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Tuberculosis vaccination needs to avoid 'decoy' immune reactions.

Running Title: **Decoy reactions in tuberculosis**

Review

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Abstract

Current search for a new effective vaccine against tuberculosis involves selected antigens, vectors and adjuvants. These are being evaluated usually by their booster inoculation following priming with Bacillus Calmette-Guerin. The purpose of this article is to point out, that despite being attenuated of virulence, priming with BCG may still involve immune mechanisms, which are not favourable for protection against active disease. It is postulated, that the responsible 'decoy' constituents selected during the evolution of pathogenic tubercle bacilli may be involved in the evasion from bactericidal host resistance and stimulate immune responses of a cytokine phenotype, which lead to the transition from latent closed granulomas to reactivation with infectious lung cavities. The decoy mechanisms appear as favourable for most infected subjects but leading in a minority of cases to pathology which can effectively transmit the infection. It is proposed that construction and development of new vaccine candidates could benefit from avoiding decoy-type immune mechanisms.

1.0 Introduction

Prophylactic vaccination had eradicated smallpox and is eminently effective for a number of previously devastating viral infections (e.g. polio, measles or rubella) which get transmitted soon after infection. Protection by these vaccines as well as following natural infection are antibody mediated. In contrast, the host response to intracellular pathogens, including *Mycobacterium tuberculosis* (Mtb) has been attributed to Darwinian selection of 'decoy' constituents with low immunogenicity and lack of protective antibodies [1]. Unlike the viral transmission by most infected subjects prior to their immune response, Mtb is transmitted following a long asymptomatic latent phase by only about a 5 % fraction of subjects who reactivate into lung cavitary disease with efficient expectoration of bacilli. The duplicity of the immune responses is reflected by inducing initially beneficial latency in the majority of infected subjects, while also conditioning the host to reactivation toward pulmonary pathology, leading to transmission of infection. The latter aim, being a mandatory requirement for pathogenicity would have led to the evolution of the required Mtb constituents. Unlike the virulence factors of other microbial pathogens being often toxins for certain host cells, it has been proposed, that Mtb evolved its antigenic and immunomodulatory constituents as 'decoys' or 'secret trumps' to mislead the infected host by high-jacking its immune responses in favour of its persistence and transmission [2,3] which fits within the more widely formulated 'damage-response framework' of microbial pathogenesis.[4]

Prophylactic vaccination against TB with Bacillus Calmette-Guerin (BCG) is known to be protective only against TB meningitis and disseminated TB in children under 5 years of age [5], but not against adult TB and it failed also in reducing the transmission of tuberculous infection. Protection by BCG vaccination has long been classified as T cell mediated, but none of the cytokine bioassays correlate reliably with protection[6,7] and early protection in mice was attributed to T cell independent recruitment of CD11b+F4/80+ monocytes into the lungs. [7] The geographic variations in BCG protection have been associated with the gut microbiome, since its protection can be reduced by either non-tuberculous mycobacteria (e.g. *M.* avium) [8], or by antibiotic induced gut dysbiosis [9]. Moreover, some degree of protection against other infections was attributed to non-specific 'trained' memory of cells of the innate immune system [10,11]. BCG vaccination is widely used in several countries, although it was never accepted in the USA, and has recently been labelled as 'antiquated and inadequate'[12]. Despite such reservations, priming by BCG carries the advantage, that it abrogates the adjuvant dependency for the booster subunit antigen inoculation [13] and has still been retained within the prime-boost paradigm for the development of some of the multistage antigen construct based new vaccines. [14] The following paragraphs discuss the decoy elements of host responses to tuberculous infection, particularly with a potential of being involved following BCG-prime/boost vaccination and proposing that their avoidance would be desirable for continuing research efforts toward an effective prophylactic vaccine against TB.

2.0 Early post-infection events

2.1. Evasion from host resistance

Tubercle bacilli escape destruction by the infected host by abrogating the initial bactericidal action of macrophages and then by subverting the antigen presenting function of dendritic cells by separate distinct mechanisms.[15,16] The survival of Mtb in infected macrophages is facilitated through repression of their apoptosis [17,18]. However the complex metabolic reprogramming of phagocytic cells[19], involving the triggering of different 'pattern recognition receptor' (PRR) mediated pathways by mycobacterial 'pathogen associated molecular patterns (PAMPs) [20,21] is beneficial not only to the host, but also to the pathogen.[22]

Thus, potentially the protective Th1 immune response is delayed by the mycobacterial glycolipoprotein (LprG) induced TLR-2 mediated inhibition of MHC-II antigen processing by dendritic cells (DC),[23] and by lipoarabinomannan (LAM) mediated blocking of the expression of the DC-SIGN receptor and induction of immunosuppressive IL-10 and CCL18 production [24].

Although BCG has reduced expression of virulence factors and activities, such as the Rv3097c-encoded lipase [25] and dephosphorylation of host proteins [26], it still produces both LAM and LprG, which inhibit the immunostimulatory CD-1 and IL-12 production and induces the immunosuppressive IL-10 even in the absence of DC-SIGN [27]. Hence, BCG retains despite its attenuated virulence, demonstrable activity *in vitro,* which is pertinent to immune evasion mechanisms and subversion of its vaccination potential. [28]
Delayed migration of antigen-bearing DCs from Mtb infected lungs of infected mice to draining lymph nodes results in the failure of Th1 cell activation and their recruitment to the lungs.[29] Furthermore, an infected phagosome coat protein TACO, preventing lysosomal transfer and degradation of mycobacteria allows their survival. [30] Notably, both Mtb and BCG survive *in vivo* within phagosomes by initially resisting delivery to lysosomes and despite their subsequent lysosomal transfer [31].

2.2 Conditioning for reactivation
Experimental models of reactivation of Mtb infection in mice ('Cornell' models) have a number of variants, involving either spontaneous relapse following non-sterilizing chemotherapy with isoniazid and pyrazinamide or a rapid increase of bacterial counts from low level persistent infection, induced by immunosuppressive agents, such as hydrocortisone or anti-CD4 T cell antibodies. [32] The duration of the spontaneous relapse is much shorter than the recrudescence from chronic infection, but neither model reflects fully the unknown circumstances, which lead to the development of clinically active TB in the small minority of infected humans.

Both Cornell models of reactivation were shown to occur not only following pathogenic Mtb, but also following intravenous BCG Pasteur strain infection.[33,34], which could be explained by the presence of a number of virulence-related constituents (discussed in the preceding paragraph. [25-28] Using the post-chemotherapy spontaneous relapse model, the essential role of an early host response conditioning for the infection relapse has been suggested by the finding, that reactivation occurred only when the chemotherapy was started not earlier than three weeks post-infection. [33] Since the innate immune mechanisms which condition the host toward reactivation remain to be unidentified, further research, could be targeted on: a) the granulocyte macrophage colony stimulating factor (GM-CSF) and DC-SIGN receptor mediated function of DCs[27]; b) the influence of mycobacterial DosR(Rv3133c) genes which had been associated with extracellular Mtb persistence[35] and c) regulation of mRNA expression in monocytes, by the Toll-interacting ubiquitin binding protein (TOLLIP), which has been associated with BCG adjuvanticity and susceptibility to tuberculosis.[36] Though the relapse was demonstrated following intravenous inoculation of BCG, a lower risk from subcutaneous vaccination may depend from the vaccine dose[37] and could apply also to subsequent pathogenic Mtb infection. However, the early conditioning for reactivation is clearly different from the later mechanisms which can be alleviated by immunotherapy[38-40] and different from the TNFα mediated exacerbation of lung granulomas without reducing the bacillary load ('Koch phenomenon'), caused by the administration of mycobacterial antigens following Mtb infection.[41]

3.0 Adaptive immune responses.

3.1.MHC-permissive epitopes.

MHC-permissive T cell recognition of mycobacterial immunodominant epitopes in the context of both class II [42-44] and class I alleles[45] has been attributed to evolutionary selection on the grounds of their higher frequency in MHC- heterozygous than homozygous T cell hybridomas, [42] hence considered favourable for the pathogen's transmission. [46] This interpretation has been confirmed by the highly conserved sequence of immunodominant epitopes between diverse Mtb strains.[47,48] Recognition of lower peptide concentrations by the higher affinity of T cell receptors for these epitopes is conducive for host survival during the latent phase of infection. However, the decoy character of these hyperconserved epitopes and their evolutionary advantage to the pathogen has been proposed to be associated with mechanisms which lead to the subsequent reactivation in the minority of infected subjects, hence leading to efficient transmission of the infection in the population. [49]

3.2. Ambiguity of the T cell functions

Despite the broadly accepted protective role of T cells the corresponding effector functions have not been clarified and a reliable protection bioassay, based on Th1 cellular or cytokine markers, has not been found.[50] Moreover, subunit vaccination can elevate Th1 cytokine levels, but without protection [51], while mucosal vaccination can protect, without elevating Th1 cytokine levels.[52] Some of this ambiguity may be due to IL-6 mediated blocking of bactericidal action of Th1 cytokines [53]. Though IFNγ can activate macrophages to become bactericidal and limits neutrophilic pulmonary inflammation,[54] it's levels failed to associate reliably with protection and adding IFNγ to tissue culture media was reported even to enhance Mtb growth in human macrophages [55]. Although protection in humans has been associated with the recognition of more antigen epitopes [56,57] and the production of several cytokines [58-62], the polyfunctional phenotype was not found fully representative of protection in all studies [63].

Protection following BCG vaccination is considered compromised by inducing T cells mostly of the terminally exhausted 'effector' phenotype, instead of the better protective 'central' memory phenotype [64] and by the initial localization of T cells to the un-infected areas of the lungs, leading to delayed granuloma formation and latent infection with a potential for reactivation. [65] Hence, the immunity imparted by BCG, despite its attenuation, may still carry the decoy advantages for the transmission of infection. However, following peptide based vaccination, CD4+T cells also failed to target the Mtb infected cells, despite reaching the lung parenchyma [66]. Notably, in a murine model of diabetes, BCG or recombinant BCG vaccination was shown to be protective against reactivation of latent lymphatic TB infection by CD4 and CD8 T cell independent mechanisms.[67] Moreover, dysregulated CD4 T cell responses may have deleterious role, reflected by their amplification of infection and lung pathology in PD-1 receptor knockout mice [68], by enhanced T cell responses in mitochondrial cytophilin D (CypD) deficient mice [69] and by their influx to sites of lung pathology, mediated by the chemotactic CXCL10 chemokine. [70]

A protective role of CD8 T cells has been demonstrated in CD4- T cell depleted mice. [71] However, their limited role for protection was argued on the grounds that CD8 T cell lines binding the TB10.44-11 immunodominant epitope failed to recognise and to inhibit the growth of Mtb-infected macrophages, although they recognised the same epitope, when macrophages were pulsed with irradiated Mtb.[72] The authors attributed the blocking of the CD8 response to TB10.44 by the live infection to the subversive decoy function of this major

epitope. The limited protective role of CD8 T cells, despite their robust response following infection is supported also by the Th2 cytokine profile human HLA-E-restricted CD8 cells [73] and by their terminally-differentiated phenotype.[74].

3.3 Antibody markers

The association of prominently elevated antibody levels to the PstS1 glycoprotein[75] with the HLA-DR2 haplotype [76,77] and with the multibacillary, infectious type of active TB qualifies the PstS1 antigen as a decoy constituent .[78] The pathogenic mechanisms may involve epitope presentation by B cells instead of dendritic cells, thus diverting T cell maturation from a protective Th1 to a pathogenic (as well as B cell helper) Th2 phenotype.[79] Thus, despite the MHC-permissive strong immunogenicity of PstS1, its tendency toward inducing a Th2 response seems discouraging for further vaccine development, without removing its Th2-stimulatory epitope moieties. The protective potential of antibodies against the α-crystallin (Acr) antigen was suggested following passive vaccination with the IgA (TBA61) monoclonal antibody combined with recombinant IFNγ and anti-IL-4 treatment.[80-82] and by *in vitro* killing of intracellular Mtb by human antibodies with a distinct glycosylation profile.[83] However, a possible inference toward active vaccination would need the removal from the antigens their potentially pathogenic IL-4 producing Th2 stimulatory moiety.

4.0 Mediators and mechanisms of pathology

The promotion of the development of destructive granulomatous lung lesions by T cell responses has been noted previously [84]. Since the initial finding of elevated IL-4 production in active TB [85], the association of Th2 cytokines with pathogenic mechanisms has been suggested by: (1) Interference with the bactericidal action of Th1 cell cytokines [86,87], involving elevated intracellular (SOCS and IRAK-M) and extracellular (IL-10 and $TGF_{\beta}RII$, IL-1Rn, and IDO) mediators.[88]; (2) Alternative activation of macrophages, which supports the persistence of Mtb [89]; (3) TNFα mediated change from apoptosis to necrosis of infected cells [90] and cAMP initiated granuloma formation [91]; (4) Inhibition of TB-resistance by intestinal microbiota induced IL-10[92] and by (5) regulatory T cell mediated suppression of protective CD4 T cells [87].

Neutrophils recruited to the infected lungs can counteract the cathelicidin and lipocalin mediated mycobactericidal action of Th1 cells [93]. Low-density neutrophils in TB lesions have deficient phagocytosis and oxidative burst [94], while necrotic neutrophils reduce TNFα and increase IL-10 production [95]. Infected macrophages upregulate the MCL1 gene expression, which interferes with Mtb killing [96], while increased Eis gene expression in Mtb-containing autophagosomes enhances Mtb survival by attenuating TLR mediated autophagy, modulating the cell death and suppressing the host innate immune defenses.[97] Subsequent translocation of Mtb-infected alveolar macrophages from airways to the lung interstitium involves the Mtb ESX-1 secretion system and MyD88/IL-1 receptor inflammasome signalling [98]. The quoted mechanisms in neutrophils and macrophages support the maturation of lung granulomas to cavitation with liquefaction, resulting in efficient transmission of the infection to susceptible hosts. The IL-1 cytokine can initially be protective against Mtb infection, but it's persistent production aggravates disease by contributing to neutrophil accumulation, which can be alleviated by co-administration of the IL-1 receptor antagonist protein with the linezolid antibiotic [99]. This example shows, that identifying decoy mechanisms can be supportive for the development of 'host directed therapies' [100]

5.0 Predominance of priming over response to challenge

The key importance of the sequence of antigen exposure and the potential for a deleterious impact was demonstrated by the finding that immunization with various mycobacterial antigens can aggravate lung pathology in Mtb pre-infected mice. [41] The 'original antigenic sin' (OAS) phenomenon, reflecting the predominance of immunity to the first priming antigen following booster immunization, has been described following infection and vaccination with influenza virus and several other infections,[101] thus overriding the antigenic specificity of the booster antigen.[102,103] The subversive role of OAS as a decoy mechanism was proposed for the generation of non-protective antibodies against HIV-1 and Ebola virus infections.[1] The OAS could also explain, why BCG vaccination showed preferential stimulation to *M. avium* antigens in children who were pre-exposed to environmental mycobacteria,[104] the failure of boosting protection by antigen challenge of BCG primed macaques,[105] and the blocking of BCG protection by prior exposure to environmental species of mycobacteria in mice,[106] and in human populations.[107] The mandatory role of the sequence of antigens for heterologous prime/boost vaccination has been documented also by the finding, that priming with the Ag85A-coding DNA yielded protection in mice when administered before, but not when given after BCG.[108]

Although the readout of the OAS phenomenon has previously involved mostly antibody responses, the recognition mechanism has been attributed to T helper cell mediated cross-reactivity between taxonomically related antigens[109]. Moreover, its impact could be even wider, due to non-reciprocal (immunodominant or cryptic) cross-recognition ('mimicry') of epitopes between taxonomically unrelated mycobacterial proteins.[110] Such relationships could contribute to the predominance of the primary antigenic exposure in the BCG-prime/heterologous boost vaccine regimen, thus sustaining the specificity and phenotype of T cell response, initiated by the primary BCG, rather than of the boosting antigen subunit. Priming to several of the BCG antigens may include also the undesirable decoy antigens which evolved for initiating a host response for the ultimate advantage of the Mtb pathogen.

6.0 New vaccine research

6.1 Subunit constructs

Current research involves 'multistage' fusion of proteins, containing both replicating and latent stage antigens [111,112], adjuvants, recombinant formulations and genetically modified BCG or attenuated Mtb [113-115], with emphasis on *in vivo* and latency expressed (IVE), DosR regulon and Rpf stage specific antigens, expressed on infected macrophages. In particular, the Rv2034 protein, strongly immunogenic in TB patients was found to be protective in HLA-DR3 transgenic mice by inducing peptide 31-50 specific IFNγ and TNFα producing CD4 T-cells as well as antibodies.[116] Significance of a cluster of 17 IVE antigens has been argued on the grounds of inducing TNFα secreting T cells in TB patients.[117] In contrast, another choice of protective antigen subunits considered their downregulated expression during infection,[118] and other functions for recombinant expression in BCG, vaccinia virus and adenovirus vectors.[119-121] Subdominant Mtb peptides of ESAT-6, though poorly recognized following Mtb infection were found to protect better than epitopes which are immunodominant during natural infection [122]. On the other hand, it has been proposed to avoid antigens which are well expressed at early stage of infection, which may lead toward an exhaustion and dysfunction of T cell responses[118,123].

Several vaccine candidates reached various stages of evaluation in clinical trials. Significant protection without BCG priming was reported three years after intramuscular vaccination with two doses of the M72/AS01$_E$ the recombinant fusion construct of the Mtb32A and

Mtb39A antigens[124]. The case for avoiding BCG comes also from the finding, that the mycobacterial wall lipase LipY (Rv3097c) has been protective in mice when given as a purified protein in adjuvant, while its recombinant overexpression in BCG abrogated protection against Mtb challenge, probably by shifting the T cell response from Th1 to Th2 phenotype.[25] These considerations go along the suggestion that learning from past 'failed' trials and diversity of research need to 'embrace risk in persuit of vaccine development'.[113]

6.2 Route of vaccination

Mucosal vaccination by respiratory route delivery attracts interest with the aim of fast localizing the protective T cells to the lungs, ready for countering inhaled Mtb infection without dependence on the recruitment of systemically primed T cells, [125,126] which may also lack CCR5 and CXCR3 chemokine receptors needed for migration to the lung.[127] Oral inoculation of BCG in the original MRC Trial was abandoned due to cervical lymphadenopathy. Though intranasal BCG caused lung pathology in mice [128], profoundly better protection by pulmonary mucosal than intradermal delivery in rhesus macaques was attributed to polyfunctional TH17 cells, interleukin-10 and immunoglobulin A [129]. Other proposed mechanisms involved the role of innate immunity in facilitating the homing of DCs and T cells[130,131] and the recruitment and retention of protective CD8 T cells in the lungs [132]. Interest in mucosal delivery is supported in a study of needle-free vaccination of mice and guinea pigs, combining oral prime with intranasal booster [52], the use of nanoparticles [133]or the use of tetramerized streptavidin core fused with biotinylated anti-DC antibodies [134]. Notably, protection without BCG priming, imparted by intranasal delivery of recombinant soluble DnaK (hsp70) antigen was attributed to lung resident IL-17 producing T cells, acting even after depleting circulating CD4 T cells [135].

6.3 Testing for protection against reactivation

Preclinical testing of novel TB vaccines in animal models is done routinely in reference to protection against primary challenge, thus ignoring that the predominant disease in adult humans is the post-primary, reactivated form of TB. However, significant contributions have been made by testing of different recombinant vaccines on the reactivation of latent TB infection in murine models which mimic the susceptibility of HIV-infected humans by anti-CD4 antibody treatment [67] or by diet-induced type-2 diabetes.[136] The substantial difference between the primary and secondary disease has recently been pointed out, while emphasizing the need to test novel vaccines in available animal models of human post-primary TB [137]. This concept is to be supported, since it would focus research on such host responses which can prevent reactivation, unlike natural infection which leads merely to latency, but with a potential for recrudescence in the minority of subjects.

There is a dilemma of choosing suitable stimuli for reactivating a persisting latent infection in animal models. Though HIV or immunosuppressive therapy are well known to reactivate human TB, the vast majority of adults develop pulmonary disease without any known immune compromise, but associated with a broad range of causes, such as poverty, malnutrition, endocrine and life-habit related factors. Decoy immune mechanisms have been indicated by the association of reactivation of chronic infection in mice with overexpressed IL-10 production [138] and by the finding of higher cortisone-induced reactivation in Bcg-resistant than Bcg-susceptible mice [34]. SIV-coinfection induced reactivation of lung TB in rhesus macaques showed elevated proinflammatory cytokines and chemokines, thus associating increased T cell activity with a decoy, rather than protective function [139].

The role of neuro-endocrine regulation by the hypothalamic-pituitary-adrenal axis was demonstrated by triggering the reactivation of TB infection by physical exertion of mice.[140] The increase in cortisol/DHEA in association with the Th1 to Th2 shift in pulmonary TB [141] also indicated the role of dysregulation of the hypothalamic-pituitary-adrenal axis.[142], while an even wider signature distinguishing between latent and active TB was identified by immunometabolomic and RNA sequence analysis [143]. Future preclinical research is desirable to select from this broad list of available approaches, which biomarkers would be most suitable for testing of vaccine candidates for protection against the reactivation form of active TB in humans.

7.0 Ethical, safety and funding issues

Protection against disseminated TB in children and also cross-protection against other microbial pathogens [144] gives ethical justification for continued neonatal BCG vaccination, established in several countries despite a reported risk of aggravated reactions in HIV co-infection [145]. However, research toward novel vaccine constructs, aiming to reduce TB transmission should reconsider if priming by BCG is suitable, in view of its potential decoy capacities, as discussed in this article. This aspect seems pertinent also to the new genetically attenuated strains of Mtb and recombinant BCG constructs, despite their rigorous conventional testing for attenuation [146]. The proposed testing of candidate vaccines for protection against TB reactivation would extend the housing of experimental animals in containment facilities, thus substantially increasing the cost of pre-clinical research.

8.0. Conclusions

The decoy reactions to tuberculous infection are represented by initial conditioning of innate immunity followed by subsequent Th2 cell cytokine responses to HLA-permissive epitopes. These host reactions are in favour of the pathogen, since following a latent infection, they lead to reactivation in a minority of subjects to a type of pathology which effectively transmits the infection. Therefore, pre-clinical testing would benefit from extended evaluation for protection against recrudescence from dormancy. The predominant influence of priming (e.g. BCG) carrying a potential risk for conditioning for reactivation, deserves a consideration for optimising future strategies for new subunit vaccines.

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References

1. Burton DR, Parren PW. Vaccines and the induction of functional antibodies: time to look beyond the molecules of natural infection? *Nat Med* 2000; **6**(2): 123-5.
2. Ivanyi J. Pathogenic and Protective Interactions in Mycobacterial Infections. *Clinics in Immunology and Allergy* 1986; **6**: 127-57.
3. Cardona PJ, Ivanyi J. The secret trumps, impelling the pathogenicity of tubercle bacilli. *Enferm Infecc Microbiol Clin* 2011; **29 Suppl 1**: 14-9.
4. Casadevall A, Pirofski LA. The damage-response framework of microbial pathogenesis. *Nat Rev Microbiol* 2003; **1**(1): 17-24.
5. Mangtani P, Abubakar I, Ariti C, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. *Clin Infect Dis* 2014; **58**(4): 470-80.
6. Mittrucker HW, Steinhoff U, Kohler A, et al. Poor correlation between BCG vaccination-induced T cell responses and protection against tuberculosis. *Proc Natl Acad Sci U S A* 2007; **104**(30): 12434-9.

7. Bickett TE MJ, Creissen E, Izzo L, Izzo A, Angulo FS, Izzo AA. Characterizing the BCG Induced Macrophage and Neutrophil Mechanisms for Defense Against Mycobacterium tuberculosis. *Frontiers in Immunology* 2020; **11**.
8. Poyntz HC, Stylianou E, Griffiths KL, Marsay L, Checkley AM, McShane H. Non-tuberculous mycobacteria have diverse effects on BCG efficacy against Mycobacterium tuberculosis. *Tuberculosis (Edinb)* 2014; **94**(3): 226-37.
9. Nadeem S, Maurya SK, Das DK, Khan N, Agrewala JN. Gut Dysbiosis Thwarts the Efficacy of Vaccine Against Mycobacterium tuberculosis. *Front Immunol* 2020; **11**: 726.
10. Uthayakumar D, Paris S, Chapat L, Freyburger L, Poulet H, De Luca K. Non-specific Effects of Vaccines Illustrated Through the BCG Example: From Observations to Demonstrations. *Front Immunol* 2018; **9**: 2869.
11. Choreno-Parra JA, Weinstein LI, Yunis EJ, Zuniga J, Hernandez-Pando R. Thinking Outside the Box: Innate- and B Cell-Memory Responses as Novel Protective Mechanisms Against Tuberculosis. *Front Immunol* 2020; **11**: 226.
12. Barber DL. The Helper T Cell's Dilemma in Tuberculosis. *Cell Host Microbe* 2017; **21**(6): 655-6.
13. Rahman MJ, Fernandez C. Neonatal vaccination with Mycobacterium bovis BCG: potential effects as a priming agent shown in a heterologous prime-boost immunization protocol. *Vaccine* 2009; **27**(30): 4038-46.
14. Fatima S, Kumari A, Das G, Dwivedi VP. Tuberculosis vaccine: A journey from BCG to present. *Life Sci* 2020; **252**: 117594.
15. Mariotti S, Teloni R, Iona E, et al. Mycobacterium tuberculosis diverts alpha interferon-induced monocyte differentiation from dendritic cells into immunoprivileged macrophage-like host cells. *Infect Immun* 2004; **72**(8): 4385-92.
16. Khan N, Vidyarthi A, Pahari S, Agrewala JN. Distinct Strategies Employed by Dendritic Cells and Macrophages in Restricting Mycobacterium tuberculosis Infection: Different Philosophies but Same Desire. *Int Rev Immunol* 2016; **35**(5): 386-98.
17. Liu CH, Liu H, Ge B. Innate immunity in tuberculosis: host defense vs pathogen evasion. *Cell Mol Immunol* 2017; **14**(12): 963-75.
18. Zhang W, Lu Q, Dong Y, Yue Y, Xiong S. Rv3033, as an Emerging Anti-apoptosis Factor, Facilitates Mycobacteria Survival via Inhibiting Macrophage Intrinsic Apoptosis. *Front Immunol* 2018; **9**: 2136.
19. Kumar R, Singh P, Kolloli A, et al. Immunometabolism of Phagocytes During Mycobacterium tuberculosis Infection. *Front Mol Biosci* 2019; **6**: 105.
20. Hossain MM, Norazmi MN. Pattern recognition receptors and cytokines in Mycobacterium tuberculosis infection--the double-edged sword? *Biomed Res Int* 2013; **2013**: 179174.
21. Stamm CE, Collins AC, Shiloh MU. Sensing of Mycobacterium tuberculosis and consequences to both host and bacillus. *Immunol Rev* 2015; **264**(1): 204-19.
22. Huynh KK, Joshi SA, Brown EJ. A delicate dance: host response to mycobacteria. *Curr Opin Immunol* 2011; **23**(4): 464-72.
23. Pecora ND, Gehring AJ, Canaday DH, Boom WH, Harding CV. Mycobacterium tuberculosis LprA is a lipoprotein agonist of TLR2 that regulates innate immunity and APC function. *J Immunol* 2006; **177**(1): 422-9.
24. Ferrara G, Bleck B, Richeldi L, et al. Mycobacterium tuberculosis induces CCL18 expression in human macrophages. *Scand J Immunol* 2008; **68**(6): 668-74.
25. Singh VK, Srivastava V, Singh V, et al. Overexpression of Rv3097c in Mycobacterium bovis BCG abolished the efficacy of BCG vaccine to protect against Mycobacterium tuberculosis infection in mice. *Vaccine* 2011; **29**(29-30): 4754-60.
26. Choudhary E, Bullen CK, Goel R, et al. Relative and Quantitative Phosphoproteome Analysis of Macrophages in Response to Infection by Virulent and Avirulent Mycobacteria Reveals a Distinct Role of the Cytosolic RNA Sensor RIG-I in Mycobacterium tuberculosis Pathogenesis. *J Proteome Res* 2020; **19**(6): 2316-36.

27. Gagliardi MC, Teloni R, Giannoni F, et al. Mycobacterium bovis Bacillus Calmette-Guerin infects DC-SIGN- dendritic cell and causes the inhibition of IL-12 and the enhancement of IL-10 production. *J Leukoc Biol* 2005; **78**(1): 106-13.
28. Gagliardi MC, Teloni R, Mariotti S, et al. Bacillus Calmette-Guerin shares with virulent Mycobacterium tuberculosis the capacity to subvert monocyte differentiation into dendritic cell: implication for its efficacy as a vaccine preventing tuberculosis. *Vaccine* 2004; **22**(29-30): 3848-57.
29. Jeyanathan M, McCormick S, Lai R, et al. Pulmonary M. tuberculosis infection delays Th1 immunity via immunoadaptor DAP12-regulated IRAK-M and IL-10 expression in antigen-presenting cells. *Mucosal Immunol* 2014; **7**(3): 670-83.
30. Ferrari G, Langen H, Naito M, Pieters J. A coat protein on phagosomes involved in the intracellular survival of mycobacteria. *Cell* 1999; **97**(4): 435-47.
31. Sundaramurthy V, Korf H, Singla A, et al. Survival of Mycobacterium tuberculosis and Mycobacterium bovis BCG in lysosomes in vivo. *Microbes Infect* 2017; **19**(11): 515-26.
32. Scanga CA, Mohan VP, Joseph H, Yu K, Chan J, Flynn JL. Reactivation of latent tuberculosis: variations on the Cornell murine model. *Infect Immun* 1999; **67**(9): 4531-8.
33. Cox JH, Ivanyi J. The role of host factors for the chemotherapy of BCG infection in inbred strains of mice. *APMIS* 1988; **96**(10): 927-32.
34. Cox JH, Knight BC, Ivanyi J. Mechanisms of recrudescence of Mycobacterium bovis BCG infection in mice. *Infect Immun* 1989; **57**(6): 1719-24.
35. Karakousis PC, Yoshimatsu T, Lamichhane G, et al. Dormancy phenotype displayed by extracellular Mycobacterium tuberculosis within artificial granulomas in mice. *J Exp Med* 2004; **200**(5): 647-57.
36. Shah JA, Musvosvi M, Shey M, et al. A Functional Toll-Interacting Protein Variant Is Associated with Bacillus Calmette-Guerin-Specific Immune Responses and Tuberculosis. *Am J Respir Crit Care Med* 2017; **196**(4): 502-11.
37. Power CA, Wei G, Bretscher PA. Mycobacterial dose defines the Th1/Th2 nature of the immune response independently of whether immunization is administered by the intravenous, subcutaneous, or intradermal route. *Infect Immun* 1998; **66**(12): 5743-50.
38. Venkataprasad N, Ledger P, Ivanyi J. The effect of glucosaminylmuramyl dipeptide injection to mice on the course of tuberculous infection and in vitro superoxide anion production. *Int Arch Allergy Immunol* 1997; **114**(1): 23-9.
39. Ha SJ, Jeon BY, Kim SC, et al. Therapeutic effect of DNA vaccines combined with chemotherapy in a latent infection model after aerosol infection of mice with Mycobacterium tuberculosis. *Gene Ther* 2003; **10**(18): 1592-9.
40. Buccheri S, Reljic R, Caccamo N, et al. Prevention of the post-chemotherapy relapse of tuberculous infection by combined immunotherapy. *Tuberculosis (Edinb)* 2009; **89**(1): 91-4.
41. Moreira AL, Tsenova L, Aman MH, et al. Mycobacterial antigens exacerbate disease manifestations in Mycobacterium tuberculosis-infected mice. *Infect Immun* 2002; **70**(4): 2100-7.
42. Vordermeier HM, Harris DP, Moreno C, Ivanyi J. Promiscuous T cell recognition of an H-2 IA-presented mycobacterial epitope. *Eur J Immunol* 1994; **24**(9): 2061-7.
43. Harris DP, Vordermeier HM, Arya A, Moreno C, Ivanyi J. Permissive recognition of a mycobacterial T-cell epitope: localization of overlapping epitope core sequences recognized in association with multiple major histocompatibility complex class II I-A molecules. *Immunology* 1995; **84**(4): 555-61.
44. Jurcevic S, Hills A, Pasvol G, Davidson RN, Ivanyi J, Wilkinson RJ. T cell responses to a mixture of Mycobacterium tuberculosis peptides with complementary HLA-DR binding profiles. *Clin Exp Immunol* 1996; **105**(3): 416-21.
45. Sundaramurthi JC, Brindha S, Shobitha SR, Swathi A, Ramanandan P, Hanna LE. In silico identification of potential antigenic proteins and promiscuous CTL epitopes in Mycobacterium tuberculosis. *Infect Genet Evol* 2012; **12**(6): 1312-8.

46. Ivanyi J. Could active case finding reduce the transmission of tuberculosis? *Lancet* 2014; **383**(9922): 1035-6.
47. Comas I, Chakravartti J, Small PM, et al. Human T cell epitopes of Mycobacterium tuberculosis are evolutionarily hyperconserved. *Nat Genet* 2010; **42**(6): 498-503.
48. Lindestam Arlehamn CS, Paul S, Mele F, et al. Immunological consequences of intragenus conservation of Mycobacterium tuberculosis T-cell epitopes. *Proc Natl Acad Sci U S A* 2015; **112**(2): E147-55.
49. Woodworth JS, Andersen P. Reprogramming the T Cell Response to Tuberculosis. *Trends Immunol* 2016; **37**(2): 81-3.
50. Dockrell HM, Smith SG. What Have We Learnt about BCG Vaccination in the Last 20 Years? *Front Immunol* 2017; **8**: 1134.
51. Majlessi L, Simsova M, Jarvis Z, et al. An increase in antimycobacterial Th1-cell responses by prime-boost protocols of immunization does not enhance protection against tuberculosis. *Infect Immun* 2006; **74**(4): 2128-37.
52. Badell E, Nicolle F, Clark S, et al. Protection against tuberculosis induced by oral prime with Mycobacterium bovis BCG and intranasal subunit boost based on the vaccine candidate Ag85B-ESAT-6 does not correlate with circulating IFN-gamma producing T-cells. *Vaccine* 2009; **27**(1): 28-37.
53. Russell DG, Sturgill-Koszycki S, Vanheyningen T, Collins H, Schaible UE. Why intracellular parasitism need not be a degrading experience for Mycobacterium. *Philos Trans R Soc Lond B Biol Sci* 1997; **352**(1359): 1303-10.
54. Nandi B, Behar SM. Regulation of neutrophils by interferon-gamma limits lung inflammation during tuberculosis infection. *J Exp Med* 2011; **208**(11): 2251-62.
55. Douvas GS, Looker DL, Vatter AE, Crowle AJ. Gamma interferon activates human macrophages to become tumoricidal and leishmanicidal but enhances replication of macrophage-associated mycobacteria. *Infect Immun* 1985; **50**(1): 1-8.
56. Friscia G, Vordermeier HM, Pasvol G, Harris DP, Moreno C, Ivanyi J. Human T cell responses to peptide epitopes of the 16-kD antigen in tuberculosis. *Clin Exp Immunol* 1995; **102**(1): 53-7.
57. Chua-Intra B, Peerapakorn S, Davey N, et al. T-cell recognition of mycobacterial GroES peptides in Thai leprosy patients and contacts. *Infect Immun* 1998; **66**(10): 4903-9.
58. Caccamo N, Guggino G, Joosten SA, et al. Multifunctional CD4(+) T cells correlate with active Mycobacterium tuberculosis infection. *Eur J Immunol* 2010; **40**(8): 2211-20.
59. Prezzemolo T, Guggino G, La Manna MP, Di Liberto D, Dieli F, Caccamo N. Functional Signatures of Human CD4 and CD8 T Cell Responses to Mycobacterium tuberculosis. *Front Immunol* 2014; **5**: 180.
60. Wilkinson KA, Wilkinson RJ. Polyfunctional T cells in human tuberculosis. *Eur J Immunol* 2010; **40**(8): 2139-42.
61. Derrick SC, Yabe IM, Yang A, Morris SL. Vaccine-induced anti-tuberculosis protective immunity in mice correlates with the magnitude and quality of multifunctional CD4 T cells. *Vaccine* 2011; **29**(16): 2902-9.
62. Lindenstrom T, Agger EM, Korsholm KS, et al. Tuberculosis subunit vaccination provides long-term protective immunity characterized by multifunctional CD4 memory T cells. *J Immunol* 2009; **182**(12): 8047-55.
63. Lewinsohn DA, Lewinsohn DM, Scriba TJ. Polyfunctional CD4(+) T Cells As Targets for Tuberculosis Vaccination. *Front Immunol* 2017; **8**: 1262.
64. Orme IM. Development of new vaccines and drugs for TB: limitations and potential strategic errors. *Future Microbiol* 2011; **6**(2): 161-77.
65. Delahaye JL, Gern BH, Cohen SB, et al. Cutting Edge: Bacillus Calmette-Guerin-Induced T Cells Shape Mycobacterium tuberculosis Infection before Reducing the Bacterial Burden. *J Immunol* 2019; **203**(4): 807-12.

66. Ernst JD, Cornelius A, Desvignes L, Tavs J, Norris BA. Limited Antimycobacterial Efficacy of Epitope Peptide Administration Despite Enhanced Antigen-Specific CD4 T-Cell Activation. *J Infect Dis* 2018; **218**(10): 1653-62.
67. Sathkumara HD, Pai S, Aceves-Sanchez MJ, Ketheesan N, Flores-Valdez MA, Kupz A. BCG Vaccination Prevents Reactivation of Latent Lymphatic Murine Tuberculosis Independently of CD4(+) T Cells. *Front Immunol* 2019; **10**: 532.
68. Barber DL, Mayer-Barber KD, Feng CG, Sharpe AH, Sher A. CD4 T cells promote rather than control tuberculosis in the absence of PD-1-mediated inhibition. *J Immunol* 2011; **186**(3): 1598-607.
69. Tzelepis F, Blagih J, Khan N, et al. Mitochondrial cyclophilin D regulates T cell metabolic responses and disease tolerance to tuberculosis. *Sci Immunol* 2018; **3**(23).
70. Blauenfeldt T, Petrone L, Del Nonno F, et al. Interplay of DDP4 and IP-10 as a Potential Mechanism for Cell Recruitment to Tuberculosis Lesions. *Front Immunol* 2018; **9**: 1456.
71. Derrick SC, Repique C, Snoy P, Yang AL, Morris S. Immunization with a DNA vaccine cocktail protects mice lacking CD4 cells against an aerogenic infection with Mycobacterium tuberculosis. *Infect Immun* 2004; **72**(3): 1685-92.
72. Yang JD, Mott D, Sutiwisesak R, et al. Mycobacterium tuberculosis-specific CD4+ and CD8+ T cells differ in their capacity to recognize infected macrophages. *PLoS Pathog* 2018; **14**(5): e1007060.
73. van Meijgaarden KE, Haks MC, Caccamo N, Dieli F, Ottenhoff TH, Joosten SA. Human CD8+ T-cells recognizing peptides from Mycobacterium tuberculosis (Mtb) presented by HLA-E have an unorthodox Th2-like, multifunctional, Mtb inhibitory phenotype and represent a novel human T-cell subset. *PLoS Pathog* 2015; **11**(3): e1004671.
74. La Manna MP, Orlando V, Prezzemolo T, et al. HLA-E-Restricted CD8+ T Lymphocytes Efficiently Control Mycobacterium tuberculosis and HIV-1 Co-Infection. *Am J Respir Cell Mol Biol* 2019.
75. Ivanyi J, Sharp K, Jackett P, Bothamley G. Immunological study of the defined constituents of mycobacteria. *Springer Semin Immunopathol* 1988; **10**(4): 279-300.
76. Bothamley GH, Beck JS, Schreuder GM, et al. Association of tuberculosis and M. tuberculosis-specific antibody levels with HLA. *J Infect Dis* 1989; **159**(3): 549-55.
77. Brahmajothi V, Pitchappan RM, Kakkanaiah VN, et al. Association of pulmonary tuberculosis and HLA in south India. *Tubercle* 1991; **72**(2): 123-32.
78. Ivanyi J. Function and Potentials of M. tuberculosis Epitopes. *Front Immunol* 2014; **5**: 107.
79. Rossi-Bergmann B, Muller I, Godinho EB. TH1 and TH2 T-cell subsets are differentially activated by macrophages and B cells in murine leishmaniasis. *Infect Immun* 1993; **61**(5): 2266-9.
80. Reljic R, Ivanyi J. A case for passive immunoprophylaxis against tuberculosis. *Lancet Infect Dis* 2006; **6**(12): 813-8.
81. Buccheri S, Reljic R, Caccamo N, et al. IL-4 depletion enhances host resistance and passive IgA protection against tuberculosis infection in BALB/c mice. *Eur J Immunol* 2007; **37**(3): 729-37.
82. Balu S, Reljic R, Lewis MJ, et al. A novel human IgA monoclonal antibody protects against tuberculosis. *J Immunol* 2011; **186**(5): 3113-9.
83. Lu LL, Chung AW, Rosebrock TR, et al. A Functional Role for Antibodies in Tuberculosis. *Cell* 2016; **167**(2): 433-43 e14.
84. Orme IM, Robinson RT, Cooper AM. The balance between protective and pathogenic immune responses in the TB-infected lung. *Nat Immunol* 2015; **16**(1): 57-63.
85. Surcel HM, Troye-Blomberg M, Paulie S, et al. Th1/Th2 profiles in tuberculosis, based on the proliferation and cytokine response of blood lymphocytes to mycobacterial antigens. *Immunology* 1994; **81**(2): 171-6.
86. Rook GA, Hernandez-Pando R, Dheda K, Teng Seah G. IL-4 in tuberculosis: implications for vaccine design. *Trends Immunol* 2004; **25**(9): 483-8.
87. Kursar M, Koch M, Mittrucker HW, et al. Cutting Edge: Regulatory T cells prevent efficient clearance of Mycobacterium tuberculosis. *J Immunol* 2007; **178**(5): 2661-5.

88. Almeida AS, Lago PM, Boechat N, et al. Tuberculosis is associated with a down-modulatory lung immune response that impairs Th1-type immunity. *J Immunol* 2009; **183**(1): 718-31.
89. Kahnert A, Seiler P, Stein M, et al. Alternative activation deprives macrophages of a coordinated defense program to Mycobacterium tuberculosis. *Eur J Immunol* 2006; **36**(3): 631-47.
90. Abebe M, Kim L, Rook G, et al. Modulation of cell death by M. tuberculosis as a strategy for pathogen survival. *Clin Dev Immunol* 2011; **2011**: 678570.
91. Agarwal N, Lamichhane G, Gupta R, Nolan S, Bishai WR. Cyclic AMP intoxication of macrophages by a Mycobacterium tuberculosis adenylate cyclase. *Nature* 2009; **460**(7251): 98-102.
92. Arnold IC, Hutchings C, Kondova I, et al. Helicobacter hepaticus infection in BALB/c mice abolishes subunit-vaccine-induced protection against M. tuberculosis. *Vaccine* 2015; **33**(15): 1808-14.
93. Martineau AR, Newton SM, Wilkinson KA, et al. Neutrophil-mediated innate immune resistance to mycobacteria. *J Clin Invest* 2007; **117**(7): 1988-94.
94. La Manna MP. *Mycobacterium tuberculosis drives expansion of low density neutrophils equipped with regulatory activities*. *Fronriers in Immunology* 2019; **10**(November).
95. Lowe DM, Demaret J, Bangani N, et al. Differential Effect of Viable Versus Necrotic Neutrophils on Mycobacterium tuberculosis Growth and Cytokine Induction in Whole Blood. *Front Immunol* 2018; **9**: 903.
96. Lam A, Prabhu R, Gross CM, Riesenberg LA, Singh V, Aggarwal S. Role of apoptosis and autophagy in tuberculosis. *Am J Physiol Lung Cell Mol Physiol* 2017; **313**(2): L218-L29.
97. Shin DM, Jeon BY, Lee HM, et al. Mycobacterium tuberculosis eis regulates autophagy, inflammation, and cell death through redox-dependent signaling. *PLoS Pathog* 2010; **6**(12): e1001230.
98. Cohen SB, Gern BH, Delahaye JL, et al. Alveolar Macrophages Provide an Early Mycobacterium tuberculosis Niche and Initiate Dissemination. *Cell Host Microbe* 2018; **24**(3): 439-46 e4.
99. Winchell CG, Mishra BB, Phuah JY, et al. Evaluation of IL-1 Blockade as an Adjunct to Linezolid Therapy for Tuberculosis in Mice and Macaques. *Front Immunol* 2020; **11**: 891.
100. Zumla A, Maeurer M, Host-Directed Therapies N, et al. Towards host-directed therapies for tuberculosis. *Nat Rev Drug Discov* 2015; **14**(8): 511-2.
101. Vatti A, Monsalve DM, Pacheco Y, Chang C, Anaya JM, Gershwin ME. Original antigenic sin: A comprehensive review. *J Autoimmun* 2017; **83**: 12-21.
102. Monto AS, Malosh RE, Petrie JG, Martin ET. The Doctrine of Original Antigenic Sin: Separating Good From Evil. *J Infect Dis* 2017; **215**(12): 1782-8.
103. Biswas A, Chakrabarti AK, Dutta S. Current challenges: from the path of "original antigenic sin" towards the development of universal flu vaccines. *Int Rev Immunol* 2019: 1-16.
104. Abrahams EW. 'Original mycobacterial sin'. *Tubercle* 1970; **51**(3): 316-21.
105. Darrah PA, DiFazio RM, Maiello P, et al. Boosting BCG with proteins or rAd5 does not enhance protection against tuberculosis in rhesus macaques. *NPJ Vaccines* 2019; **4**: 21.
106. Demangel C, Garnier T, Rosenkrands I, Cole ST. Differential effects of prior exposure to environmental mycobacteria on vaccination with Mycobacterium bovis BCG or a recombinant BCG strain expressing RD1 antigens. *Infect Immun* 2005; **73**(4): 2190-6.
107. Price DN, Kusewitt DF, Lino CA, McBride AA, Muttil P. Oral Tolerance to Environmental Mycobacteria Interferes with Intradermal, but Not Pulmonary, Immunization against Tuberculosis. *PLoS Pathog* 2016; **12**(5): e1005614.
108. Romano M, D'Souza S, Adnet PY, et al. Priming but not boosting with plasmid DNA encoding mycolyl-transferase Ag85A from Mycobacterium tuberculosis increases the survival time of Mycobacterium bovis BCG vaccinated mice against low dose intravenous challenge with M. tuberculosis H37Rv. *Vaccine* 2006; **24**(16): 3353-64.

109. Ivanyi J. Recall of antibody synthesis to the primary antigen following successive immunization with heterologous albumins. A two-cell theory of the original antigenic sin. *Eur J Immunol* 1972; **2**(4): 354-9.
110. Harris DP, Vordermeier HM, Roman E, et al. Murine T cell-stimulatory peptides from the 19-kDa antigen of Mycobacterium tuberculosis. Epitope-restricted homology with the 28-kDa protein of Mycobacterium leprae. *J Immunol* 1991; **147**(8): 2706-12.
111. Aagaard C, Hoang T, Dietrich J, et al. A multistage tuberculosis vaccine that confers efficient protection before and after exposure. *Nat Med* 2011; **17**(2): 189-94.
112. Khademi F, Yousefi A, Derakhshan M, Najafi A, Tafaghodi M. Enhancing immunogenicity of novel multistage subunit vaccine of Mycobacterium tuberculosis using PLGA:DDA hybrid nanoparticles and MPLA: Subcutaneous administration. *Iran J Basic Med Sci* 2019; **22**(8): 893-900.
113. Voss G, Casimiro D, Neyrolles O, et al. Progress and challenges in TB vaccine development. *F1000Res* 2018; **7**: 199.
114. Andersen P, Scriba TJ. Moving tuberculosis vaccines from theory to practice. *Nat Rev Immunol* 2019; **19**(9): 550-62.
115. Kaufmann SHE. Vaccination Against Tuberculosis: Revamping BCG by Molecular Genetics Guided by Immunology. *Front Immunol* 2020; **11**: 316.
116. Commandeur S, van den Eeden SJ, Dijkman K, et al. The in vivo expressed Mycobacterium tuberculosis (IVE-TB) antigen Rv2034 induces CD4(+) T-cells that protect against pulmonary infection in HLA-DR transgenic mice and guinea pigs. *Vaccine* 2014; **32**(29): 3580-8.
117. Coppola M, Villar-Hernandez R, van Meijgaarden KE, et al. Cell-Mediated Immune Responses to in vivo-Expressed and Stage-Specific Mycobacterium tuberculosis Antigens in Latent and Active Tuberculosis Across Different Age Groups. *Front Immunol* 2020; **11**: 103.
118. Moguche AO, Musvosvi M, Penn-Nicholson A, et al. Antigen Availability Shapes T Cell Differentiation and Function during Tuberculosis. *Cell Host Microbe* 2017; **21**(6): 695-706 e5.
119. Velmurugan K, Grode L, Chang R, et al. Nonclinical Development of BCG Replacement Vaccine Candidates. *Vaccines (Basel)* 2013; **1**(2): 120-38.
120. Perera PY, Derrick SC, Kolibab K, et al. A multi-valent vaccinia virus-based tuberculosis vaccine molecularly adjuvanted with interleukin-15 induces robust immune responses in mice. *Vaccine* 2009; **27**(15): 2121-7.
121. Haddadi S, Vaseghi-Shanjani M, Yao Y, et al. Mucosal-Pull Induction of Lung-Resident Memory CD8 T Cells in Parenteral TB Vaccine-Primed Hosts Requires Cognate Antigens and CD4 T Cells. *Front Immunol* 2019; **10**: 2075.
122. Olsen AW, Hansen PR, Holm A, Andersen P. Efficient protection against Mycobacterium tuberculosis by vaccination with a single subdominant epitope from the ESAT-6 antigen. *Eur J Immunol* 2000; **30**(6): 1724-32.
123. Lindenstrom T, Moguche A, Damborg M, Agger EM, Urdahl K, Andersen P. T Cells Primed by Live Mycobacteria Versus a Tuberculosis Subunit Vaccine Exhibit Distinct Functional Properties. *EBioMedicine* 2018; **27**: 27-39.
124. Tait DR, Hatherill M, Van Der Meeren O, et al. Final Analysis of a Trial of M72/AS01E Vaccine to Prevent Tuberculosis. *N Engl J Med* 2019; **381**(25): 2429-39.
125. Basile JI, Liu R, Mou W, Gao Y, Carow B, Rottenberg ME. Mycobacteria-Specific T Cells Are Generated in the Lung During Mucosal BCG Immunization or Infection With Mycobacterium tuberculosis. *Frontiers in Immunology* 2020; **11**(2551).
126. Diogo GR, Reljic R. Development of a new tuberculosis vaccine: is there value in the mucosal approach? *Immunotherapy* 2014; **6**(9): 1001-13.
127. Beverley PC, Sridhar S, Lalvani A, Tchilian EZ. Harnessing local and systemic immunity for vaccines against tuberculosis. *Mucosal Immunol* 2014; **7**(1): 20-6.
128. Tree JA, Williams A, Clark S, Hall G, Marsh PD, Ivanyi J. Intranasal bacille Calmette-Guerin (BCG) vaccine dosage needs balancing between protection and lung pathology. *Clin Exp Immunol* 2004; **138**(3): 405-9.

129. Dijkman K, Sombroek CC, Vervenne RAW, et al. Prevention of tuberculosis infection and disease by local BCG in repeatedly exposed rhesus macaques. *Nat Med* 2019; **25**(2): 255-62.
130. Lai R, Jeyanathan M, Shaler CR, et al. Restoration of innate immune activation accelerates Th1-cell priming and protection following pulmonary mycobacterial infection. *Eur J Immunol* 2014; **44**(5): 1375-86.
131. Woodworth JS, Cohen SB, Moguche AO, et al. Subunit vaccine H56/CAF01 induces a population of circulating CD4 T cells that traffic into the Mycobacterium tuberculosis-infected lung. *Mucosal Immunol* 2017; **10**(2): 555-64.
132. Santosuosso M, McCormick S, Roediger E, et al. Mucosal luminal manipulation of T cell geography switches on protective efficacy by otherwise ineffective parenteral genetic immunization. *J Immunol* 2007; **178**(4): 2387-95.
133. Stylianou E, Paul MJ, Reljic R, McShane H. Mucosal delivery of tuberculosis vaccines: a review of current approaches and challenges. *Expert Rev Vaccines* 2019; **18**(12): 1271-84.
134. Dong H, Stanek O, Salvador FR, et al. Induction of protective immunity against Mycobacterium tuberculosis by delivery of ESX antigens into airway dendritic cells. *Mucosal Immunol* 2013; **6**(3): 522-34.
135. Chuang YM, Pinn ML, Karakousis PC, Hung CF. Intranasal Immunization with DnaK Protein Induces Protective Mucosal Immunity against Tuberculosis in CD4-Depleted Mice. *Front Cell Infect Microbiol* 2018; **8**: 31.
136. Sathkumara HD, Muruganandah V, Cooper MM, et al. Mucosal delivery of ESX-1-expressing BCG strains provides superior immunity against tuberculosis in murine type 2 diabetes. *Proc Natl Acad Sci U S A* 2020; **117**(34): 20848-59.
137. Hunter R, Actor J. The pathogenesis of post-primary tuberculosis. A game changer for vaccine development. *Tuberculosis (Edinb)* 2019; **116S**: S114-S7.
138. Turner J, Gonzalez-Juarrero M, Ellis DL, et al. In vivo IL-10 production reactivates chronic pulmonary tuberculosis in C57BL/6 mice. *J Immunol* 2002; **169**(11): 6343-51.
139. Bucsan AN, Chatterjee A, Singh DK, et al. Mechanisms of reactivation of latent tuberculosis infection due to SIV coinfection. *J Clin Invest* 2019; **129**(12): 5254-60.
140. Brown DH, Miles BA, Zwilling BS. Growth of Mycobacterium tuberculosis in BCG-resistant and -susceptible mice: establishment of latency and reactivation. *Infect Immun* 1995; **63**(6): 2243-7.
141. Quiroga MF, Angerami MT, Santucci N, et al. Dynamics of adrenal steroids are related to variations in Th1 and Treg populations during Mycobacterium tuberculosis infection in HIV positive persons. *PLoS One* 2012; **7**(3): e33061.
142. Vecchione MB, Eiras J, Suarez GV, et al. Determination of dehydroepiandrosterone and its biologically active oxygenated metabolites in human plasma evinces a hormonal imbalance during HIV-TB coinfection. *Sci Rep* 2018; **8**(1): 6692.
143. Duffy FJ, Weiner J, 3rd, Hansen S, et al. Immunometabolic Signatures Predict Risk of Progression to Active Tuberculosis and Disease Outcome. *Front Immunol* 2019; **10**: 527.
144. Higgins JP, Soares-Weiser K, Lopez-Lopez JA, et al. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. *BMJ* 2016; **355**: i5170.
145. Hesseling AC, Schaaf HS, Hanekom WA, et al. Danish bacille Calmette-Guerin vaccine-induced disease in human immunodeficiency virus-infected children. *Clin Infect Dis* 2003; **37**(9): 1226-33.
146. Walker KB, Brennan MJ, Ho MM, et al. The second Geneva Consensus: Recommendations for novel live TB vaccines. *Vaccine* 2010; **28**(11): 2259-70.

AUTHOR CONTRIBUTIONS STATEMENT

Being the single author, I confirm writing this Review Article.