Chapter 4

The Role of Antibodies in the Defense Against Tuberculosis

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1. Introduction

Throughout history tuberculosis (TB) has been a health problem for humanity. In the beginning of civilization, when human population densities were sparse, this disease may have been fairly harmless. However, with the increase in population densities, probably from the 17th to 19th centuries, TB took epidemic proportions [1].

Bacille Calmette Guérin (BCG) is effective to prevent miliary and meningeal TB in infants [1]. The reports about the efficacy of this vaccine for the prevention of adults pulmonary TB are contradictory and the consensus is that the protection conferred by BCG against this form of TB is questionable [1]. The wide use of BCG vaccination has been unable to prevent nearly two million deaths associated with TB that are produced every year. Currently the World Health Organization no longer recommends BCG vaccination in children born from HIVpositive mothers which complicate the implementation of BCG vaccination programs [2]. The implementation of standard drug treatment for TB is difficult in the areas of the highest incidence of the disease. Treatment is further complicated by the limited effectiveness of the current therapeutic schemes against drug resistant strains of TB [3-5].

Nowadays there is an increasing realization of the need for new animal models to test vaccine efficacy in more realistic scenarios, overcoming the limitations of current models in use. In addition, the elucidation of the significance of antibody-mediated defense against intracellular pathogens, in particular against *Mycobacterium tuberculosis*, constitutes an exciting new approach to improve the rational design of new vaccines, therapies and diagnostics.

2. Specific antibodies: Players in the defense against TB

In order to develop improved vaccines and new methods for controlling TB, an important element is the discovery of markers to measure the effectors' mechanisms of the protective immune response against *M. tuberculosis*.

For many years Cell-Mediated Immunity (CMI) was viewed as the exclusive defense mechanism against intracellular pathogens. The Th1/Th2 classical paradigm prevailed for a long time and the development of vaccines followed this theory [6]. Based on this theory, only intracellular pathogens could be effectively controlled by granulomatous inflammation induced by a Th1 response, whereas a Th2 response induces antibody production that controls extracellular pathogens and parasites. However, the question of what constitutes a true demarcation between "extracellular and intracellular" pathogens is important in this regard. During their infectious cycle, intracellular pathogens could be found in the extracellular space and *vice versa*. In the specific case of *M. tuberculosis*, it can be localized extracellularly at the beginning of the infection in the upper respiratory tract as well as during advanced stages of the disease, after rupture of granulomatous lesions occur [7]. This facultative intracellular pathogen was shown to have an extracellular phase [7] [8] that may include replication [7] which in turn could potentially be targeted by specific antibodies.

There are several prokaryotic and eukaryotic intracellular pathogens for which antibody have been shown to modify the course of infection by different mechanisms, as reviewed extensively by Casadevall and colleagues [9, 10, 11]. In the case of *Erhlichia* spp., specific antibodies were shown to mediate protection [12], possibly by blocking cellular entry or promoting the expression of proinflammatory cytokines. [13,14]. A combination of both humoral and cellular immune mechanisms could be the optimal choice controlling certain intracellular pathogens,.In this regard, de Valliere and colleagues reported that human antimycobacterial antibodies enhanced Cell-Mediated Immune responses to mycobacteria that are beneficial to the host [15].

3. Epidemiological evidence of antibody mediated protection

There is accumulating evidence, in the last few decades, regarding the effect of antibodies in the context of development of pulmonary or disseminated TB. Children with low serum IgG against sonicated mycobacterial antigens and LAM, or those who could not mount antibody responses to these antigens were predisposed to dissemination of *M. tuberculosis* [16].

M. leprae reactive salivary IgA antibodies were suggested to be important in a mucosal protective immunity [17]. In study carried out among the Mexican Totonaca Indian population, the presence of high antibody titers to Ag87 complex antigens were observed in patients with non-cavitary TB and in patients who were cured with anti-TB chemotherapy. In contrast, patients without such antibodies had a poor outcome of the disease [18].

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4. Experimental studies

4.1. Animal models

An important criterion for the evaluation of the role of specific antibodies in the protection against TB is the use of animal models. Currently, there is no optimal model to re-produce the infection as it occurs in humans [19].

The geographical location, genetic factors of the host, the presence of environmental mycobacteria and other concomitant infections like helminthiasis, are factors that have to be considered when designing animal experiments [20]. Several animal models have been used to evaluate different aspects of mycobacterial infection and disease. A crucial aspect is the delivery of mycobacterial inoculum. In this regard, several routes of inoculation have been employed experimentally, including intravenous, intraperitoneal, intranasal, intratracheal and aerosol [21, 22].

The study of the distribution of monoclonal and polyclonal antibody formulations in different organs and tissues of mice after administration by different routes, including the use of backpack models have been reported [23-27]. Each model has its advantages and drawbacks.

For example, the backpack model is very useful for the evaluation of the protective role of IgA, but poses ethical problems in long term experiments due to the increase in tumour size over time produced by the inoculated hybridoma [28]. In prophylactic and therapeutic models, antibody formulations have been administered via the intranasal [29], intravenous [30] and intraperitoneal [26] routes and combined with cytokines and antibiotics [31, 32] before and/or after the infectious challenge. The administration of *M. tuberculosis* pre-coated with antibodies [27, 33] in different models of infection has also contributed to understanding the interactions between host and microbe.

Another approach has been the use of knockout mice models for IgA [34] polymeric immunoglobulin receptor (pIgR) [34] and B cells [35,36,37,38], as will be discussed later.

4.2. Experimental studies with antibodies

A substantial number of studies utilizing anti mycobacterial antibodies have been conducted as far back as the end of the 19th century. These experiments can be grouped into several categories: serum therapies, mouse polyclonal antibodies, human polyclonal antibodies (including commercial human gamma globulins), secretory human IgA (hsIgA) and studies with monoclonal antibodies.

4.2.1. Serum therapies

Serum therapy experiments were conducted from the second half of the 19th century (reviewed in [39,40]). Immune sera was generated by immunizing animals with different mycobacterial fractions and administered either to animals or humans [39,40] The results obtained were either beneficiary, variable, inconclusive or contradictory, [39,40]. These variable results led to the perceived minor role of antibodies in the defense against *M. tuberculosis*.

What factors could have led to the heterogeneity in study results? Recognizable differences in the methods used for serum preparations and their administration, as well as the lack of appropriate experimental controls probably accounted in part for the studies outcomes. Furthermore, it is important to recognize that immune serum is a polyclonal preparation that includes antibodies with multiple specificities and isotypes. Consequently, polyclonal sera may contain antibodies of different subclasses and functional categories that can affect theoutcome of infection. For example, IgG3 murine monoclonal antibodies protected against *M.tuberculosis* [27] but failed to protect against *Cryptococcus neoformans* [41]. An IgG3 non-protectivemonoclonal antibody to *C. neoformans*, became protective upon subclass switching to IgG1 [41]. In addition to intrinsic factors associated with the antibody structure, other parameters such as the genetic background of the microbe and the immunocompetence of the host could alter the outcome of antibody protection experiments.

For some microorganisms, such as *Samonella typhimurium* and *C. neoformans*, passive antibody therapy efficacy depends on the mouse strain used [42, 43]. In the same way, some microbial strains are more susceptible to the effects of antibodies [44].

The animal model used is another important parameter that varies between different experiments cited in the literature [45]. Timing, the route of infection, the magnitude of the infecting inoculum are some additional variables that could affect antibody protection studies [46].

Despite their variability, the results obtained with serum therapy were valuable, demonstrating some beneficial effect of serum on the course of TB in humans, mainly in cases of early or localized TB [45]. Moreover, it was demonstrated that long periods of treatment were necessary to achieve a sustained effect [45].

4.2.2. Polyclonal mouse antibodies

A recent study re-examined the usefulness of immune serum in the context of a therapeutic vaccine against TB [32]. This vaccine, named RUTI, is generated from detoxified *M. tuberculosis* cell fragments that facilitate a balanced T helper response to a wide range of antigens along with intense antibody production [47]. Local accumulation of specific CD8+ T cells and a strong humoral response after immunization are characteristic features of RUTI that contribute to its protective properties. In that study, immune serum was generated by immunizing mice with RUTI [32]. Severe Combined Immunodeficiency (SCID) mice were inoculated with *M. tuberculosis* and treated with chemotherapy for 3–8 weeks. After chemotherapy they were treated for up to 10 weeks with intraperitoneal injections of the generated immune serum. Mice treated with immune serum from RUTI vaccinated animals showed significant decreases in lung CFU in addition to reduced extent of granulomatous response and abscess formation [47]. These results indicate that protective serum antibodies can be elicited by vaccination, and that antibodies may be usefully combined with chemotherapy [32, 47, 48].

4.2.3. Human gammaglobulins

4.2.3.1. Specific human polyclonal antibodies

Evidence for the stimulatory role of specific polyclonal antibodies on cellular immunity in experimental mycobacterial infections was reported by de Valliere and colleagues in 2005 [15].

In this study, serum samples containing specific antimycobacterial antibodies were obtained from volunteers vaccinated twice with BCG by the intradermal route. Significant titres of IgG antibodies against lipoarabinomannan (LAM) were detected in the sera. BCG internalization into phagocytic cells was significantly increased in the presence of these BCG induced antibodies as were the growth inhibitory effects of neutrophils and macrophages on mycobacteria. Furthermore, these antibodies induced significant production of IFN-γ by CD4+ and CD8+ T cells [15].

4.2.3.2. Commercial immunoglobulin formulations

Human Intravenous Immunoglobulin (IVIG) has been used to treat individuals with immune deficiencies and patients with inflammatory, autoimmune and infectious conditions [49, 50, 51]. Several groups tested the effect of human immunoglobulin preparation on mycobacterial infection. Roy and colleagues showed that treatment of *M. tuberculosis* infected mice with one cycle of IVIG led to the substantially lower bacterial loads in the spleen and lungs following its administration either at early or at late stage of infection 52]. The effect of the administration of a commercial preparation of human immunoglobulin (hIg) in a mouse model of intranasal infection with BCG was evaluated by Olivares and colleagues [33]. This group demonstrated the passage of specific antibodies to saliva and lung lavage following the intranasal or intraperitoneal administration of human hIg to mice. This treatment inhibited BCG colonization of the lungs of treated mice. A similar inhibitory effect was observed after infection of mice with hIg -opsonized BCG [33].

The same formulation was evaluated also in a mouse model of intratracheal infection with *M. tuberculosis*. Animals receiving human hIg intranasally 2h prior to intratracheal challenge demonstrated a significant decrease in lung bacillary load as compared with non-treated animals [29]. When *M. tuberculosis* was pre-incubated with hIg prior to challenge the same effect was observed [29].

The protective effect of the hIg formulation was abolished following pre-incubation with *M. tuberculosis* [29]. These results are suggestive of a potential role for specific human antibodies in the defense against mycobacterial infections.

Taken together, these studies provide support for the potential use of immunoglobulins against *M. tuberculosis*.

4.2.3.3. Human secretory IgA

Human secretory IgA (hsIgA) is the major class of antibody associated with immune protection of the mucosal surfaces [53]. Colostrum volume is above 102 mL in humans during the first three days after delivery [54]. The high percentage of (hsIgA) in human colostrum [55] strongly suggests its important role in passive immune protection against gastrointestinal and respiratory infections [56]. In one study performed by Alvarez and colleagues, hsIgA from human colostrum was obtained by anion exchange and gel filtration chromatographic methods, using DEAE Sepharose FF and Superose 6 preparative grade, respectively [57].

hsIgA was administered intranasally to BALB/c mice, and the level of this immunoglobulin in several biological fluids was determined by ELISA. The results showed the presence of this antibody in the saliva of animals that received the hsIgA, at all time intervals studied. In tracheobronchial lavage, hsIgA was detected at 2 and 3 hours after inoculation in animals that received the hsIgA [58]. Similar studies were performed by Falero and colleagues with monoclonal antibodies of IgA and IgG class [59]. Following demostration that hsIgA could be detected in several biological secretions after intranasal administration, the protective effect of this formulation against *M. tuberculosis* challenge was evaluated. Mice challenged with *M. tuberculosis* preincubated with hsIgA showed a statistically significant decrease in the mean number of viable bacteria recovered from the lungs compared to control mice and to the group that received the hsIgA before challenge with *M. tuberculosis*. Moreover, an increased level of iNOS production was also reported (Alvarez et al., mannuscript in preparation). Consistently with this result, a better organization of granulomatous areas with foci of lymphocytes and abundant activated macrophages were observed in the lungs of mice that received *M. tuberculosis* pre-incubated with hsIgA and sacrificed at 2 months postchallenge. Untreated animals, however, showed an increased area of bronchiectasis and atelectasis as well as fibrin deposits, accumulation of activated macrophages and lymphocytes.

The pneumonic areas were more prominent in the untreated animals than in the groups treated with hsIgA and *M. tuberculosis* pre-incubated with hsIgA (Alvarez et al., manuscript in preparation)

4.2.4. Monoclonal antibodies

Since the first report on the use of the monoclonal antibody Mab 9d8 against *M. tuberculosis* [27], many similar studies have been reported [40]. This IgG3 monoclonal antibody (Mab) generated against arabinomannan (AM) capsular polysaccharide, increased the survival of intratracheally infected mice when the *M. tuberculosis* Erdman strain was pre-coated with it [27]. In this study, a longer survival associated with an enhanced granulomatous response in the lungs was found as compared to controls receiving an isotype-specific non-related Mab [27]. Another Mab, SMITB14, directed against the AM portion of LAM prolonged the survival of intravenously infected mice associated with reduced lung CFU and prevention of weight loss [60]. In this study, the authors demonstrated that protection was independent of the antibody Fc portion, because the F(ab')2 fragment also conferred a similar protective effect [60]. In another study, mice receiving the Mab 5c11 (an IgM antibody that recognizes other mycobacterial arabinose-containing carbohydrates in addition to AM) intravenously prior to Mannosylated lipoarabinomannan (ManLAM) administration, showed a significant clearance of Man-LAM and redirection of this product to the hepatobiliary system [26]. This study strongly supports an indirect effect of certain antibodies on the course of mycobacterial infection, altering problably the pharmacokinetics of mycobacterial components and contributing to protection against TB [26].

Heparin Binding Hemagglutinin Adhesin (HBHA) is a surface-exposed glycoprotein involved in the mycobacterial binding to epithelial cells and in mycobacterial dissemination [62]. Monoclonal antibodies 3941E4 (IgG2a) and 4058D2 (IgG3) directed against HBHA were used

to coat mycobacteria before administration to mice. In this study, spleen CFUs was reduced while lung CFUs did not [63]. These results suggest that binding of these antibodies to HBHA can impede mycobacterial dissemination.

The protective efficacy of a monoclonal antibody, TBA63 and IgA anti-Acr administered intranasally before and after the intranasal or aerosol challenge with *M. tuberculosis* was demonstrated in a study by Williams and colleagues [64]. In another series of experiments carried out by López and colleagues, the protective effect of this Mab administered intratracheally before an intratracheal challenge with virulent mycobacteria was evaluated. At 21 days post-infection, pre-treatment of mice with TBA63 caused a significant decrease in viable bacteria in the lungs compared to control mice or those treated with the Mab against the 38-kDa protein (TBA86) [65]. Consistent with the reduction of viable bacteria following treatment with TBA63, the area of peribronchial inflammation was also statistically smaller in this group compared to the control group [65].

When the lungs of mice were histologically examined, granulomas were better organized in the infected animals that had received TBA63 than in controls or mice treated with TBA86. The reduction of CFU in lungs of the treated group was associated with milder histopathological changes, as indicated by the organization of the granulomas and less pneumonic area. The fact that this Mab promotes granuloma formation in mice infected intratracheally with *M. tuberculosis* strongly suggests the close interaction between antibody mediated immunity and cell-mediated immunity to induce protection against intracellular pathogens (66).

The 16 kDa protein (Acr antigen) has been defined as a major membrane protein peripherally associated with the membrane [67] carrying epitopes restricted to tubercle bacilli on the basis of B-cell recognition [68, 69]. The Acr antigen is present on the surface of tubercle bacilli and is highly expressed in organisms growing within infected macrophages, allowing it to be potentially targeted by specific antibodies either inside infected cells as well as extracellulary. A novel immunotherapy, combining treatment with anti-IL-4 antibodies, IgA antibody against 16 kDa protein and IFN-γ, showed the potential for passive immunoprophylaxis against TB. In genetically deficient IL-4-/- BALB/c mice, infection in both lungs and spleen was substantially reduced for up to 8 weeks. Administration of rIL-4 to IL-4-/- mice with increased bacterial counts to wild-type levels and make mice refractory to protection by IgA/IFN-γ [70].

More recently, Balu and colleagues reported that intranasal administration of a human IgA1 Mab, obtained using a single-chain variable fragment derived from an Ab phage library with high affinity for hspX and the human FcαRI (CD91) IgA receptor together with recombinant mouse IFN-γ significantly inhibited pulmonary infection with *M. tuberculosis* H37Rv in mice transgenic for human CD91 but not in the CD91-negative controls. These results suggested that binding to CD91 was necessary for the IgA-imparted passive protection [71]. When the Mab was incubated with human whole-blood or monocyte cultures it inhibited H37Rv infection.

Inhibition of the infection by the antibody was synergistic with human rIFN-γ in purified human monocytes cultures but not in whole blood cultures [71]. The demonstration of the role of FcαRI (CD91) in human IgA mediated protection contributes to understanding the mecha-

nisms involved as well as for using this knowledge for the future development of new immunotherapies for TB [71].

4.2.5. Transgenic mice

Mouse models with deficiency in antibody production can provide useful information for understanding certain roles of antibodies in protection against mycobacterial infections. Rodríguez and colleagues reported that after immunization of IgA deficient (IgA-/-) and wild type mice by the intranasal route with the mycobacterial surface antigen PstS-1, IgA-/- mice were more susceptible to BCG infection than IgA+/+ mice [34]. Cytokine response analysis demonstrated reduction in the IFN-γ and TNF-α production in the lungs of IgA-/- as compared with IgA+/+ mice, suggesting that IgA may play a role in protection against mycobacterial infections in the respiratory tract. Furthermore, these authors demonstrated that immunized pIgR-/- mice were more susceptible to BCG infection than immunized wild-type mice [34].

In an attempt to elucidate whether antibody-mediated immunity has a special role in the defense against TB, different experiments with B cell knockout mice were performed by several authors. In 11016, Vordermeier and colleagues developed an infection model of TB in μ chain knockout Ig- mice. Organs from *M. tuberculosis* infected IgG- mice had three to eight fold elevated counts of viable bacilli compared with those from normal mice. This result suggested that B cells play a role in the containment of murine tuberculous infection [35]. In another study B cell KO mice and controls were infected by aerosol with *M. tuberculosis*. They were subsequently given chemotherapy to destroy remaining bacilli and then re-challenged by aerosol exposure. There were not differences in the ability of animals to control this second infection, indicating that, in this low dose pulmonary infection model, any local production of antibodies neither impeded nor enhanced the expression of specific acquired resistance [36].

In another series of experiments the role of B cells was evaluated during early immune responses to infection with a clinical strain of *M. tuberculosis* (CDC 1561). In this study, despite comparable bacillary loads in the lungs, B cell KO mice had a less severe pulmonary granuloma formation and delayed dissemination of bacteria from lungs to peripheral organs. Additional analysis of lung cells demonstrated higher numbers of lymphocytes, particularly CD8+ T cells, macrophages, and neutrophils in wild-type and reconstituted mice as compared with B cell KO mice. These results demonstrate that less severe granuloma formation and delayed dissemination of mycobacteria found in B cell KO mice were dependent on B cells, (not antibodies, at least in this study) and were associated with modification of cellular infiltrate in the lungs [37]. This latter result differs from a study carried out by Maglione and colleagues in which B cell-/- mice demonstrated exacerbated immunopathology corresponding with enhanced pulmonary recruitment of neutrophils following aerosol challenge with *M. tuberculosis* Erdman strain [38]. Infected B cell-/- mice demonstrated increased production of IL-10 in the lungs, while IFN- γ, TNF-α, and IL-10R were not significantly different from those of wild type mice [38].. B cell-/- mice demonstrated enhanced susceptibility to aerosol infection of 300 CFU of *M. tuberculosis* with elevated bacterial burden in the lungs but not in the spleen or liver [38].

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Together these studies suggest that B cells may have an important role in host defense against *M. tuberculosis*.

5. Mechanisms of action

The various effects of antibodies demonstrated in the studies analyzed, suggest that different mechanisms of action are involved in the effect of monoclonal and polyclonal antibodies on *M. tuberculosis*. Secretions found on mucosal surfaces contain significant levels of Igs, particularly, IgA. IgA has both direct and indirect functional roles for combating infectious agents such as viruses and bacteria that cross the mucosal barrier [72]. Moreover, experimental evidence suggests that IgA associated with the pIgR may neutralize pathogens and antigens intracellularly during their transport from the basolateral to the apical zone of epithelial cells [73,74]. In addition, IgA may interact with Gal-3 (an intracellular binding β-galactosidase lectin), and interfere with the interaction of mycobacteria with the phagosomal membrane, resulting in the decrease of bacterial survival and replication in the phagosome [75].

Antibodies may be critical, during the extracellular phases of intracellular facultative pathogens. They may act by interfering with adhesion, by neutralizing toxins and by activating complement. Moreover, antibodies may be able to penetrate recently infected cells, bind internalised pathogens, and enhance antigen processing (76). Antibodies may also play a crucial role in modulating the immune response by activating faster secretion of selected cytokines that in turn, contribute to more efficient and rapid Th1 response [76,77], increasing the efficacy of co-stimulatory signals, enhancing Antibody Dependent Cellular Cytotoxicity (ADCC) and the homing of immune cells to the lungs after the respiratory infection [10,78, 79, 80, 81, 82, 83].

Examples of relevant potential action mechanisms of antibodies against *M. tuberculosis* were discussed by Glatman-Freedman [40].

6. Potential uses of antibodies against TB

Future applications of antibody formulations for the control of TB may include several possibilities including treatment, prevention and diagnosis.

6.1. Treatment

Antibody based therapy could potentially be useful in several scenarios. They could be used to shorten the standard treatment period of patients with uncomplicated TB when coupled with standard chemotherapy. However, they would be particularly important in the treatment of patients infected with Multidrug Resistant (MDR) and Extensively Drug Resistant (XDR) strains, in combination with the standard treatment.

6.2. Prophylactic use

Prophylactic use of antibodies could be applied in recent contacts of TB patients, with special attention to risk groups [84]. In this regard, successful prophylactic use of antibodies in exposed individuals has been shown in the case of several other pathogens such as varicella, tetanus, Respiratory Synsicial Virus (RSV), rabies and Hepatitis B [85, 86]

6.3. Vaccines

The induction of specific protective antibody responses by vaccination, either alone or as an addition to the stimulation of cell mediated immunity could be a novel strategy for the development of new generation of prophylactic and therapeutic vaccines against TB.

The prevailing past dogma that discounted the role of antibodies in host protection against TB has resulted in a limited study of B cell immunodominant epitopes as targets for protective immunity [87].

6.3.1. Polysaccharide conjugate vaccines

Polysaccharide conjugate vaccines are considered to elicit specific protective antibody responses against a variety of pathogens [88]. However, the polysaccharide conjugate vaccine against *Salmonella typhi* [89] demonstrates the feasibility of this kind of vaccines for the prevention of infectious diseases caused by intracellular pathogens. In the case of *M. tuberculosis*, several authors reported the use of polysaccharide conjugated vaccine candidates [61, 90, 91, 92].

All these vaccine candidates induced the production of specific IgG [61, 90, 91, 92] and some of them conferred variable levels of protection [61, 91] which validate this strategy as one of the potential avenues for the development of new generation of vaccines against tuberculosis

6.3.2. Identifying other B-cell immunodominant epitopes

With the development of bioinformatics tools for bacterial genome analysis, it has been possible to predict *in silico* microbial regions that trigger immune responses relevant for protection and vaccine development. A candidate experimental vaccine based on proteoliposomes from *M. smegmatis* is currently in development [93].

In one study, bibliographic search was used to identify highly expressed proteins in active, latent and reactivation phases of TB [94]. The subcellular localization of the selected proteins was defined according to the report on the identification and localization of 1044 *M. tuberculosis* proteins using two-dimensional, capillary high-performance liquid chromatography coupled with mass spectrometry (2DLC/MS) method [95] and using prediction algorithms. Taking into consideration the cell fractions potentially included in the proteoliposome, from the previously identified proteins, the ones located in the cell membrane and cell wall, as well as those which are secreted and homologous to those of *M. smegmatis* were selected.

The regions of the selected proteins containing promiscuous B and T cell epitopes were determined [94]. Thus the *M. smegmatis* proteoliposomes were predicted to contain multiple

B and T epitopes which are potentially cross reactive with those of *M. tuberculosis*. It is important to note that there could be conformational B epitopes and additional epitopes related with lipids and carbohydrates included in the proteoliposomes that could reinforce the humoral cross reactivity.

Considering the results of the *in silico* analysis, proteoliposomes of *M. smegmatis* were obtained and their immunogenicity was studied in mice [93]. In addition to cellular immune effectors recognizing antigens from *M. tuberculosis*, cross reactive humoral immune responses of several IgG subclasses corresponding with a combined Th1 and Th2 pattern against antigenic components of *M. tuberculosis* were elicited. These findings were in concordance with the *in silico* predictions [93, 94]. It is interesting to note that differences in the pattern of humoral recognition of lipidic components was dependent on the characteristics of the adjuvant used, which could have relevance for the development of vaccines which includes lipidic components [93]. Currently studies are underway to evaluate the protective capacity of *M. smegmatis* proteoliposomes in challenge models with *M. tuberculosis* in mice.

Bioinformatics tools for prediction of T and B epitopes were also employed for the design of multiepitopic constructions, which were used to obtain recombinant BCG strains. Based on this prediction, B cell epitopes from ESAT-6, CFP-10, Ag87B and MTP40 proteins were selected and combined with T cell epitopes of the 87B protein and fused to Mtb8.4 protein [96].

A significant IgG antibody response against specific B cell epitopes of ESAT-6 and CFP-10 was obtained in mice immunized with the recombinant strain. After studying the specific response of spleen cells by lymphoproliferation assay and detection of intracellular cytokines in CD4 + and CD8 + subpopulations, the recognition of T epitopes was also observed. The response showed a Th1 pattern after immunization with this recombinant strain (Mohamud, R, et al. manuscript in preparation). In another series of experiments, recombinant BCG strains expressing several combinations of multiepitopic constructions were used to immunize BALB/c mice subcutaneously and challenged intratracheally with the *M. tuberculosis* H37Rv strain. Recombinant BCG strains expressing T epitopes from 87BAg fused to Mtb8.4 protein and BCG expressing a HSP62 T cell epitope plus different combinations of B cell epitopes from 87BAg, Mce1A, L7/L12, 16 kDa, HBHA, ESAT6, CFP10 and MTP40 and combinations of B cell epitopes alone produced significant reductions in lung CFU compared to BCG (Norazmi MN, et al. manuscript in preparation).

6.3.3. Diagnosis

Although no serological assays are currently recommended for diagnosis of TB [97], largely due to the possibility of false results and thus incorrect treatments, for many other pathogens, serological diagnostic tests has been of great value, particularly in poor countries. In some cases, antibody responses can constitute useful correlates of protection [98]. In the specific case of TB, several studies of the antibody response have been reported [99]. There is a substantial amount of variability in antibody response to TB [100]. This variability has been attributed to several factors. Some of these factors are associated with the pathogen (strain variation, microenvironment and growth state of bacteria) and others are related to the host, primarily previous exposure to related antigens and host genetics [99].

However, it is important to consider that only a small fraction of the genomic regions of *M. tuberculosis* encoding proteins has been explored. Currently, novel immunoassay platforms are being used to dissect the entire proteome of *M. tuberculosis*, including reacting protein microarrays with sera from TB patients and controls [101,102]. These studies could lead to the discovery of new antigens that may constitute suitable diagnostic markers and tools for the identification of protection correlates.

7. Concluding remarks

The cumulative work reviewed above with regard to the use of antibody formulations and vaccines suggest that antibodies if present at the right moment at the site of infection could provide protection against *M. tuberculosis*. This concept leads the way to the development of a new generation of vaccines. Such vaccines could work by eliciting specific IgA and/or IgG antibodies that could recognize and intercept the pathogen at the port of entry, primarily the mucosal surfaces, inactivating bacterial components essential for the microbial survival in the host, activating complement for direct lysis of the cells, opsonizing bacteria to promote their capture by phagocytic cells and inducing stimulation of specific cellular immune responses [103, 104].

Various antibody formulations could potentially be used as immunotherapeutic agents in combination with the conventional treatment and in the management of patients affected by Multidrug Resistant (MDR) and Extensively Drug Resistant (XDR) strains.

The study of the role of specific antibodies in the defense against tuberculosis opens new possibilities for future development of new vaccines, diagnostics tools and therapies against this pathogen. It is likely that new discoveries will arise from the ongoing studies in this area that will expedite the introduction of new strategies in the fight against tuberculosis.

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