18

Towards a New Challenge in TB Control: Development of Antibody-Based Protection

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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), the only licensed vaccine against TB, has been shown to be effective in preventing meningeal and miliary TB in children. However, the efficacy of this vaccine in preventing adult pulmonary TB is questionable. Despite widespread vaccination with BCG, nearly 2 million people die each year from TB. Furthermore, the World Health Organization no longer recommends BCG vaccination of children with HIV or HIV-positive mothers due to safety concerns, leaving many infants without any protection against this disease. While drug therapies exist to combat TB infection, the implentation of suitable treatment is often difficult in the countries hardest hit by the disease and a fact complicated by the limited effectiveness of the current therapeutic schemes at treating drug resistant strains of TB [2-4].

Nowadays there is an increasing realization of the need of new animal models to test vaccine efficacy in more realistic scenarios overcoming the limitations of the current models in use. In addition, the elucidation of the significance of humoral 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.

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2. Reshaping the classical paradigm

In order to develop improved vaccines and new methods for the control of TB, an important element is the discovery of markers to measure the effector mechanisms of the protective immune response against *M. tuberculosis*. For many years Cellular Mediated Immunity (CMI) was attributed as the exclusive defence mechanism against intracellular pathogens. The Th1/Th2 classical paradigm prevailed for a long time and directed the development of vaccines according to this theory [5].

Based on this point of view, only intracellular pathogens could be effectively controlled by granulomatous inflammation induced by a Th1 response whereas a Th2 response induces antibody production that control extracellular pathogens and parasites. However, the question arises of what really constitutes the true demarcation between "extracellular and intracellular"? In the infectious cycle of several intracellular pathogens, they 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 in advanced stages of the disease after the rupture of granulomatous lesions [6]. In the case of *Erhlichia* spp specific antibodies could mediate protection against [7], possibly by blocking cellular entry or promoting the expression of proinflammatory cytokines [8;9]. It has been demonstrated that this obligate intracellular pathogen has also an extracellular phase that may include replication which could be targeted by specific antibodies [10].

For certain viral pathogens, the induction of Antibody Mediated-Immunity is sufficient to prevent infection, as has been clearly demonstrated by the almost complete eradication of smallpox with the use of vaccines that elicited antibody-mediated immunity [11]. 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 [12-14]. Nowadays, it is well established that an efficient combination of both humoral and cellular immune mechanisms could be the best choice to control certain diseases produced by intracellular pathogens [15;16].

In 2005, de Valiere and colleagues reported for the first time that human antimycobacterial antibodies stimulates the Th1 response instead of diminishing it, as was thought previously [17].

3. Protective role of antibodies: Epidemiological evidence

There is accummulated evidence in the last few decades on the influence of antibodies in the development of pulmonary or disseminated TB. Children with low serum IgG against sonicated mycobacterial antigens and LAM, or those who cannot mount antibody responses to these antigens, were predisposed to dissemination of the bacteria [18]. In another report, Kamble and colleagues reported that *M. leprae* reactive salivary IgA antibodies could be quite important in a mucosal protective immunity [19]. In one study carried out on the Mexican Totonaca Indian population, the presence of high antibody titers to Ag85 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 [20].

4. Experimental studies

4.1 Animal models for the evaluation of the role of antibodies in TB infection

One 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 reproduce the infection as it occurs in humans [21]. Several animal models have been used to evaluate different aspects. One crucial aspect is the delivery of inoculums, where several routes of inoculation have been employed as intravenous, intraperitoneal, intranasal, aerosol and intratracheal [22]. 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 [23].

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 [24-26]. 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 [27].

In prophylactic and therapeutic models, antibody formulations have been administered via the intranasal, intravenous and intraperitoneal routes and combined with cytokines and antibiotics [28,29] before and/or after the infectious challenge.

The administration of *M. tuberculosis* pre-coated with antibodies [30,31] in different models of infection have also contributed to understanding the interactions between host and microbe.

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

4.2 Experimental studies with antibodies

A great number of studies involving antibodies as inoculum have been conducted as far back as the end of the 19th century. These experiments can be grouped in 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. Immune sera was generated by immunizing animals with different microbial fractions and administered either to animals or humans. The results obtained were either variable, inconclusive or contradictory, due to differences in the methods of serum preparation or its administration, and the lack of appropriate experimental controls [32]. These controversial results led to the perceived minor role of antibodies in the defence against intracellular pathogens.

Why these results were considered ¨controversial¨? Immune serum is a polyclonal preparation that includes antibodies to multiple specificities and isotypes; consequently, polyclonal sera may contain blocking antibodies [33] and antibodies of different functional categories that can affect the outcome of infection. For example, IgG3 murine monoclonal antibodies protects against *Streptococcus pneumoniae* and *M. tuberculosis* but fails to protect against *C. neoformans* [34]. Moreover, results from animal studies are not always reflective of the Ig isotype function in humans. Besides intrinsic factors associated to 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 (*Legionella pneumophila* and *C. neoformans*), passive antibody therapy efficacy depends on the mouse strain used [35]. In the same way, some microbial strains are more susceptible to the effects of antibodies. The animal model used is another important parameter that varies between different experiments cited in the literature. Timing, the route of infection, the magnitude of the infecting inoculum and the variables to measure efficacy are some of the critical parameters in antibody protection studies [36].

Despite its controversial nature, 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 [37]. Moreover, it was demonstrated that long periods of treatment were necessary to achieve a sustained effect [38].

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 [39]. This vaccine, called 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. Local accumulation of specific CD8+ T cells and a strong humoral response after immunization are characteristic features of RUTI, features that contribute to its protective properties. In this study, immune serum was generated by immunizing mice with RUTI. Severe Combined Immunodeficiency (SCID) mice were infected 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 immune serum. Mice treated with immune serum from RUTI vaccinated animals showed significant decreases in lung CFU as well as reduction in the extent of granulomatous response and abscess formation in comparisson with controls. These results suggest that protective serum antibodies can be elicited by vaccination, and that antibodies may be usefully combined with chemotherapy [29,40].

4.2.3 Human gammaglobulins

4.2.3.1 Human polyclonal antibodies

The first evidence of the stimulatory role upon cellular immunity of specific antibodies in experimental mycobacterial infections was reported by Valiere and colleagues in 2005. 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 volunteers. Moreover, BCG internalization into phagocytic cells was significantly increased in the

presence of BCG induced antibodies as were the inhibitory effects of neutrophils and macrophages on mycobacterial growth. Furthermore, these antibodies induced significant production of IFN-γ by CD4+ and CD8+ T cells [17].

4.2.3.2 IgG formulations

Roy and colleagues demonstrated that the treatment of *M. tuberculosis*-infected mice with a single cycle of human intravenous Ig resulted in substantially reduced bacterial loads in the spleen and lungs when administered either at early or at late stage of infection [41].

The effect of the administration of a commercial preparation of human gammaglobulins in a mouse model of intranasal infection with BCG was evaluated by our group. We demonstrated the passage of specific antibodies to saliva and lung lavage following the intranasal or intraperitoneal administration of human gammaglobulins to mice. This treatment inhibited BCG colonization of the lungs of treated mice. A similar inhibitory effect was observed after infection of mice with gammaglobulin-opsonized BCG [42]. The same formulation was evaluated also in a mouse model of intratracheal infection with *M. tuberculosis*. Animals receiving human gammaglobulins intranasally 2h before intratracheal challenge showed a significant decrease in lung bacilli load compared to non-treated animals. When *M. tuberculosis* was pre-incubated with the gammaglobulin before challenge the same effect was observed. The protective effect of the gammaglobulin formulation was abolished after pre-incubation with *M. tuberculosis* [30]. These results suggest a potential role of specific human antibodies in the defence against mycobacterial infections.

Taken together these studies provide consistent support for the potential use of gammaglobulins and their beneficial immunomodulatory effects in tuberculosis. The results of certain knockout mouse studies and the gammaglobulin experiments indicate that B cells and their products mediate protection against *M. tuberculosis* [43-45]. However, the important question that remains is whether B-cell responses can be augmented to improve immunity against *M. tuberculosis* through immunotherapy or vaccination.

4.2.3.3 Purified human secretory IgA

Human secretory IgA (hsIgA) is the major class of antibody associated with immune protection of the mucosal surfaces [46]. Colostrum volume is above 100 mL in humans during the first three days after delivery [47]. The high percentage of (hsIgA) in human colostrum [48] strongly suggests its important role in passive immune protection against gastrointestinal and respiratory infections [49]. In one study performed by our group, hsIgA from human colostrum was obtained by anion exchange and gel filtration chromatographic methods, using DEAE Sepharose FF and Superose 6 preparative grade, respectively [50]. 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 [51]. Similar studies were performed by Falero and colleagues with monoclonal antibodies of IgA and IgG class [52]. 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 of the group that received *M. tuberculosis* pre-incubated with hsIgA sacrificed at 2 months post-challenge. 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., mannuscript in preparation)

4.2.4 Studies performed with monoclonal antibodies

Since the first report on the use of the monoclonal antibody Mab 9d8 against *M. tuberculosis*, many similar studies have been reported [53;54]. This IgG3 monoclonal antibody (Mab) generated against arabinomannan (AM) capsular polysaccharide, increased thesurvival of intratracheally infected mice when the *M. tuberculosis* Erdman strain was pre-coated with it. 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 [31].

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. 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 [55].

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 ManLAM and redirection of this product to the hepatobiliary system. 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 [56].

Heparin Binding Hemagglutinin Adhesin (HBHA) is a surface-exposed glycoprotein involved in the mycobacterial binding to epithelial cells and in mycobacterial dissemination [57]. Monoclonal antibodies 3921E4 (IgG2a) and 4057D2 (IgG3) directed against HBHA were used to coat mycobacteria before administration to mice. In this study, spleen CFUs were reduced while lung CFUs did not [58]. These results suggest that binding of these antibodies to HBHA impede mycobacterial dissemination.

The protective efficacy of a monoclonal antibody, TBA61, IgA anti-Acr administered intranasally before and after the intranasal or aerosol challenge with *M. tuberculosis* was demonstrated in a previous work [59]. 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 TBA61 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 (TBA84). Consistent with the reduction of viable bacteria following treatment with TBA61,

the area of peribronchial inflammation was also statistically smaller in this group compared to the control group [60].

When the lungs of mice were histologically examined, granulomas were better organized in the infected animals that had received TBA61 than in controls or mice treated with TBA84. 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 (61). Some of the results obtained in the evaluation of TBA61 monoclonal antibody under different conditions are listed in the Table 1.

MAb, delivery route and inoculation regime	**Challenge**	**Days selected for Organ Harvesting**	**Parameter measured**		**References**
			CFU reduction	**Histopathology**	
TBA61 i.n (-3h, +3h, 6h) TBA61 i.n (-3h) TBA61 i.n (+3h) TBA61 i.n (-3h, +3h)	H37Rv i.n, aerosol	9 days	Significant reduction of CFU post-challenge	nd	59
TBA61 i.n + IFN-γ i.n (-3h, -2h, +2h, +7h)	H37Rv i.n, aerosol	9, 21 and 28 days	Significant reduction of CFU post-challenge	Significant reduction of the granulomatous area in the lungs of treated as, compared to untreated mice	28
TBA61 i.t (-3h)	H37Rv i.t	24h, 72h, 21 days	Significant reduction at 21 days post-challenge	Less interstitial and peribronchial inflammation. Well-organized granuloma	60

Table 1. Results from different experimental approaches involving a monoclonal antibody against *M. tuberculosis* 16 kDa protein (TBA61). Note: i.n: intranasal; i.t: intratracheal

The 16 kDa protein (Acr antigen) has been defined as a major membrane protein peripherally associated with the membrane [62] carrying epitopes restricted to tubercle bacilli on the basis of B-cell recognition [63,64]. 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. Reconstitution of IL-4-/- mice with rIL-4 increased bacterial counts to wild-type levels and making mice refractory to protection by IgA/IFN-γ [65].

More recently, Balu and colleagues reported a novel human IgA1 Mab, constructed using a single-chain variable fragment clone selected from an Ab phage library. The purified Mab monomer revealed high binding affinities for the mycobacterial α-crystallin Ag and for the human FcαRI (CD89) IgA receptor. Intranasal inoculations with the monoclonal antibody and recombinant mouse IFN-γ significantly inhibited pulmonary H37Rv infection in mice transgenic for human CD89 but not in CD89-negative littermate controls, suggesting that binding to CD89 was necessary for the IgA-imparted passive protection. The Mab added to human whole-blood or monocyte cultures inhibited luciferase-tagged H37Rv infection although not for all tested blood donors. Inhibition of the infection by the antibody was synergistic with human rIFN-γ in cultures of purified human monocytes but not in whole-blood cultures. The demonstration of the mandatory role of FcαRI (CD89) for human IgA-mediated protection is important for understanding the mechanisms involved and also for translating this approach towards the development of passive immunotherapy for TB [66].

In all the studies analyzed, it is possible to assert that different mechanisms of action of monoclonal and polyclonal antibodies are involved in the protection against TB. Some of these mechanisms will be discussed later in this chapter.

4.2.5 Studies performed in transgenic mice

Mouse models with deficiency in antibody production can be useful in understanding certain roles of the antibodies in protection against mycobacterial infections. However, knockout mouse studies can lead to premature conclusions regarding the role of a particular component of immunity, if not interpreted carefully. Additionally, experimental conditions can have marked effects on the results.

Rodríguez and colleagues reported that IgA deficient (IgA-/-) mice and wild type non-targeted littermate (IgA+/+) were immunized by intranasal route with the mycobacterium surface antigen PstS-1. These authors showed that IgA-/- mice were more susceptible to BCG infection compared to IgA+/+ mice, as revealed by the higher bacterial loads in the lungs and bronchoalveolar lavage (BAL). More importantly, analysis of the cytokine responses revealed a reduction in the IFN-γ and TNF-α production in the lungs of IgA-/- compared to 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 [67].

In the attempt to elucidate whether humoral immunity has a special role in the defence against TB, different experiments with B cell knockout mice were performed by several authors. In 1996, 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 [68]. In another study, B cell gene disrupted mice (B cell KO) and controls were infected by aerosol with *M. tuberculosis* to allow the latter group to generate an antibody response in the upper respiratory tract. They were subsequetly given chemotherapy to destroy remaining bacilli and then re-challenged by aerosol exposure. The results of this study, however, revealed no differences in the ability of animals to control this second infection, indicating that, in this low dose pulmonary infection model at least, any local production of antibodies neither impeded nor enhanced the expression of specific acquired resistance [69].

In another series of experiments the role of B cells during early immune responses to infection with a clinical isolate of *M. tuberculosis* (CDC 1551) was evaluated. In this study, despite comparable bacterial loads in the lungs, less severe pulmonary granuloma formation and delayed dissemination of bacteria from lungs to peripheral organs were observed in BKO mice. Additional analysis of lung cell populations revealed greater numbers of lymphocytes, especially CD8+ T cells, macrophages, and neutrophils in wild-type and reconstituted mice than in BKO mice. Thus, less severe lesion formation and delayed dissemination of bacteria found in BKO mice were dependent on B cells, (not antibodies, at least in this study) and were associated with altered cellular infiltrate to the lungs [70].

This latter result differs to the study carried out by Maglione and colleagues in which B cell-/- mice had exacerbated immunopathology corresponding with elevated pulmonary recruitment of neutrophils upon aerosol challenge with *M. tuberculosis* Erdman strain. Infected B cell-/- mice showed increased production of IL-10 in the lungs, whereas IFN-γ, TNF-α, and IL-10R remain unchanged from wild type. B cell-/- mice had enhanced susceptibility to infection when aerogenically challenged with 300 CFU of *M. tuberculosis* corresponding with elevated bacterial burden in the lungs but not in the spleen or liver [43].

Together these studies reveal that B cells may have a greater role in the host defence against *M. tuberculosis* than previously thought.

5. Possible mechanisms of action

Secretions found on mucosal surfaces contain significant levels of Igs, particularly, IgA. This immunoglobin has direct and indirect functional roles to combat infectious agents such as viruses and bacteria that cross the mucosal barrier. Moreover, experimental evidences suggest that the IgA associated with the pIgR may neutralize pathogens and antigens intracellularly during their transport from the basolateral to the apical zone of epithelial cells [71,72]. In addition, as demonstrated previously, 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 [73].

As reported by several authors, antibodies may be critical, at least during the extracellular phases of intracellular facultative pathogens. Antibodies may act by interfering with adhesion, neutralizing toxins and activating complement. Moreover, antibodies may be able to penetrate recently infected cells and bind to the internalised pathogen, increasing the antigen processing (74). It is well accepted that antibodies 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 [74,75], increasing the efficacy of co-stimulatory signals, enhancing Antibody Dependent Cellular Cytotoxicity responses (ADCC) and the homing of immune cells to the lungs after the respiratory infection [13,76-81].

Examples of relevant action mechanisms of antibodies have been discussed by Glatman-Freedman [82].

6. Potential applications

Future applications of antibody formulations for the control of TB may include treatment of patients infected with Multidrug Resistant (MDR) strains, combination with the standard treatment in order to achieve faster therapeutic effects, and administration to recent contacts of TB patients with special attention to risk groups [85].

On the other hand, the induction of specific antibody responses by vaccination in addition to the stimulation of cell mediated immunity could be a novel strategy for the development of new generation prophylactic and therapeutic vaccines against TB.

The prevailing dogma about the uncertain role of antibodies in the protection against TB has somewhat limited the study of B cell immunodominant epitopes which have been mainly related with the development of serodiagnosis assays [86]. Consequently, little information is available on B cell epitopes that could potentially contribute to protection or therapy. 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.

Our group is currently developing a candidate experimental vaccine based on proteoliposomes from *M. smegmatis*. In one study, bibliographic search was used to identify highly expressed proteins in active, latent and reactivation phases of TB [87]. 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 [88] 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 [87]. Thus the *M. smegmatis* proteolipomes 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 [89]. 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 [87,89]. 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 [89]. 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, Ag85B and MTP40 proteins were selected and combined with T cell epitopes of the 85B protein and fused to 8.4 protein [90]. 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 85BAg fused to Mtb8.4 protein and BCG expressing a HSP60 T cell epitope plus different combinations of B cell epitopes from 85BAg, Mce1A, L7/L12, 16 kDa, HBHA, ESAT6, CFP10 and MTP40 and combinations of B cell epitopes alone produced significant reductions in lung CFU compared with BCG (Norazmi MN, manuscript in preparation).

The cumulative works reviewed above related with the use of antibody formulations and vaccines suggest that antibodies if present at the right moment at the site of infection can provide protection against *M. tuberculosis*. This concept opens the way to the development of a new generation of vaccines that elicit specific IgA and/or IgG antibodies able to protect at the port of entry against the infection and directed to bacteria in the infected tissues.

An antibody-based vaccine could be implemented against TB. Such antibodies should recognize the pathogen immediately after its entry into the host, mainly at the mucosal surfaces, where these antibodies must be strategically induced [91]. This vaccine has to induce IgA and IgG antibodies that can inactivate bacterial components essential for the microbial survival in the host, activate complement for direct lysis of the cells, opsonize bacteria to promote their capture by phagocytic cells and induce stimulation of specific cellular immune responses.

Although no serological tests for diagnosis of TB are recommended [92], due to the generation of false results as well as incorrect treatments, for many other pathogens, the availability of serological diagnostic tests has been of great value, in particular in poor countries. In some cases, it constitutes the best protection correlate [93].

In the specific case of TB, several studies of the antibody response have been developed [94]. A number of factors have been described to contribute to the variation of antibody response during the disease. Some of these factors are associated to the pathogen (strain variation,

micro-environment and growth state of bacteria). Not less important are the factors related to the host, mainly the previous exposure to antigen and host genetics [95].

On the other hand, 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 [96,97]. These studies could lead to the discovery of new antigens that may constitute a suitable diagnostic marker as well as to the identification of correlates of protection.

The study of the role of specific antibodies in the defense against tuberculosis is opening new possibilities for the future development of new vaccines, diagnostics and therapies against the disease. It is envisaged 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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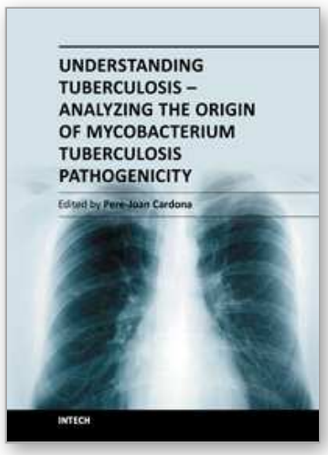

Understanding Tuberculosis - Analyzing the Origin of Mycobacterium Tuberculosis Pathogenicity

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Mycobacterium tuberculosis in an attempt to understand the extent to which the bacilli has adapted itself to the host and to its final target. On the other hand, there is a section in which other specialists discuss how to manipulate this immune response to obtain innovative prophylactic and therapeutic approaches to truncate the intimal co-evolution between Mycobacterium tuberculosis and the Homo sapiens.

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