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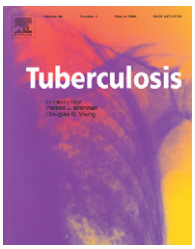

Consequence of prior exposure to environmental mycobacteria on BCG vaccination and diagnosis of tuberculosis infection

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Summary
The protective efficacy of *Mycobacterium bovis*-bacille Calmette Guérin (BCG) against tuberculosis (TB) is variable in both humans and cattle. Exposure to environmental mycobacteria is thought to result in inappropriate priming of host immune responses. To investigate the impact of environmental mycobacteria on BCG efficacy, cattle were infected with *M. avium*, vaccinated with BCG, challenged with *M. bovis* and skin tested prior to necropsy.
Elevated levels of IFNγ were evident in *M. avium*-exposed animals before and after BCG vaccination with a bias towards avian purified protein derivative (PPD-A), suggesting that *M. avium* primed host immune responses. Exposure to *M. avium* also resulted in a higher frequency of circulatory IFNγ-producing cells in response to PPD antigens at the time of *M. bovis* challenge. After *M. bovis* inoculation, the IFNγ response to bovine PPD (PPD-B) increased compared to pre-challenge levels, indicating that all animals had been exposed to *M. bovis*. Skin test responses indicated 2/6 *M. avium*-BCG-*M. bovis* animals as reactors and 2/6 as inconclusive compared with 6/6 BCG-*M. bovis* animals as reactors. *M. avium*-exposed animals also had fewer lesions and the number of tissues containing viable *M. bovis* at post-mortem was significantly lower ($P<0.02$ compared with BCG-*M. bovis* animals), with two of the animals described as skin test negative with no visible lesions or viable bacteria. Thus, exposure of cattle to environmental mycobacteria such as *M. avium* prior to BCG vaccination did not dampen BCG-specific immune responses and resulted in lower TB pathology. However, the PPD-A bias associated with *M. avium* exposure is likely to undermine current TB diagnostic tests and the IFNγ test in cattle.

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Introduction

The attenuated *Mycobacterium bovis*-bacille Calmette Guérin (BCG) strain is the only vaccine against tuberculosis (TB) currently available for routine use in humans. Protection afforded by BCG has been shown to be variable[1,2] and is particularly poor in tropical regions where it is proposed that a high prevalence of environmental mycobacteria contributes to low BCG efficacy.[3–5] The most commonly isolated environmental mycobacteria are those originating from *M. avium* complex, as determined by high reactivity to avian purified protein derivative (PPD) in tuberculin skin tests.[6] Other possible causes for the large discrepancy in BCG efficacy have been proposed including differences in BCG vaccine strains,[7] the age of the vaccinate[8] and methodological differences.[9]

The impact of environmental mycobacteria on BCG efficacy has been illustrated in the guinea pig, whereby exposure to environmental mycobacteria resulted in low level protection against *M. bovis* that was not enhanced by subsequent BCG vaccination.[10] Exposure to environmental mycobacteria generates a cross-reactive immune response that has been proposed to block BCG activity.[11] Studies in mice suggest that environmental mycobacteria may have a direct antagonistic effect on BCG vaccination resulting in a marked reduction in antigen-specific IFNγ; an essential component of BCG-vaccine induced resistance to TB.[11–13]

Humans and cattle share close similarities in terms of pathogenicity and host immune responses to mycobacteria.[14,15] Thus, experimental studies in cattle exploit a disease model in its natural host and may inform the development of effective vaccines and diagnostics for human, as well as bovine TB. Trials in New Zealand suggested that the reduction in the level of protection afforded by BCG was associated with a previous exposure to environmental mycobacteria.[16,17] Likewise, the enhanced protection against *M. bovis* observed in neonatal calves vaccinated at birth with BCG[18] may be due to the naivety of the animals to environmental mycobacteria. Conflicting evidence suggests that infection of cattle with *M. avium* may prime immune responses to subsequent BCG vaccination[19] and that exposure of cattle to *M. avium* may, in itself, induce low level protection against *M. bovis*.[20] It is unclear whether *M. avium* actually primes the immune response, or if it merely induces responses to common mycobacterial antigens that are consequentially boosted by BCG. Here, we aimed to determine the effect of pre-exposure to environmental mycobacteria, namely *M. avium* on the kinetics of the BCG specific immune response and on the protection afforded by BCG against *M. bovis*. These results indicate that prior exposure to *M. avium* does not reduce the efficacy of BCG vaccination, but may interfere with host immune responses to mycobacteria, which could compromise diagnosis of infection.

Materials and methods

Exposure to *M. avium*, BCG vaccination and *M. bovis* challenge

British Holstein-Friesian calves (*Bos taurus*) were bred from the bovine TB-free herd at the Institute for Animal Health, Compton, Berkshire, UK. Animals were aged four to six months at time zero. The experiment was approved by the local ethics committee according to national UK guidelines.

Twelve calves were inoculated subcutaneously with 10^6 CFU *M. avium* strain D4ER[19] and 12 with 7H9 control medium. After 12 weeks, *M. avium* calves and six control calves were inoculated subcutaneously with 10^6 CFU BCG strain Pasteur.[18] After a further 12 weeks, all calves with the exception of six of the *M. avium*-BCG calves were challenged intranasally with 10^4 CFU virulent *M. bovis* strain AF 2122/97.[18] Post-mortems were performed 12 weeks later. Thus, the four calf groups were: (1) *M. avium*-BCG, (2) *M. avium*-BCG-*M. bovis*, (3) BCG-*M. bovis* and (4) *M. bovis*, as summarised in Table 1.

Antigens

PPDs from PPD-A and PPD-B were obtained from the Tuberculin production unit at Veterinary Laboratories Agency (VLA), Weybridge, UK.

Immunological assays

Blood was collected into heparin (10 U/ml). For cytokine assays, blood was incubated for 24 h with PPD-A, PPD-B (20 μg/ml final concentration) or *M. bovis*-specific antigens: ESAT-6, CFP-10 (5 μg/ml final concentration) as described previously.[18] The supernatants were removed after centrifugation and stored at $-20\,^{\circ}$C until assayed. IFNγ (pg/ml) concentration was determined by enzyme-linked immunosorbent assay (ELISA) using recombinant bovine standards as described previously.[21] Samples were measured in duplicate with variability between duplicates of less than 5%.

Table 1 Calf group vaccination and challenge timetable.

Group	10^6 CFU *M. avium* (s.c.)	10^6 CFU BCG (s.c.)	10^4 CFU *M. bovis* (i.n.)	Skin test and post-mortem
1. *M. avium*-BCG	0[a]	12	–	36–37
2. *M. avium*-BCG-*M. bovis*	0	12	24	36–37
3. BCG-*M. bovis*	–[b]	12	24	36–37
4. *M. bovis*	–	–	24	36–37

[a]Week of experiment.
[b]7H9 control medium given.