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Review

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Cattle as Experimental Model to Study Immunopathogenesis of Tuberculosis

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Abstract

Various animal models are used to study the immunology, genetics and molecular biology of tuberculosis (TB) as well as for testing the new vaccines and drugs. Mice are widely used to study the immunology of chronic TB infection, while guinea pigs are used for aerosol TB infection and rabbits are used to study the lung cavitations. Cattle are natural host to *Mycobacterium bovis* infection, which act as a connecting link between the small laboratory animals and human counterparts for testing the vaccine efficacy. By using cattle as an experimental model, the disease outcome is understood through natural infection with *M. bovis* and a comparison can be made with *M. tuberculosis* infection. In this manuscript, the utility of cattle in understanding the progression of disease and the immunological correlates to evaluate the protective efficacy of vaccines are described.

Keywords: Cattle; Bovine tuberculosis; *Mycobacterium bovis*; *Mycobacterium tuberculosis*; Vaccine; Immunopathogenesis; Immunological correlates

Introduction

Bovine TB, caused by *Mycobacterium bovis* remains a global problem. Besides considerably affecting the economy of food industry, cattle infected with *M. bovis* poses higher risk for human health. The live attenuated *M. bovis* bacillus Calmette-Guérin (BCG) is the only available vaccine against TB, which has varying protective efficiency in both humans and cattle [1,2]. It is found unsafe in HIV-infected infants and its use for such individuals has not been recommended [3]. Hence, there is a requirement to devise new vaccines, which shall be safe in HIV-infected infants and other immunocompromised individuals and efficacious for all forms of TB in every age group as well as for cattle. Cattle are natural hosts to *M. bovis* infection, which share > 99% genetic identity with *M. tuberculosis* and induce similar host response and disease profile upon infection [4]. The bovine TB pathogenesis is similar to human TB in many features and is considered as a role model for human TB [5]. This review is primarily focused to update insights on the important findings pertaining to immunopathogenesis of TB using cattle as an experimental model.

Human TB caused by *M. bovis* (zoonotic disease) is contemplated sporadic in developed countries, but in developing countries including India, this disease is ill-defined. A high prevalence of TB cases has been documented in professionals exposed to *M. bovis* infected cattle. Interspecies spread of disease is common and poses a serious health issue in such setting [6]. A single bacillus within a droplet nucleus may be adequate to establish an infection in bovine lung and the disease is restricted in the respiratory tract [7,8]. In cattle, both low and high dose infection with *M. bovis* are established [4]. Similar to human TB, bovine pathogenesis primarily entails lungs as well as regional lymph nodes but the upper respiratory tract is also involved [9].

Earlier attempts to design *M. bovis* infection models were established via subcutaneous and intravenous challenges, which lead to severe systemic infection, but could not replicate the pathology of natural infection. Similarly, experimental oral infection with high doses [10,11] revealed intestinal lesions. However, the main focus has been to design experimental models via the respiratory tract, either by direct inoculation or indirect challenge, with the latter achieved by keeping animals in contact with a source of infection (e.g. infected cattle, wildlife, etc.). Indirect infection models can replicate pathology of natural infection, but the in-contact models are variable in efficiency [10]. Direct inoculation of *M. bovis* into the nasal cavity, tonsil or the trachea is highly effective in eliciting infection, though the pathology could be atypical of natural infections. Buddle et al. [12] observed pathology of natural infection via intra-tracheal inoculation with 10^3 colony-forming units (CFU) of *M. bovis*, but not with higher doses.

A correlation was found between the dose used and the elicited pathology via intra-nasal inoculation in cattle [13]. Palmer et al. [14] attained natural infection-like disease in cattle after aerosol exposure (up to 10^3 CFU *M. bovis*). Furthermore, Neill et al. [15] earlier reported that nasal secretion and lung lavage samples taken from skin test negative cases were actually culture-positive thus supporting the potential for cattle-to-cattle spread by aerogenous routes. To determine the minimum infective dose of *M. bovis* essential to elicit specific immune responses and generate pathology in cattle, calves were infected via intratracheal route with different doses ranging from 1-10^3 CFU of *M. bovis* [8], however, no differences in the sizes of the tuberculin skin test (TST) reactions and the times taken to achieve a positive interferon-gamma (IFN-γ) were observed for different doses [8].

The conditions under which infected cattle transmit infection to other cattle are poorly understood, but that depends on aerosol generation, numbers of bacilli excreted and herd density. In an earlier transmission study [16], sentinel calves exposed to *M. bovis*-contaminated pasture could not develop lesions. It was documented that the environmental contamination from infected calves had little impact on cattle to cattle spread [10,17]. However, O'Reilly and Costello [18] did not detect transmission from skin test positive animals (96% of which were culture-positive) to in-contact animals

thus suggesting that there was a lower rate of cattle to cattle transmission in outdoor conditions in comparison to animals sharing a confined airspace [19]. On the other hand, Costello et al. [20] observed transmission from skin test positive cattle to four of ten native cattle. Transmission of *M. bovis* infection from cattle infected intranasally under experimental conditions to native animals has also been documented [13,21].

Experimental infection with *M. bovis* have shown that all the major T-cell subsets (γδ, CD4 and CD8 T cells) are involved [10,22]. Of utmost importance in response to *M. bovis* infection is the development of a Th1 type IFN-γ immune response. In experimentally infected *M. bovis* cattle, CD4 memory T cells appear to be the dominant cell population producing IFN-γ. CD8 memory T cells are also identified as important producers of IFN-γ [23]. CD4 T cells contribute to the inhibition of intracellular mycobacterial growth, whereas CD8 T cells lyse *M. bovis*-infected macrophages [24]. Cattle express NK cell marker NKp46 and their NK cells also produce IFN-γ to stimulate macrophages for augmented killing of *Mycobacterium* [25]. Furthermore, γδ T cells are involved in both early stages of *M. bovis* infection and early lesion formation [22], where they have an important role in bacterial killing via IFN-γ production [10]. Notably, γδ T cells constitute 10-20% of circulating T cells of adult cattle, but make up to 55% in neonate calves in comparison to 1-10% of adult humans [26]. As infection progresses, changes in the balance of the anti-mycobacterial immune response are associated with a more prominent Th0 immune profile including the development of antibody responses, reduced cell-mediated immune (CMI) responses and a widespread pattern of disease [27-29].

Following inhalation, bacteria get deposited in the lung bronchioles and alveoli from where they are taken by alveolar macrophages, eliciting the expression of cytokines [30,31]. Both pro-inflammatory as well as anti-inflammatory cytokines are expressed by the alveolar macrophages and stimulate innate immune cells [31]. The adaptive immunity is put into when dendritic cells containing tubercle bacilli move from the lung to the regional lymph nodes, stimulating naïve T-cells via antigen presentation. Such T-cells move to the infection site of lungs, and in combination with epithelioid macrophages and multinucleated giant cells form a characteristic tubercle lesion known as granuloma [32]. The salient features of Cattle as an experimental model for studying TB pathogenesis are summarized in Table 1. The promising TB vaccines can be tested (for safety and efficacy) in calves prior to testing in costlier non-human primates. Some of the major challenges in cattle TB research include difficulties of keeping these large animals in biosafety level-3 (BSL3) facilities.

Histopathological characteristics	Necrosis and caseation present; cavitation absent
Natural host	M. bovis, which share > 99% epitopes with M. tuberculosis
Available immunological reagents	Moderately large range
Disease Dissemination	Natural transmission
Vaccine trials	Experimental challenge and natural transmission model for vaccine studies
Latent TB Infection studies	Poorly studied
Genetics studies	Well-defined lineages (Bos taurus, Bos indicus), cross-breeds and inbred herds
Advantages	Connecting link between small laboratory animals and non-human primates
Disadvantages	Absence of cavitation, use of M. bovis instead of M. tuberculosis, higher cost of maintenance

Table 1: Salient features of cattle to study TB pathogenesis.

Granuloma and Host-pathogen interactions

The host-pathogen interactions within granuloma determine the disease outcome. Based on microscopic evaluation of cellular composition of granulomas, Wangoo et al. [33] and later Palmer et al. [34] categorized the morphological distinct granulomas into four stages as stage I (initial), stage II (solid), stage III (necrotic) and stage IV (necrotic and mineralized). Such classification of granuloma and the diverse cytokine production at different stages of granuloma may facilitate to understand bovine TB pathogenesis.

To understand the pertinency of aero genic transmission for bovine TB, Johnson et al. [35] documented granuloma formation and its distribution in cattle infected with low doses of *M. bovis* via aerosol route (1-10^3 CFU). Interestingly, the degree of lesion growth and granuloma distribution were similar for the lowest dose (1 CFU) and the highest dose group (10^3 CFU). The mRNA expression of pro (IFN-γ and TNF-α) as well as anti-inflammatory cytokines (IL-4 and IL-10) were gradually increased in cattle infected with different doses of *M. bovis* [35]. Several cytokines have been evaluated recently by immunohistochemical method in different stages of granuloma in lungs and lymph nodes of *M. bovis* infected (via aerosol route) cattle [36]. Animals with advanced stage IV granulomas revealed high reactivity to IFN-γ and TGF-β in caseous necrosis areas, whereas animals with stage III granuloma also revealed high reactivity to IFN-γ, but moderate reactivity to TNF-α, IL-10 and TGF-β. The stage I and stage II granulomas were found in few bovines, and exhibited low cytokine production.

ESAT-6 (Rv3875) and CFP-10 (Rv3874) secreted proteins of *M. tuberculosis* complex are involved in the phagolysosomal escape of bacilli and in granuloma formation. Upon TB infection, multi-nucleated giant cells are induced intended at comprising mycobacteria and in tissue culture systems, while signal regulatory protein (SIRP)α is necessary for multi-nucleated giant cell formation. Such interactions between SIRPα and ESAT-6/CFP-10 complex were examined by Waters et al. [37] with specimens collected from *M. bovis* infected calves. The capability of ESAT-6/CFP-10 to distend SIRPα cells, bind them as well as to elicit multi-nucleated giant cells expressing SIRPα has been documented [37]. In humans, giant cells in TB granulomas

are shown to express various cytokines, chemokines and enzymes for the formation and maintenance of granuloma. To assess the role of cytokine production by giant cells in tuberculoid granulomas of *M. bovis* infected calves, *in situ* hybridization technique; RNAScope was employed [38]. Interestingly, very early and late stage granulomas produced distinct levels of TGFF-β, IL-17A and IL-10. An inter-connection was also found between the cytokine levels and the cell size or number of nuclei of giant cells thus suggesting that giant cells within granulomas actively contribute to TB immunopathogenesis. In a similar manner, cytokine production was evaluated by *in situ* RNAScope assay in the granulomas of lungs and caudal mediastinal/tracheobronchial lymph nodes of *M. bovis* infected cattle via aerosol route [30]. Though there was morphological alikeness, disparities were observed in the late stage granulomas of the lung in comparison to tracheobronchial lymph nodes for the production of IFN-γ, TGF-β, IL10, IL-17A, IL- 22, etc. In addition, disparities were observed in tracheobronchial versus caudal mediastinal lymph node granulomas for IFN-γ, IL-10, IL-17A, TGF-β, etc. production thus suggesting that morphology of granuloma is not a true index of granuloma function.

Vaccines tested in cattle

Many reports are available on TB vaccines for cattle. The main hindrance for the limited use of TB vaccines in cattle is that the protection may not be complete and the vaccine sensitized animals respond to conventional TB diagnostic tests (IFN-γ and skin testing). This issue could be resolved by using the DIVA tests (differentiate infected from vaccinated animals). New vaccine candidates are available for testing but there is a shortage of BSL3 facilities to conduct the costly trials in cattle. Calmette and Guérin documented in 1911 that high doses of BCG induced protection in cattle against *M. bovis* challenge and trials were conducted across the world to evaluate the BCG efficacy [2,39]. However, the results of field trials were disappointing and that could be due to different BCG strains used, very high doses of BCG inoculated, previous disclosure to atypical mycobacteria, etc. [40]. Several challenge models for vaccination studies have employed a dose of 10^3 - 10^4 CFU of *M. bovis* injected via intratracheal, endobronchial or aerosol route, thus replicating the natural disease [14,41,42]. Against *M. bovis* challenge, BCG vaccination via subcutaneous or oral routes repeatedly revealed decreased pathological and bacteriological results, though sterilizing effect was not documented. BCG was found to be efficacious when injected subcutaneously at low doses (10^3 to 10^6 CFU) [41] or at higher doses (10^8 CFU) via oral route [43], and by different substrains (Pasteur and Danish) [44,45]. The neonatal calves of < 1 month vaccinated with BCG elicited higher protective immunity than 6 month old calves vaccinated with BCG [46,47] and that could be due to high frequency of circulating γδ T and NK cells in those neonates, which could result into a strong innate response and also inoculation of BCG prior to exposure with atypical mycobacteria. The prior exposure with atypical mycobacteria could mask the protection elicited by BCG in calves [48], while exposure to *M. avium* could induce some protection against *M. bovis* challenge, thus masking the subsequent immunity elicited by BCG [49]. In fact, BCG immunity could not last longer, as protection was elicited in those calves vaccinated at 1 month of age and challenged 12 months later with *M. bovis*, whereas no protection was demonstrated in calves challenged with *M. bovis* after 24 months [50].

Notably, calves vaccinated as neonates and then revaccinated 6 weeks later revealed reduced protection in comparison to neonates vaccinated [46]. However, such revaccinated calves showed very strong antigen-specific IFN-γ responses, thus indicating that BCG was still actively replicating in those animals and BCG revaccination was contraindicated due to strong pre-existing immune response. Interestingly, BCG revaccination at 2 years after initial vaccination (when immunity was waned) could boost protective immunity against *M. bovis* challenge, whereas revaccination with TB protein vaccines could not boost such protection [51] thus suggesting further to optimize the duration of revaccination schedule to induce long-term protective immunity. Several vaccines that are recently been evaluated for their efficacies in cattle include live attenuated mycobacteria, which could replace BCG and subunit vaccines such as DNA, protein, and virus-vectored vaccines [40] and that could be used to boost BCG induced immunity. The live attenuated mycobacterial vaccines include modified BCG strains, *M. bovis* auxotrophs, and mutants of *M. tuberculosis* and *M. bovis* [40]. For example, a BCG strain was developed that overexpressed Ag85B and cattle vaccinated with this strain showed lesser histopathological lesion scores in the lungs after *M. bovis* challenge than the parent BCG [52]. On the other hand, the subunit vaccines themselves could not induce protection against *M. bovis* challenge, though a synergistic effect was observed when such vaccines were used in combination with BCG. DNA vaccines, by themselves, also revealed lower protection against *M. bovis* challenge, though some protection was observed when combined with DNA encoding costimulatory molecules or adjuvant [53,54]. When DNA vaccines were employed in prime-boost regimes with BCG, better protective immunity was noticed than with BCG alone [24,55,56]. Similarly, TB protein vaccines alone induced negligible protection in cattle, but when co-administered with BCG at adjacent sites, they showed better protection than BCG alone [57,58]. The main problem of protein vaccines is the difficulty of inducing strong CMI responses in cattle, even when they are co-administered with toll-like receptors agonists.

Priming with BCG and boosting with modified vaccinia virus Ankara (MVA) expressing 85A (MVA85A) or adenovirus 5 (Ad5) expressing Ag85A (Ad5-85A) revealed a superior protection as compared to BCG alone in animals challenged with *M. bovis* [42]. Dean et al. [59] and previously Vordermeier et al. [42] also observed similar findings by boosting BCG-primed cattle with Ad5-85A or MVA85A. In fact, Ag85A itself is immunogenic in nature and is considered as a good applicant for boosting CMI responses primed by BCG [60,61]. A prime-boost approach has also been adopted in cattle (sensitized to atypical mycobacteria) by injecting DNA vaccines encoding Hsp65, Hsp70 and Apa for priming, succeeded by a BCG booster and then *M. bovis* challenged [62], which revealed augmentated protective immunity in comparison to BCG alone. An attenuated *M. bovis* Ravenel strain RD1 deletion mutant (DeltaRD1) has been constructed, inoculated and later challenged in calves with low dose of *M. bovis* through aerosol route. Four-five months after challenge, DeltaRD1- mutant as well as BCG-vaccinated animals showed reduced TB-associated pathology in lungs and associated lymph nodes in comparison to non-vaccinated calves. The DeltaRD1 strain might be useful for bovine TB vaccine programs, notably if additional mutations are needed to improve safety and better immunogenicity [37]. Interestingly, a study has been undertaken by Buddle et al. [63] to find out if BCG vaccination post-challenge could induce protective effect on early *M. bovis* infection in cattle. In comparison to non-vaccinated animals, BCG vaccination after challenge with *M. bovis* produced no change in coarse pathology and histopathology, but elicited high mRNA expression of pro-inflammatory mediators such as IFN-γ, IL-12p40, IL-17A, CXCL10,

iNOs, TNF-α, etc. in the lung lymph nodes thus suggesting that one should be cautious for using high BCG doses.

Immunological correlates of protection studies in cattle

Identification of appropriate immunological correlates for protective immunity in bovine TB could expedite designing the efficacious vaccines as well as improved diagnostics and therapeutic strategies; but to find out such a correlate is a daunting task. The most commonly utilized assays for the diagnosis of bovine TB are the TST and IFN-γ that determine CMI responses to *M. bovis* infection. However, these assays have limitations as all the infected animals are not identified. A number of vaccination approaches have documented that IFN-γ responses alone are not essentially associated with protective immunity and parallel IL-4 production or antibody responses are also induced. IFN-γ responses to ESAT-6, antibody responses following TST and antigen-specific IL-4 mRNA expression coordinate with the progression of disease and indirectly give a notion of protection [64].

Strikingly, Lyashchenko and co-workers [65] analyzed humoral responses to several mycobacterial antigens such as MPB-64, MPB-70, MPB-83, ESAT-6, etc. in BCG-vaccinated cattle. Following *M. bovis* infection, the comparative TST strongly boosted IgG, IgG1, and IgG2 antibody responses, particularly against MPB83 and MPB70 proteins in unvaccinated cattle, but failed to boost such responses, or did so only weakly, in BCG-vaccinated calves. The skin test-induced increase in MPB83-specific IgG responses positively correlated with the bacterial loads as well as ESAT-6-induced in vitro IFN-γ responses, thus suggesting their possible role as markers for vaccine efficacy after challenge with *M. bovis* [43]. Buddle et al. [43] demonstrated that low doses of BCG given orally did not induce TST response, IFN-γ response or protection in cattle against *M. bovis* challenge, whereas in the BCG vaccine group (inoculated by subcutaneous route) where the protection was observed, no correlation was observed between the protection and TST/IFN-γ responses. While comparing IFN-γ-induced protein 10 (IP-10) and IFN-γ responses in cattle infected with *M. bovis* via aerosol route, Waters et al. [2] could not establish the role of IP-10 response as an immunological marker for bovine TB. However, a single intradermal tuberculin test could identify them as TB reactors or non-reactors, and the IFN-γ and IP-10 production were evaluated by ELISA [66]. A good association between IP-10 and IFN-γ elicitation has also been documented. Moreover, distinct release of IP-10 in response to protein purified derivative (PPD) from *M. bovis* and *M. avium* could differentiate reactor and non-reactor animals with high sensitivity (100%) and specificity (97%) thus suggesting that IP-10 could be a useful diagnostic biomarker of *M. bovis* challenge in cattle.

Similarly, a 6 h cytokine flow cytometric IFN-γ (CFC) assay has recently been developed [67] for studying bovine TB immunopathogenesis and to evaluate IL-1β as a biomarker to be utilized along with the IFN-γ CFC assay to improve the diagnostic accuracy for bovine TB. Notably, *M. bovis*-infected animals showed a higher number of IFN-γ producing CD4+ T cells as well as plasma IL-1β than animals exposed to atypical mycobacteria or uninfected controls and the two readouts had a significant correlation among themselves. Furthermore, Cattle exposed to *M. bovis* are found to have positive reaction to the bovine PPD (BPPD) skin test, but some animals are found to be negative to BPPD as they could be resistant to such infection. The macrophages from TB-infected cattle showed replication of *M. bovis*; whereas macrophages from healthy, exposed cattle (in contact, for a long-time with high TB prevalence but BPPD negative) showed two-fold lesser bacterial burden, higher production of nitric oxide as well as lesser IL-10 production [68].

Witchell et al. [69] earlier described the role of IL-10 in *M. bovis* infected cattle and its association as a biomarker for disease progression. However, Thacker and coworkers [70] documented that early immune responses in *M. bovis* challenged calves might play an important role in establishing the pathological outcomes of disease due to differential expression of Th1 and Th2 cytokines. While using BCG as a priming vaccine, Whelan et al. [62] compared the boosting abilities of Ad5-85Ainjected via endobronchial or intradermal route and showed that Ad5-85A delivered through either route could induce almost similar peripheral blood antigen specific IFN-γ responses. Moreover, bronchoalveolar lavage cells also produced similar antigen-specific IFN-γ response [62]. The evaluation of vaccine-driven central memory T-cell generation as an indicator of the outcome of heterologous prime-boost vaccine strategies has been demonstrated with cultured IFN-γ ELISpot assay [37,42,71], in contrast to conventional, *ex vivo* ELISpot assay, which does not specifically evaluate the central memory T-cell responses. Instead of using the traditional BPPD, DIVA tests have been developed with *M. tuberculosis* complex specific antigens (ESAT-6 and CFP-10) and are used in the TST or IFN-γ assay [72]. Following transcriptome studies, a protein, Rv3615c, has also been included in DIVA tests to raise the sensitivity. The evaluation of IFN-γ DIVA test using ESAT-6, CFP-10 and Rv3615c proteins in BCG-vaccinated, *M. bovis*-challenged and BCG-vaccinated, non-challenged cattle, has been documented with high sensitivity (96.0%) and specificity (95.53%) [73].

Several Th17-associated cytokine genes including IL-17A, IL-17F, IL-22, etc. have been shown to be up-regulated (in response to BPPD) in *M. bovis*-infected cattle [74]. Moreover, IFN-γ and IL-17A production are interlinked thus suggesting the utility of Th17-associated cytokines as potential biomarkers of protection in bovine TB. To decipher the immune responses associated with enhanced protection by Ad5-85A, Metcalfe et al. [75] compared the immune cells of BCG-primed Ad5-85A-boosted cattle with those from BCG-vaccinated cattle. Boosting BCG-primed cattle with Ad5-85A increased the numbers of Ag85A-specific CD4+ T cells, which also correlated with the protective immunity (with reduced pathology), but avidity was not enhanced and there was no expansion of Ag85A-specific CD4+ T cell repertoire.

During the last decade, research in TB immunopathology has identified IL-17A and IL-22 as the major effector cytokines required for the detection and clearance of tubercle bacilli. IL-17A is elicited immediately after BCG inoculation of mice and also participates in granuloma formation [76,77]. Although less well-studied, IL-22 has been shown to induce protection. IL-22 produced from NK cells could inhibit *M. tuberculosis* growth inside the human macrophages by increasing phagolysosomal fusion [78,79]. In fact, in cattle vaccine/challenge protocols, enhanced IL-17A and IL-22 production observed after vaccination, but before challenge are correlated with the success of vaccine (impediment of pathology) following successive *M. bovis* challenge [42,52,80]. However, excessive production of IL-17A may contribute to pathology [81]. Indeed, enhanced IL-22 and IL-17A mRNA expression in *M. bovis*-infected cattle following elicitation with BPPD has also been reported [82] thus proposing that evaluation of these cytokines could be useful biomarkers in bovine infection. To investigate the precise cell populations involved in production of these cytokines, Steinbach et al. [83] reported higher IL-22 and IL-17A protein production (in response to BPPD) in *M. bovis* infected cattle,

in comparison to non-infected cattle. In cattle infected with *M. bovis*, BPPD specific IL-17A and IL-22 responses were found in CD4+ T cells and γδ T cells. However, IL-22/IL-17A double producers were restricted primarily to γδ T cells, hence authenticating the earlier gene transcription reports [74,79]. These observations might be useful for further understanding the immunopathology of bovine TB and to produce more accurate immunodiagnostic reagents.

In the 4th global forum on TB vaccines, held in Shanghai, China (2015), the status of BCG vaccine and future directions for its improvements were discussed [84]. When protection is observed for a vaccine in preclinical studies, it goes through the necessary gating guidelines and is further selected for clinical trial. However, the failure of a novel TB vaccine i.e. BCG boosted with MVA85A to elicit protection in a recent phase 2b efficacy trial [85,86] has raised concerns, notably about the rigorousness of the go/no-go decisions used for this practice [84]. It has been suggested that high throughput, genomic screening for TB immunogens and the exploitation of non-classical pathways of TB antigen presentation such as HLA-E pathway could lead to the rational designing of novel vaccines. Identification of appropriate biomarkers of protection would serve as a major step to select vaccine candidates, as would the designing of human challenge TB model.

In conclusion, cattle represent a useful model to study immunopathoogenesis of bovine TB, which may assist to understand human TB pathology. The classification of granuloma into different stages and the diverse cytokine production at different stages may facilitate understanding bovine TB pathogenesis. Several vaccines are tested in cattle, which act as interlink between the small animals and non-human primates. Antigen-specific post-vaccination T cell central memory immune responses could serve as a potential predictor of vaccine efficacy. Although IFN-γ is considered as the major protective cytokine, detection of IL-17A and IL-22 seems to be important biomarkers as correlate of protection, in addition, to Il-1β, IL-10, IP-10 and IL-4 detection. Identification of such biomarkers may have plausible influence to screen new vaccines in cattle.

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