations could be used to boost BCG vaccine efficacy.

Interestingly, in recent protein-prime BCG-boost experiments in mice we have shown that adjuvant itself combined with BCG was sufficient to increase the levels of protection over that induced by BCG alone (Logan et al 2005). This echoed the observation in the DNA/BCG prime boost studies (Hogarth et al 2006) and demonstrated the potential for improving the efficacy of BCG via adjuvant-induced mechanisms. However, the period between vaccination and challenge was considerably shorter than in the cattle experiment described above, and the duration of this adjuvant effect needs to be determined in future studies.

Recently, we have shown that a different adjuvant system ('Triple adjuvant' composed of ISA70, Quil A, DEAE-dextran), when used to immunize cattle with our model mycobacterial antigen Rv3019c, induced strong antigen specific IFN-γ responses that could be boosted with BCG (Vordermeier unpublished data).

Viral–BCG prime-boost strategies

As mentioned previously, the best heterologous prime-boost combination reported so far has been BCG followed by recombinant virus (McShane et al 2001, 2004; McShane & Hill 2005). To evaluate this strategy, an immunogenicity study has been performed to assess the efficacy of various combinations of BCG and recombinant modified virus Ankara (MVA) or fowlpox virus expressing the mycobacterial protein Ag85A (MVA85A).

Table 1 Pathological and microbiological parameters of protection against experimental infection with *M. bovis*. Reproduced from Skinner et al (2003) with authors' permission

Group	No. of animals with lung lesions/total no.	No. of animals with lymph node lesions/total no.	Mean lung score[a]	Mean lymph node score[b]	Mean no. of lymph nodes with lesions per animal	No. of *M. bovis* culture-positive animals/total no.	No. of retropharyngeal and thoracic lymph nodes *M. bovis* positive/total no.	Mean log CFU of *M. bovis* for retropharyngeal and thoracic lymph nodes
Nonvaccinated	9/12	12/12	2.67 ± 0.56	3.75 ± 0.33	2.42 ± 0.42	12/12	32/72	1.89 ± 0.43
DNA	8/12	12/12	2.92 ± 0.67	4.00 ± 0.33	2.58 ± 0.29	12/12	35/72	2.03 ± 0.44
DNA/BCG	3/12[c]	5/12[d]	0.42 ± 0.26[d]	1.08 ± 0.43[e]	1.08 ± 0.49[c]	9/12	22/72	1.36 ± 0.34[d]
BCG	5/12	7/12[c]	1.33 ± 0.54	1.92 ± 0.56[d]	1.33 ± 0.41	10/12	21/72	1.51 ± 0.42

[a]Lung lesion scores: 0, no lesions; 1, 1–9 lesions; 2, 10–29 lesions; 3, 30–99 lesions; 4, 100–199 lesions; 5, ≥200 lesions. [b]Lymph node lesion score of the most severely affected node for each animal based on the following scale: 0, no lesions; 1, 1–5 small lesions (diameter, 1–4 mm); 2, 5–19 small lesions; 3, ≥20 small lesions; 4, medium-size lesion (diameter, 5–9 mm); 5, large lesion (diameter, ≥10 mm). [c]Significantly different from the value for the nonvaccinated group ($P < 0.05$). [d]Significantly different from the value for the nonvaccinated group ($P < 0.01$). [e]Significantly different from the value for the nonvaccinated group ($P < 0.001$).

Table 2 Pathological findings following challenge of calves with *M. bovis*[c]. Reproduced from Wedlock et al (2005b) with authors permission

Vaccine group	No. of animals with macroscopic lesions/total no. of animals		Mean lung score[a] ± s.e.m.	Mean total LN score[b] ± s.e.m.	Mean no. of LNs (± s.e.m.) with macroscopic tuberculosis lesions per animal
	Lung lesions	LN lesions			
No vaccine	9/10	10/10	3.40 ± 0.48	7.10 ± 1.04	2.50 ± 0.22
CFP/Emulsigen/CpG	10/10	10/10	4.30 ± 0.21	8.60 ± 1.30	3.00 ± 0.37
CFP/Emulsigen/CpG + BCG	4/9*	4/9*	1.56* ± 0.76	2.22* ± 1.06	1.11* ± 0.46
BCG	5/10	8/10	2.00 ± 0.76	3.50* ± 1.17	1.70 ± 0.40

[a]Lung lesion scores: 0, no lesions; 1, 1–9 lesions; 2, 10–29 lesions; 3, 30–99 lesions; 4, 100–199 lesions; 5, ≥200 lesions. [b]Total lymph node (LN) lesion scores for individual animals based on scores for individual nodes: 0, no lesions; 1, 1–19 small lesions (diameter, 1–4 mm); 2, ≥20 small lesions; 3, medium-sized lesion (diameter, 5–9 mm); 4, large lesion (diameter, ≥10 mm). *Significantly different from nonvaccinated group ($P < 0.05$).

Prime-boost protocols using recombinant MVA85A and BCG in either order resulted in significantly higher frequencies of Ag85-specific IFN-γ secreting cells than the two viral vectors used in combination, or BCG used alone. The most promising combination was BCG priming followed by one MVA85A boost (Vordermeier et al 2004).

Therefore, the observation in laboratory animals and primates that heterologous prime-boost strategies based on recombinant MVA and BCG induced superior cellular immune responses compared with BCG alone has now been confirmed in cattle (Vordermeier et al 2004). Studies are currently under-way to assess the protective efficacy of this protocol.

Recombinant adenoviruses can be used as alternative viral vectors to attenuated poxviruses. We have recently established that a heterologous prime-boost protocol based on the use of BCG to prime the immune response followed by boosting with recombinant adenovirus expressing Ag85A also results in strong IFN-γ responses as well as strong central memory responses compared with immunization with either BCG, adenovirus-85A–BCG, or adenovirus-85A–adenovirus-85A homologous prime-boosting (Vordermeier et al 2006). This boosting effect was statistically significant, and strong central memory responses were induced after applying this protocol. We were able to challenge a proportion of the BCG–adenovirus-85A vaccinated calves with *M. bovis* and determine the degree to which they were protected against infection. Interestingly, the degree to which individual animals were protected seemed to correlate directly with the number of spot-forming cells in cultured IFN-γ ELISPOT assays (Reece et al 2004; Vordermeier unpublished data) (Table 3). These preliminary results therefore prioritize the further assessment of central memory responses as potential surrogates of protection.

Table 3 Effector and central memory IFN-γ responses before challenge

Animals	Pathology score	IFN-γ ELISPOT (Ag85A, SFC/10^6 cells)	
		Peak ex-vivo, week 7	Cultured (before challenge, week 19)
A	0	1160	12850
B	10	1745	1675
C	0	875	4975

Animals A–C were vaccinated with BCG followed by adenovirus-85A boosting, and then challenged with *M. bovis*. Pathology scores were established at post-mortem. Corresponding pathology scores of three unvaccinated control animals: 8, 15, 15 ($P = 0.025$ compared with vaccinated calves, Wilcoxon ranked sum test). IFN-γ responses were determined at week 7 (peak ex-vivo ELISPOT responses post-boost), and week 19, before challenge at week 21 (cultured ELISPOT). SFC, spot-forming cells.

Neonatal vaccination

From a practical perspective, vaccination of neonatal calves with BCG would be advantageous. This approach would overcome problems associated with early exposure to infection or pre-sensitization to environmental mycobacteria. To investigate whether neonates could be vaccinated with BCG, a study was performed whereby groups of calves were vaccinated with BCG either within eight hours of birth, at six weeks of age, or within eight hours of birth and again at six weeks of age (Buddle et al 2003). All animals were challenged with *M. bovis* four months later. Calves vaccinated at birth or at six weeks of age were protected despite the fact that by six weeks the animals had already become sensitized to environmental mycobacteria. Thus, neonatal BCG vaccination is a viable option.

When BCG was given at birth 0/10 animals had lung or lymph node lesions and in the group where BCG was given at six weeks, 0/9 animals had lung lesions and 1/9 animals had lung lymph node lesions. In contrast, animals vaccinated at both birth and six-weeks of age were less protected than those vaccinated only at birth (lung lesions: 3/10, lymph node lesions: 4/10). In the control group all animals had lesions after experimental challenge both in the lung and in lung

associated lymph nodes (Buddle et al 2003). Thus, it is encouraging that BCG protected very young calves from the development of tuberculous lesions. Significantly, protection was not complete even in the calves vaccinated once with BCG because *M. bovis* was isolated from a proportion of the vaccinated animals (Buddle et al 2003), further emphasizing the need to improve BCG vaccine efficacy in cattle. Although this time period has to be re-defined more closely in future studies, previous studies have suggested that the protection imparted by BCG in cattle wanes significantly 12–15 months following vaccination. Thus, BCG vaccination will have to be boosted, possibly after approximately 12 months. This booster vaccination(s) is likely to be part of a heterologous prime-boost strategy as discussed above as it has been shown that BCG vaccine efficacy cannot be boosted by homologous boosting with BCG itself.

Strategy for vaccine development

We have summarized the progress made by ourselves and others in vaccine development, to show the current position of research. We now outline the next phase of our strategy to build on these advances for the development of a cattle vaccine.

Evaluation of antigens identified by bioinformatic antigen mining

The genomes of *M. tuberculosis* (Cole et al 1998), *M. bovis* (Garnier et al 2003), and BCG Pasteur (Sanger Institute 2006) have now been sequenced. Systematic comparative genome analysis (Cockle et al 2002; Aagaard et al 2003) combined with high-throughput peptide screening in the BOVIGAM IFN-γ assay can therefore be performed to identify antigens that are recognized by BCG vaccinated and *M. bovis*-infected cattle. Such antigens constitute promising candidates for use as subunit vaccines or to boost BCG. Using this approach we have recently identified a number of immunogenic proteins that could be useful as vaccine antigens in cattle. These include proteins belonging to the ESAT-6 protein family (Gey Van Pittius et al 2001; Pallen 2002), such as Rv3019c and Rv0288 (Aagaard et al 2003).

To prioritize antigens identified by comparative genomics and expression analysis for further study in murine protection models, we intend to use T cells from BCG vaccinated cattle that have subsequently been challenged with *M. bovis* to identify promising antigen candidates. Our working hypothesis is based on the observation that BCG vaccinated calves that were protected against *M. bovis* challenge developed transient cellular immune responses very early after *M. bovis* challenge at a time when no or only weak responses were detected in unvaccinated controls (Vordermeier et al 2002; Vordermeier & Hewinson unpublished data). We postulate that these responses were caused by the recognition of protective antigens, and therefore one of the criteria that we would apply to the selection of subunit vaccine candidates would be their recognition by T cells from BCG vaccinated animals during this early time period after *M. bovis* challenge.

Determination of protective efficacy of subunit vaccine candidates in mice

Due to financial constraints and the limited availability of large animal biohazard containment level 3 facilities, it is possible to conduct only a small number of challenge experiments in cattle. We have demonstrated that vaccines which gave superior protection to BCG in cattle also gave superior protection in mice (Hogarth et al 2006). Futhermore, we found that immune responses induced by vaccination and infection in mice and cattle were similar (data not shown). We propose to test promising vaccine candidates in mice as a pre-screen, before testing the best candidates in cattle challenge experiments.

Selection of potent adjuvants for protein delivery

As discussed previously, adjuvants selected on their immunogenicity/efficacy in small animal models are not necessarily effective in cattle. An adjuvant that induces optimal cell-mediated responses in cattle can only be achieved by screening adjuvants directly in cattle; the identification of such adjuvants is of high priority.

Adjuvants of several formulation types will be screened in cattle for their ability to induce suitable cell-mediated immune responses using a model mycobacterial protein. Those adjuvants which induce relevant responses will then be tested in a cattle challenge model.

Evaluation of immunogenicity and protective efficacy in cattle of vaccines developed for human tuberculosis that are now entering clinical trials in man

The development of novel vaccines against bovine tuberculosis has to some degree closely followed that of the tuberculosis vaccine effort in man. As described above, there is an increasing alignment between the human and bovine tuberculosis vaccine programmes (Hewinson et al 2003) and strong links are in place between the two programmes. To optimize synergy between the two programmes, it will be important to carry out detailed comparative studies of the performance of vaccine candidates. Several candidates are currently undergoing or will enter phase I clinical trials in 2006 (reviews in Ginsberg (2002) and Reed at al (2003)): a heterologous prime-boost strategy combining BCG priming with boosting with modified MVA85A (Goonetilleke et al 2003; McShane et al 2004); a recombinant fusion protein (Mtb72f) applied in AS02 adjuvant (Skeiky et al 2004); an ESAT-6/Ag85B fusion protein (Weinrich Olsen et al 2001) in IC31 adjuvant; a recombinant BCG strain over-expressing the mycobacterial antigen Ag85B, rBCG30 (Horwitz et al

2000; Horwitz & Harth 2003); and a recombinant BCG expressing listeriolysin, hly + BCG (Grode et al 2005).

Clearly these vaccines represent ideal candidates for comparative testing in the cattle model. Comparison of the immunogenicity and protective efficacy of these candidates in the cattle model with results in man and nonhuman primates could provide insights into the extent to which vaccine studies can be extrapolated between species. In addition, these studies would generate information that will inform subsequent rounds of candidate production, and may identify one or more candidates suitable to take forward as a cattle vaccine.

Further approaches

In addition to these objectives, promising results have been obtained from vaccinating neonatal calves with BCG and this is an area which should be pursued (Buddle et al 2003; Hope et al 2005). Finally, experimental systems to measure vaccine efficacy in a natural transmission setting are needed to assess whether 'laboratory' advances will have any significant impact in the field.

Conclusion

We have presented an overview of the current status of bovine tuberculosis in the UK and the problems underlying the use of the only currently available tuberculosis vaccine, BCG. By reviewing current advances in the field and progress by ourselves, collaborators and the wider tuberculosis community we hope to show the current position of research towards a vaccine against bovine tuberculosis, and show the process by which we have developed our current strategy for the development of a cattle vaccine.

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