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Original Mycobacterial Sin, a consequence of highly homologous antigens?

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ABSTRACT

The role of antigens shared between Mycobacteria in *in-vivo* cross-reactive immune responses in host animals, have been reported to be responsible for reduced BCG vaccination efficacy as well reduced specificity of routine immunological diagnostic tests. This presents with significant disease control challenges in humans and animals. The present review highlights the results of previous studies on the effect of pre-sensitization to environmental mycobacteria on either pathogenic mycobacteria and/or *M. bovis* BCG, in experimental animals. It also takes an in-depth view into assessing the genetic similarities and relationships between atypical mycobacteria and *Mycobacterium tuberculosis* complex (MTBC) and how they might explain the immunological imprint of environmental mycobacteria in directing the hosts' immune response upon subsequent exposure to other classes of mycobacteria. The outcome of this review suggests that genetic closeness between particular atypical mycobacteria and MTBC usually indicate a higher level of homology for certain shared protective antigens. This ultimately results in a higher level of cross reactive immune responses as compared with other atypical mycobacteria that are further away genetically. This would explain the different effects of environmental mycobacteria on MTBC that have been reported in the different studies. In other words the direction of the host immune system in response to exposure to MTBC would depend on the type of environmental mycobacteria that was encountered in the initial exposure. We also explain these mycobacterial interactions in the context of the phenomenon of "Original Mycobacterial Sin". The effects of these inevitable mycobacterial interactions on field diagnosis and control by vaccination and how to circumvent them are discussed.

1. Introduction

The world acclaimed means of prevention of human tuberculosis has been through neonatal vaccination with *M. bovis* BCG, however, evidence of lowered efficacy of BCG vaccination has been reported in adults living in non tuberculous mycobacteria (NTM) prevalent areas as compared with those in non-prevalent areas (Black et al., 2003; Weir et al., 2003). This was observed as failure of protection of the BCG vaccine in adult humans exposed to NTM which was suspected to be due to the modulation of host immunity by the NTM prior to vaccination. Conversely, the protective ability of BCG is not compromised when neonatal vaccination is done on infants from the same geographic location.

Non tuberculous mycobacteria, otherwise regarded as mycobacteria other than tuberculosis type (MOTT) and in some instances referred to as atypical or environmental mycobacteria are facultative intracellular bacteria and are ubiquitous with specific niches in the environment (Lin et al., 2009), although, rarely solely responsible for causing infections, they are often implicated as opportunistic infections in immunocompromised hosts. Environmental mycobacteria are not obligate pathogens, rather, true inhabitants of the environment and they can be found as saprophytes, commensals, and symbionts (Vaerewijck et al., 2005). They generally include both slow-growing (i.e., colony formation requiring 7 days or more) and rapidly growing (i.e., colony formation in less than 7 days) species (Primm et al., 2004). Although in general NTM are regarded as non-pathogenic some of them have been reported to be pathogenic i.e. *M. kansasii, M. marinum, M. avium* Complex (MAC) comprising of a group of related *Mycobacteria* including, *M. szulgai*, (Griffith, 2002, 2010; Hughes et al., 2005) resulting in sporadic infections in humans and animals. Occasionally, TB like lesions are reported to be caused by these pathogens as seen in infection with *M. kansasii*. In addition, a number of these organisms have also been described as being responsible for cross reactive immune responsiveness especially with common diagnostic reagents, leading to false positive results.

The question now arises about the relevance of these supposed

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pathogenic/opportunistic NTM such as the members of the MAC and *M. kansasii* and their immunological interference in host animals in relation to the pathogenic mycobacteria. In the same light, the question can be asked, if NTM are generally non-pathogenic, how come some subtypes of certain species have been adapted as human pathogens no matter how sporadic or infrequently they are reported as environmental species? Effects of pre-sensitization to NTM has revealed a degree of increased resistance to tuberculosis as has been reported in humans living in NTM prevalent areas (Black et al., 2001a) as well as in cattle (Hope et al., 2005a). This review highlights the results of investigations of the efficacy of BCG vaccination in the absence or presence of NTM sensitization. It will further address (cross-reactive) antigens presumed to be responsible for the interference in immune responsiveness experienced in vivo during subsequent mycobacterial infections. Different animal species as well as different NTM species used in these experiments will be considered. In summary, the nature of the immunological imprint induced by environmental mycobacteria and the way they direct their hosts' immune system preceding exposure to other classes of mycobacteria will be reviewed in detail as well as the challenges experienced with particular reference to vaccination and diagnosis.

1.1. Genetic relationships between NTM and pathogenic mycobacteria

Previously, based on deletions observed, it was assumed that the tuberculous mycobacteria evolved from a common ancestor *M. prototuberculosis/M. canetti* and later *M. bovis*, following several deletion events (Brosch et al., 2000, 2002). Recent genomic data have provided immense information regarding the genetic nature of mycobacteria as a species (a number of NTM genomes have been sequenced using the next generation sequencing) which allowed the identification of several genetic events that have occurred within the mycobacterial genome (Veyrier et al., 2011). These genetic events suggest a more appropriate pathway of evolution, suggesting that MTBC evolved from NTM on the basis of concurrent genomic deletions as well as gene acquisition via horizontal gene transfer (HGT) (Veyrier et al., 2011)with *M. tuberculosis* becoming a more specialized/professional pathogen.

Gene acquisitions via HGT have been reported to lead to the enrichment of selected gene families critical to the physiology of *Mycobacterium indicus* pranii (MIP), an NTM which is the progenitor of the MAC (Saini et al., 2012). Gene acquisition was also described in *M. tuberculosis* complex relative to *M. kansasii* (Veyrier et al., 2011) and very broadly by an environmental bacillus which exchanged genetic material with other bacterial species, including Proteobacteria (Becq et al., 2007). Focusing on a particular gene responsible for type VII secretion, encoded in the RD1 region in *M. kansasii*, it was possible to confirm this relationship as there was a high level of homology in the structure of this gene in both organisms (Abdallah et al., 2007). Another example is the phenolic glycolipids (PGLs), which are common to slow growing mycobacteria. The PGL are expressed on a phthiocerol dimycoserate (DIM) backbone, which is found in all mycobacteria, and it was found that genomic comparison of a series of HGT events occurring at the PGL's locus also indicated evolution from *M. kansasii* to *M. tuberculosis* (Veyrier et al., 2011). A similar explanation of the relationship between MIP and MAC also suggests that *MIP* is likely an organism at a unique phylogenetic time point just preceding of the opportunistic mycobacterial species of MAC as evident in the selection of certain categories of genes which has resulted in the reduced habitat diversity of pathogenic bacteria, and their consequent ability to specialize in specific hosts (Saini et al., 2012). This new series of fast advancing genomic information will ultimately form our general basis for understanding relationships between mycobacteria and, as a result of, possibly, partially yet to be elucidated, shared antigens between these types of mycobacteria.

Using the ESAT 6 and CFP 10 as examples of MTBC antigens with several shared orthologues with several other NTM (Gey Van Pittius et al., 2001; Gey van Pittius et al., 2002), the possibility exists for certain NTM to induce potent cross reactive immune responses to these antigens and this has been reported by several workers in experimentally infected animals (Arend et al., 2005; Vordermeier et al., 2007). Further evaluation of the genomes of other atypical mycobacteria will improve the knowledge of the genetic relationships between NTM and MTBC, and further aid to elucidate genetic similarities and dissimilarities between these two groups of mycobacteria. The knowledge will also be useful in obtaining potential candidate antigens for vaccination and diagnosis. It is therefore reasonable to assume that, despite the genetic events which may have occurred; a significant number of genes with potential immunomodulatory abilities may still be shared between the two groups of organisms, and if studied in greater depth using proteomic analysis, these antigens will be identified and may further improve studies that focus on immunological cross reactivity between mycobacteria.

1.2. Interference between NTM and tuberculous mycobacteria

1.2.1. Review of existing hypotheses and current thinking on the effect of NTM on MTBC

In humans living in the tropics, who have been identified as fairly resistant to tuberculosis infection, it was apparently difficult to confirm tuberculosis using the tuberculin skin test (Mantoux) (Black et al., 2002, 2003; Weir et al., 2004, 2006). These effects have been attributed to the genetic similarity in antigens shared between tuberculous mycobacteria and NTM with particular reference to the immunogenic antigens, as they are more easily studied. The mechanisms of these phenomena can be embedded in two hypotheses, both of which are an indication of the recall of memory responses to prior antigenic stimulation. The first one is the "masking" hypothesis as proposed by Palmer and colleagues (Palmer and Long, 1966) which suggests that pre-exposure to environmental mycobacteria offers some level of protective immunity to TB and subsequent BCG vaccination does not boost the immunity. The precise mechanism by which this occurs is not known however, it possibly occurs because prior immune reactivity towards NTM prevents BCG bacilli multiplication and subsequently immune recognition of the BCG bacilli (Palmer and Long, 1966).

The second hypothesis, suggests that pre-existing immune responses, induced by NTM as a results of prior exposure to antigens common to mycobacteria block the replication of BCG resulting in rapid elimination of BCG. Brandt and coworkers (Brandt et al., 2002) hypothesized from the mouse model using NTM isolated in Malawi that prior exposure to environmental mycobacteria can lead to a broad immune response to be recalled after vaccination with BCG. Their results were based on a mouse model which follows strictly on the "priming" effect that NTM have on the immune system, and not on the presence of the live bacilli or continuous multiplication of the NTM bacilli. They established the "priming" effect because they treated the mice with antibiotics to effectively clear the organisms from the host, prior to BCG vaccination, and therefore interaction with BCG was most likely due to circulating antibodies or a recall of memory responses. The outcome of the Brandt experiment, which was a non-protective BCG vaccination, was therefore strongly based on the immunological bias created by the NTM as opposed to what naturally occurs when animals are perpetually exposed to NTM. Other studies in which NTM were not cleared with antibiotics suggests that pre exposure to NTM may not actually reduce the efficacy of BCG vaccination but will rather contribute to BCG protection (Thom et al., 2008). This may be consistent with what is observed in nature, in which consistent exposure to NTM is the norm, hence the immune response in this case is under a different kind of pressure. Interestingly, in a recent study in humans, it was discovered that vaccinating at 10 weeks of age as compared to at birth enhances BCG-specific T cell response when measured at 1 year of age (Kagina et al., 2009). This 10 week delay presumably exposes these infants to NTM in the environment with the

corresponding immune priming prior to BCG vaccination.

The ability of an NTM to persist in the host including the level of homology of shared protective antigens between the NTM and BCG vaccine are primary factors which may determine the extent of immune interference of BCG vaccination in hosts that have been sensitized to NTM (Brandt et al., 2002). More recent investigations further support Brandt and co-workers (Brandt et al., 2002) by confirming clearance of BCG bacilli as a result of *M. avium* pre sensitization in mice models (Demangel et al., 2005; Young et al., 2007). They also demonstrated that immune responses to fast growing NTM like *M. scrofulaceum*, *M. vaccae*, resulted in the persistence of BCG in the spleen of mice (Demangel et al., 2005). In comparison, investigations of Young et al. (2007) also gave evidence to suggest that the more quickly an NTM grows, the quicker it is eliminated from the host. This was described explicitly when two strains of *M. avium* (slow and fast growing strains) were compared in the context of their effect on BCG efficacy in mice (Demangel et al., 2005). It was observed that the fast growing NTM induced significant up-regulation of cell surface activation markers such as major histocompatibility complex II, CD80, and CD86 and further induced the release of pro-inflammatory monokines (interleukin-1β [IL-1β], IL-6, and TNF-α) on in vitro-derived dendritic cells and in dendritic cell-macrophage respectively following direct in vitro contact of cells with bacteria. The slow growing one (IS*901*-positive strain) on the other hand had none of these effects (Young et al., 2007). Genetically, most fast growing NTM are more distantly related to BCG or MTBC, compared with slower growing NTM relatives (Veyrier et al., 2009, 2011). The slow growing NTM i.e. *M. kansasii* are known to possess a high level of homology in certain antigens i.e. ESAT-6 family of antigens, with tuberculous mycobacteria and this is evidenced in immunological cross reactivity that has been reported (Vordermeier et al., 2007). It has even been postulated that *M. kansasii* may actually be more closely related to pathogenic mycobacteria i.e. *M. tuberculosis* than previously thought (Veyrier et al., 2011). In the light of this, and given the knowledge of the varying abilities of NTM to persist in the host, differential immune responses particularly after BCG vaccination are anticipated, and should be investigated in depth.

1.3. Review of experimental scenarios of NTM sensitization prior to BCG vaccination and challenge by tuberculous mycobacteria

The masking hypothesis is supported by the findings in cattle studies in which pre-exposure to NTM led to BCG vaccination failure in cattle (Buddle et al., 2002, 2005a,b), evidenced as a significantly lower degree of protection against *M. bovis* challenge in a study where there was a high level of sensitization to NTM compared to other studies where animals had little exposure to NTM. Similar findings were observed in experiments conducted in mice (Flaherty et al., 2006; Young et al., 2007) and in both of these instances, *M. avium* was used. Whilst the mice were experimentally infected with *M. avium*, the cattle study by Buddle (2002), was based on BCG vaccination of cattle with high IFN-γ response to PPD A, one may attribute the ineffectiveness of the vaccine to *M. avium* sensitization.

In other experiments in cattle, BCG efficacy was not impaired by NTM sensitization (Howard et al., 2002; Hope et al., 2005a; Thom et al., 2008) despite using *M. avium* (strain used in PPD A production). In this case however, the cattle were experimentally sensitized to NTM compared with a bias towards PPD A as seen by the IFN-γ assay by natural infection. These findings contradict the two afore mentioned hypotheses because when a single subcutaneous administration of *M. avium* (strain D4ER, the strain used in preparing PPD-A) was used experimentally to sensitize the cattle, it did not result in a reduced efficacy of BCG vaccination. This *M. avium* pre-sensitization was observed not to affect the protective cover of BCG vaccination on *M. bovis* challenge, rather it primed the beneficial immune response of BCG vaccination, thus leading to lower TB pathology in the number of affected tissues in the group of cattle which were pre-sensitized with *M. avium* compared with the group that received only BCG vaccination as well as the group with only *M. bovis* challenge (Hope et al., 2005a; Thom et al., 2008). The closest finding similar to this was reported in a mouse model, in which, despite the absence of immune responses to BCG vaccination, there was no significant difference in BCG efficacy in sensitized miceafter *M. avium* pre-sensitization compared with non-sensitized mice (Demangel et al., 2005). Variations in the effect of NTM on BCG clearance or persistence need to be carefully evaluated before arriving at a sustained conclusion on the effect of NTM pre-sensitization on BCG vaccination.

In cattle, effective prophylactic vaccination against bTB may provide a better control option than a test and slaughter control approach in countries that cannot afford such, as well as in countries that have wildlife reservoirs of *M. bovis* infection (Buddle, 2001a,b). Preliminary results of BCG vaccination in cattle is similar to the picture presented in humans living in NTM prevalent areas, in which neonatal vaccination of calves is more effective than adult vaccination (Buddle et al., 2003). In addition it must be noted that vaccination at birth provides the best level of protection compared with later time periods in the neonatal life (Buddle et al., 2003; Hope et al., 2005b). It was observed that giving a boost following an initial vaccination at birth was shown to result in an immunopathological response in neonatal calves. It was observed in this study that there was no significant difference in protection between calves vaccinated with BCG within 24 h of age and those vaccinated at 6 weeks of age while those vaccinated within 24 h of birth and revaccinated at 6 weeks had a lower degree of protection. The lower degree of protection in the revaccinated group is therefore more likely to have been related to the fact that revaccination of calves which were already strongly responding to BCG vaccination may have triggered an inappropriate immune response for protection. However another study by Parlane et al. (2014) demonstrated that BCG given 2 years post-initial vaccination boosted protection against challenge with *M. bovis*. The combined outcome of these two studies for BCG vaccination is crucial in a vaccination strategy for *M. bovis*, furthermore, time dependent wane of BCG immune responses *in-vivo* and persistent underlying exposure to NTM over the two year period are some factors that may impact on the boost of the vaccination 2 years later. It may be argued that exposure to NTM may offer some prime boost effect due to exposure to similar but non-identical antigens found in NTM. This therefore suggests that instituting a BCG vaccination policy for calves at birth and a subsequent follow up after a defined interim period should significantly improve control attempts for bTB in heavily endemic countries (Buddle et al., 2002).

Different immune responses are associated with different species (Demangel et al., 2005) or strains of mycobacteria and this is often associated with virulence status of these different mycobacteria as seen by a boost of BCG vaccinated mice when pre-exposure was by *M. vaccae* and *M. scrofulaceum* (Demangel et al., 2005). Earlier studies by Palmer and Long (Palmer and Long, 1966) also demonstrated that prior exposure of guinea pigs to *M. fortuitum, M. avium or M. kansasii* imparted 15%, 50% and 85% as much protection against *M. tuberculosis* relative to BCG alone, but when BCG was combined with either NTM, there was no more protection observed compared with BCG alone. This suggests that the virulence of these different NTM directly relates to the strength of the immune response which is generated towards these NTM in vivo. It could also mean that, considering the presence of ESAT-6 and CFP-10 in *M. kansasii*, a protective type of immune response is triggered when the host is exposed to them. Thus, suggesting that prior contact with certain environmental mycobacteria may efficiently prime protective immune responses to *M. tuberculosis* antigens with a subsequent positive effect on BCG efficacy when the vaccine boosts the beneficial immunological stimulation imparted (Demangel et al., 2005) this was also seen in experimental cattle infections (Hope et al., 2005a; Thom et al., 2008). These observations clearly highlight the effect of cross-sensitization by NTM as a potential cause of the failure of some BCG vaccination programs for humans and livestock.

Besides interfering with the protective cover of BCG vaccination, pre-sensitization to NTM have also been reported to influence the diagnosis of cattle infected with *M. bovis* because both skin test and IFN-γ diagnostic tests were compromised (Hope et al., 2005b) as well as in humans (Black et al., 2001b; Weir et al., 2003). In contrast to the fact that experimental cattle vaccination after pre-exposure of calves to *M. avium* may not alter the protective cover of BCG vaccination, a significant effect on diagnosis using the skin test and IFN-γ assays seen as elevated levels of IFN-γ to PPD-A and PPD-B in *M. avium* sensitized compared to naïve vaccinated calves were observed (Hope et al., 2005a,b; Thom et al., 2008). Young et al. (2007) showed that immune responses to two strains of *M. avium* (WAg 206 and WAg 207) varied, whilst WAg 206 was capable of compromising the protection provided by BCG in guinea pigs following *M. bovis* challenge, WAg 207 was not. In cattle, exposure to NTM has far reaching effects on skin test and can be interpreted based on the prevalence of BTB in the herd. The positive predictive value of a PPDB antigen specific response in the skin test was found to be low in low prevalence herds hence, the identification of a large number of non-infected animals as being positive (Aagaard et al., 2010) evidenced as a high frequency of false PPDB skin test responders and this is probably due to exposure of the animals to non-tuberculous mycobacteria (Amadori et al., 2002). In low prevalence herds, the percentage of skin test positive animals that could be confirmed by ESAT-6 and CFP10 *ex vivo* was only 15–18% meaning that the tuberculin skin test incorrectly identified up to 80% of the animals as being infected (Aagaard et al., 2010).

Purified protein derivative contains a crude mixture of antigens of the mycobacterial organism and stimulating a host animal to PPDB may result in animals exposed to NTM to have measurable skin test responses as well as IFN-γ responses, albeit not as high as animals truly infected with *M. bovis*. Vordermeier and coworkers (Vordermeier et al., 2007) described cross reactivity to ESAT 6 and CFP 10 peptides derived from *M. bovis* and *M. kansasii*, (isolated previously from cattle and later experimentally infected). They were also able to identify a number of NTM species from the tissues of cattle that were slaughtered under the test and slaughter policy in Great Britain of which *M. kansasii* accounted for nearly half of the NTM that were isolated. The isolation of NTM from tissues of cattle in an area known to have a low NTM prevalence, about 0.8% over a period of 2 years, emphasizes the importance of NTM and their possible impact on *M. bovis* diagnosis in endemic areas by extrapolation. The species prevalence of NTM in Sub Saharan Africa is generally unknown, but the relevance is becoming increasingly important in diagnostic assays, because of the increase in false positive cases to the SICT and Bovigam assays in cattle. The implications of NTM cross reactivity for disease control in bovine TB cannot be overemphasized. Another study showed that inclusion of ESAT-6 can markedly improve the specificity of the IFN-ɣ test for re-testing skin test-positive animals (Buddle, 2001a,b). An ESAT-6-based IFN-ɣ test could be particularly useful to reduce the false positive rate, yet still maintain an acceptable level of sensitivity (Table 1).

1.4. Just what is original antigenic sin?

Original antigenic sin (OAS) as a phenomenon was first described when exposure to a novel influenza virus strain leads to production of antibodies against a related viral strain encountered in the past (Kim et al., 2009). These cross-reactive antibodies are produced at the expense of antibodies against novel epitopes in the current strain (Powers et al., 2010). OAS is most often remarkable when intermediate antigenic relatedness exists between pathogens; as well as when they are antigenically complex and when sequential exposure intervals are long. This ultimately results in the on-going selection and expansion of lymphocyte clones with heightened antibody avidity at key cross-reactive epitopes (Kim et al., 2009).

The OAS seen in T cells is like the B cell variety, since it is antigen driven, it is specific for epitope and their variants, and it therefore is demonstrable for a number of pairs of related protein epitopes. The resultant effect thereof is the impaired clearance of the second encountered virus. The cellular basis of T cell original antigenic sin, as for the B cell variety, has been attributed to T cells primed by the original epitope and sufficiently cross-reactive with the modified epitope to be expanded (Liu et al., 2006). The precise mechanism by which OAS occurs in T cells is not clearly defined but three models (McMichael, 1998) have been described thus 1) During OAS, weak cross-reactive subsequent second epitope reactivates the larger number of memory CTLs against the initial epitope better than how it activates the small number of naive T cells that are specific for the second epitope. 2) OAS occurs due to deactivation of cells presenting the second antigen by primary CTLs that cross-react against the second antigen. 3) Thirdly OAS may occur because CTLs that respond to the variant epitope encounter “a form of T cell antagonism” observed as CTL clones reacting suboptimally to the variant epitope thus leading to partial activation of the CTL and subsequently leading to anergy.

This OAS phenomenon has been observed in T cells against lymphocytic choriomeningitis virus (LCMV) viral variants (Klenerman and Zinkernagel, 1998). In this case, initial exposure to a strain LCMV and then subsequently to a second LCMV strain generated cytotoxic T lymphocytes specific to only the first strain. This observation explains the reason why vaccines that deliver mutant epitopes at different times have the potential to induce OAS hence interfering with vaccine efficacy (Welsh and Fujinami, 2007). The OAS phenomenon in the context of mycobacteria interactions can be defined thus, “when a given antigen, shared between mycobacteria is initially encountered in a non-pathogenic or non-‘danger’ setting i.e. (NTM pre-sensitization), it may lead to a mild, non-inflammatory response and the generation of specific T cells to its epitopes, but when a more pathogenic mycobacterium bearing a similar antigen is subsequently encountered, a cross-reactive immune response will be reactivated to the epitope of shared antigen encountered in the initial exposure as well as a recall of T cells to the epitopes of the initial shared antigen”.

Singh and co-workers (Singh et al., 2002) hypothesized in their model that the large population of pre-existing memory T cells may cross-bind the APLs on MHC I dendritic cells in the draining lymph nodes, this then blocks the access to the APCs by low frequency naive T cells. This model utilized OVA/OVANT whilst OVANT is a genetically modified form of the full length OVA gene. This model also showed that splenocytes from mice immunized with OVA plasmid produced OVA-specific CTL, CD8+/IFN-γ production, as well as OVA tetramer binding, but negligible OVANT-specific responses were recorded (Singh et al., 2002). In the same experiment, mice immunized with OVA plasmid and then boosted with OVANT plasmid showed high OVA-specific CD8 responses, but no OVANT-specific CD8 responses. However, reciprocal immunizations demonstrated that OVANT plasmid priming reduced subsequent OVA-specific CD8 responses. This suggests that sequential delivery of the OVA and OVANT antigen presenting lymphocytes generate potent form of OAS.

These results were similar to what was seen in the study of OAS in LCMV (Klenerman and Zinkernagel, 1998) but differ from that model in that immune responses against the first epitope are not boosted by a second immunization with the mutant epitope. Functional cross-reactivity was also not observed between epitopes of OVA- and OVANT-specific CD8 T cells. The OVA/OVANT system clearly indicates that boosting of primary responses by the counter epitope likely does not occur because these epitopes reciprocally antagonize or inhibit each other’s T cells. Klenerman and Zinkernagel (1998). Either of these models presented by Singh et al. (2002) and Klenerman and Zinkernagel (1998) may provide insight into the cross reactive immune responses between shared antigens which are common to NTM, BCG vaccine and MTBC and it will be useful to fully understand these processes in a bid to design a suitable vaccine for bTB in livestock and wildlife.

Table 1
List of experimental studies and surveys carried out highlighting the observations of mycobacterial interactions in different animal species.

Reference	*Animal species*	*NTM*	*BCG*	*MTBC*	*Remark*
(Lozes et al., 1997)	Mice-BALB/c and C57BL/6	*M. intracellulare, M. avium, M. scrofulaceum*	BCG	*M. tuberculosis*	*M. scrofulaceum* may alter BCG protection against *M. tuberculosis* in genetically predisposed subjects
(Brandt et al., 2002)	Mice- CBA/J and C57BL/6J	*M. avium* (ATCC 15769), *M. scrofulaceum*, (ATCC 19275), *M. vaccae* (ATCC 15483	BCG Danish 1331	*M. tuberculosis (Edman*	NTM presensitization lead to rapid recall of broad immune response to BCG vaccination which controlled the replication of the BCG bacilli
(Demangel et al., 2005)	Mice C57BL/6 mice	*M. avium* (ATCC 15769), *M. scrofulaceum* (ATCC 19275), and *M. vaccae* (ATCC 15483)	BCG:RD1 and BCG	*M. tuberculosis* (H37Rv)	Inhibitory effect of NTM on BCG depends on the extent of cross recognition of antigens shared with BCG vaccine as observed when BCG:RD1 persisted longer than BCG despite *M. avium* presensitization.
(Flaherty et al., 2006)	Mice	*M. avium*	BCG	*M. tuberculosis*	BCG was initially administered followed by *M. avium*. BCG efficacy on tuberculosis infection was impaired in mice that had *M. avium* treatment
(Young et al., 2007)	Mice	*M. avium* WAg 206 and WAg 207.	*M. bovis* BCG (Pasteur strain 1172)	none	Persistence of NTM strains (WAg206 over WAg207) in host, resulted in significant differences in the ability of both NTM in the down regulation of IFN-γ and promotion of a Th1-Th2 shift, with the most persistent NTM having a more adverse effect
(Buddle et al., 2002)	Cattle	High PPD A sensitivity sensitization	*M. bovis* BCG Pasteur 1173P2	*M. bovis* challenge strain, 83/6235, *M. bovis* WAg500 and WAg501	NTM sensitivity adversely affected BCG efficacy, however vaccination with attenuated *M. bovis* strains led to improved protection
(Howard et al., 2002)	Cattle	*M. avium* strain D4ER	*M. bovis* BCG Pasteur	none	Prior exposure to environmental mycobacteria does not necessarily inhibit the immune response to BCG rather it primes the immune system of calves making the detection of *M. bovis* BCG specific immune responses to be masked by reactivity to common antigens.
(Hope et al., 2005a)	Cattle	*M. avium* strain D4ER	none	*M. bovis*	Responses to *M. avium*, although providing some immunity, may mask diagnosis of *M. bovis* infection, even when specific antigens are employed
(Thom et al., 2008)	Cattle	*M. avium* strain D4ER	*M. bovis* BCG Pasteur	*M. bovis*	Exposure of cattle to *M. avium* prior to BCG vaccination did not dampen BCG-specific immune responses but also resulted in lower TB pathology.It however undermined current TB diagnostic tests and the IFN gamma test in cattle based on avian and bovine PPD

1.5. OAS and cross reactive immune responses tomycobacterial antigens

Cross reactive immune responses between NTM and BCG bacilli resulting in the generation of a non-protective immune response can therefore be further explained by the phenomenon of the "original antigenic sin". The OAS in mycobacterial infections requires a cursory look at some shared mycobacterial antigens that have been reported to be immunogenic. This is noted to manifest practically in the field diagnosis of bTB in NTM prevalent areas when truly infected animals respond weakly to the bovine PPD in skin test and the Bovigam assay. Observations from certain studies (Amadori et al., 2002; Vordermeier et al., 2007) suggest that immune responses to shared antigens originating from NTM lead to a weaker immune response to similar antigens found in MTBC and *M. bovis* BCG This cross reactivity may be due to a notable anamnestic response to common epitopes expressed by environmental mycobacteria and sustained by cross-reacting γδ-T cells (Hoft et al., 1998; Vordermeier et al., 2007).

For instance 2 antigens encoded in the RD-1 region, ESAT-6 and CFP-10 which are often mis-regarded as "*M. tuberculosis* specific antigens", are found in the genomes of other mycobacteria including the fast growing NTM (Gey Van Pittius et al., 2001; Gey van Pittius et al., 2002; Vordermeier et al., 2007). In *M. kansasii* ESAT-6 bears 95% sequence homology at the amino acid level differing only at two to three amino acid residues whilst with CFP-10, about five to seven residues varied in the different subtypes of *M. kansasii* (Vordermeier et al., 2007).

Given the level of similarity and postulated closeness of *M. kansasii* and MTBC (Veyrier et al., 2009, 2011), high genetic similarities at the RD1 encoded antigens and complete cross reactivity may seem plausible, however, there are reports indicating that *M. smegmatis* which may not be as close to the MTBC as *M. kansasii*, is also known to share many antigens in common with the MTBC and actually secretes the ESAT-6 antigen as it has orthologues of the ESAT-6 proteins in its genome (Vordermeier et al., 2007). Besides secreted proteins, cross reactivity has been encountered at proteoliposomes from *M. smegmatis* where it was shown to induce humoral based immune cross-reactivity against *M. tuberculosis* antigens in mice (Rodriguez et al., 2011).

The secreted ESAT-6 like protein esxR (TB10.3) Rv3019, has also been described to discriminate between TB group (confirmed TB cases, individuals recovered from TB and individuals exposed to TB without evidence of clinical TB infection), NTM exposed and BCG vaccinated individuals in different classes of patients; (Ahmed et al., 2012). However, in a study in humans in Western Cape South Africa, less than 50% of all the respondents, responded to this antigen Golakai (2008). TB 10.4 (Rv0288) has also been described as a strong antigen which is useful for early detection as of *M. tuberculosis* infections (Aagaard et al., 2003). It is also strongly recognised by TB exposed individuals, as well as by TB exposed cattle (Aagaard et al., 2003). However, in animals that were sensitized to NTM based on the single intradermal skin test, this antigen TB 10.4, despite 79% homology to its *M. avium* ortholog, did not result in any IFN-γ response in these animals (Aagaard et al., 2003). Located just outside the RD1 region is a protein called the Rv3615c which is similar in size and sequence homology to CFP-10 and ESAT-6. It is an Esx-1 secreted protein C (EspC), hence a potential target of cellular immunity in tuberculosis infections (Sidders et al., 2008). EspC was found to be at least as immunodominant as ESAT-6 and CFP-10 in both active and latent TB infection in humans. Using this antigen in cattle experiments as reported by Sidders and co-workers (Sidders et al., 2008), indicated IFN-γ responses in *M. bovis*-infected cattle (11 of 30 cattle [37%] [$P < 0.01$]) but not in naïve or BCG-vaccinated animals. This is because although the gene is present in BCG, its protein is not secreted as the secretion of Rv3615c is dependent on the Esx-1 secretion system which is found in the RD1 region and is absent from BCG (Sidders et al., 2008). Furthermore, in a significant proportion of infected cattle that did not respond to the well-characterized mycobacterial antigens ESAT-6 and CFP-10, this antigen resulted in IFN- γ responses (Sidders et al., 2008). One important Heat shock proteins (HSP) of Mycobacteria is the HspX protein (Rv2031c or TB 16.2). Gamma interferon release by T cells stimulated with HspX is significantly higher in *M. tuberculosis*-exposed individuals than in *M. tuberculosis* unexposed BCG vaccinees. The 16-kDa protein was reported to be strongly recognised at 126 and 154 days post infection in a cattle experiment (Aagaard et al., 2003), in 75% of the tested samples.

2. Conclusion

The phenomenon of OAS, which is observed as suboptimal immune response to prior exposure to an antigen when it later encounters a related antigen (Pan, 2011) cannot be more relevant in bTB control using BCG vaccine in NTM prevalent areas. This review describes why pre-sensitization to certain NTM species elicit a negative effect after BCG vaccination which is consistent with the OAS phenomenon as well as why pre-sensitization to other types of NTM does not result in OAS, but rather a boost in BCG vaccination is observed. This review further highlights reported hypotheses based on outcomes of experimental studies on mycobacterial interactions which do not take into full account the myriad of possibilities that exist in in-vivo immunological responses to multiple mycobacterial exposure.

Firstly, prolonged persistent exposure to multiple species of NTM has been noted to result in BCG inefficacy. This indeed is the most probable occurrence in which animals and humans are exposed in regions where NTM are abundant. However, the nature and type of NTM isolated found in these areas will actually determine the outcome of the efficacy of BCG vaccination preceded by NTM sensitization. To this end, a thorough study of NTMs in regions with high TB or bovine TB burden is central to an efficient vaccination campaign.

Young et al. (2007) indicated that the "nature" of the NTM used could also in part be responsible for the strength of the immune system priming observed. We define the "nature" as level of virulence, antigenic homology and genetic similarity of the NTM, relative to MTBC. How close an NTM is to the MTBC genetically, and this is sometimes evidenced in higher homology of the orthologues of the shared antigens, directly translates to stronger priming of the immune system. Persistence of NTM in host animals, is strongly correlated with virulence capabilities of these NTM and this is generally linked to the presence of virulence genes i.e. RD1 gene in these organisms such as is seen with *M. kansasii* which has been isolated in animal tissues. Persistence of BCG in the host also follows the same rationale, in that effective clearing of BCG is observed when the hosts have been exposed to certain types of NTM, but modification of BCG by inclusion of the RD1 gene, improves BCG persistence in the host and ultimately confers better protection in the event of tuberculosis infection.

The overall effect of NTM sensitization on BCG vaccination is multifactorial and whilst variable effects have been observed in different classes of experimental animals, enough evidence exists to assume that immune modulation occurs and to a significant extent is directed by the shared antigens between the NTM and MTBC and the particular context of the NTM. Further evidence also allows for the conclusion that to overcome this immunomodulation, BCG as a vaccine has to be improved upon by the inclusion of specific antigens which improve its efficacy without compromising the discrimination of vaccinated from unvaccinated animals. In the same vein, the use of more specific antigens in diagnostic tests will ultimately help overcome the incidence of false positives especially in NTM prevalent areas. This review paper has identified some critical gaps and valid points in the studies on mycobacterial interaction in host animals that will be useful for future research considerations.

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