The defense mechanisms against TB are extremely complex and interconnected and some of them, potentially important, are still unknown

The Friends – Nelson Domínguez
Mixed/paper
(62 x 80 cm)

'…there is no school inside,
it is always the same day,
the same night always,
time has not yet been invented,
the sun has not aged,
it is always and never the same,
it has never rained and it always rains,
everything is happening and never happens.'

A Draft of Shadows (Poem) – Octavio Paz

Nelson Domínguez
Granma, Cuba

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The Role of Antibodies against Tuberculosis

Aharona Glatman-Freedman

Introduction

The role antibodies could play in host-defense against TB has captured the interest of investigators for more than a decade now [reviewed in (1–3)]. Although the scientific and medical literature contain evidence of multiple past attempts to demonstrate a protective role of antibodies (taking place over more than a century), [reviewed in (1–3)] the mixed results of those studies led to the eventual abandonment of the search for protective antibodies. As a result, cell-mediated immune mechanism (CMI) has been traditionally considered the sole immune mechanism against TB. However, the overwhelming prevalence of TB around the world, the need for prolonged and complex therapy together with the emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) MTB strains (4) and the limited effect of the BCG vaccine (5), encouraged investigators to examine novel approaches for vaccine development against TB. With new scientific tools that have become available over the past several decades, scientists set out to re-evaluate the role of antibodies.

The Historical Perspective

Attempts to identify effective antibodies against TB can be divided into several phases: experiments with serum therapy which took place from the late nineteenth century through the beginning of the twentieth century (1, 3), studies with polyclonal antibodies which started in the 1930s and has continued primarily

through the 1990s , [reviewed in (1)] and experiments with monoclonal antibodies (mAbs) which were initiated in the 1990s [reviewed in (3, 6)].

The Use of Serum Therapy against TB

Attempts to develop effective serum therapy against TB occurred in parallel with the successful development of serum therapy against infectious agents such as *Streptococcus pneumoniae*, *Group A streptococcus*, and *Neisseria meningitides* (7). Immune serum against TB was generated by immunizing animals with whole cell mycobacteria, mycobacterial fractions, or mycobacterial culture filtrate [reviewed in (1)]. The resulting sera were administered to animals and humans and most were reported to provide benefit to their recipients [reviewed in (1)]. However, evaluation of those studies has been difficult. Most reports lacked sufficient details about the diagnostic criteria for TB, about the preparation of sera or its administration, or about the inclusion of appropriate controls [reviewed by (1)]. In addition, comparative analysis between the studies presents a particular challenge due to the different antigens and animal species used by various investigators to prepare immune sera [reviewed by (1)].

Reports provided by two investigators, Paul Paquin and Carl Fisch, constituted an exception. The accounts of their experiments were notable with respect to their level of scientific rigour and detail. Fisch conducted animal experiments using immune horse serum (1, 6, 8). Most of his experiments were performed using guinea pigs, where the efficacy of serum administered before, simultaneously to, or after infection was examined (8). Immune serum prolonged survival of tuberculous animals irrespective of time of administration with respect to infection, as compared to control animals. The beneficial effect of the serum was dose-dependent and prolonged survival even when administered as late as 14 days after infection (8).

Paquin conducted experiments in humans at the end of the nineteenth century. His studies, published between 1895 and 1897, were the most detailed human studies done with TB immune serum reported at that period (9–13). Paquin studied the efficacy of immune horse serum on hospitalized TB patients whose diagnosis was confirmed by the presence of bacilli in the sputum, and compared their outcome to hospitalized pulmonary TB patients not receiving immune serum (10, 11, 14). Following two months of therapy, improvement was reported for 82 per cent of the patients as demonstrated by reduction in cough, hemoptysis, sputum bacillary load, and increase in appetite, weight, lung vital capacity, and survival. In addition, all patients treated with serum were alive, six months after the beginning of therapy, compared to more than 30 deaths occurring among controls during the first four months of the study (10). Long-term follow-up of 252 patients treated with Paquin's serum reported an overall good outcome (12), with 86 per cent of individuals showing clinical

improvement (to various degrees), and only 14 per cent mortality. Although no controls were reported in this follow-up study, this mortality rate is significantly lower than the 50 per cent mortality from untreated TB (15).

Although the majority of studies reported during that time did not meet present-day criteria of rigour and detail, [reviewed in (1)] their results were valuable and several common denominators were shared by many of them. Firstly, most studies reported some beneficial effect of serum on the course of TB in humans. Secondly, immune serum appeared to be more effective in cases of early as well as localized TB as compared with patients with prolonged or chronic disease (16–21). Thirdly, long periods of treatment were frequently necessary to achieve a continued effect (10, 11, 16, 22).

Overall, the studies conducted with serum therapy did not demonstrate consistent efficacy against TB. This inconsistency led to controversy about the role antibodies play in defense against TB. Coupled with the negative results reported by Albert Calmette and Edward Trudeau (23–25), leading investigators of their time, the inconsistency helped shape the scientific view that antibody-mediated immunity has limited or no effect on the course of MTB infection.

The Experience with Polyclonal Antibody Preparations

A new era in the study of antibody role against TB started in the 1930s. This new period was marked by the ability to measure serum antibody concentration, for which the technology was available by that time. Several categories of study were noted at that period, namely, serological studies, passive antibody administration studies, and in vitro studies (1). Overall, studies reported during that time period provided mixed evidence regarding the role of antibody-mediated immunity against TB, while interest in CMI against TB gained dominance.

Serological Studies

Serological studies can constitute a valuable tool in the study of antibody role in protection against TB, if a direct correlation is found between the presence of specific antibodies and decreased susceptibility to the development of disease. Multiple studies attempting to identify a correlation between the presence of serum antibodies to MTB and clinical TB were published between the 1930s and the 1980s (with some efforts continuing later on as well). In both animals and humans, some investigators found a correlation between antibody titres to MTB and improved outcome of clinical disease (26–33), while others did not (34–36). Certain studies suggested that target antigens were important, with antibodies to mycobacterial polysaccharide(s) being identified as potentially significant in affecting the course of infection (26, 27, 30, 32, 33). In this regard, antibodies to lipoarabinomannan (LAM) of the IgG class were associated with a protective effect against disseminated TB in one study (27).

Animal Experiments

Specific technology developed in the twentieth century allowed scientists to study the effect of different serum fractions. Certain serum or body fluid fractions were demonstrated to prolong survival or affect the growth of mycobacteria (37, 38). Specifically, human serum gamma-globulin fractions were found to be protective in mice (37). Some reports that did not demonstrate antibody-mediated protection led investigators to speculate that protective antibodies were missing from the sera used (39, 40). Furthermore one study found a disease-enhancing effect of antibodies on infection with *M. bovis* BCG (41).

In vitro Experiments

In vitro experiments are important in that they can suggest potential mechanisms by which antibodies exert their biological effect. In this regard, certain studies have demonstrated enhancement of cellular activity against MTB by immune serum either via promoting phagosome-lysosome function (42), or by enhancing mycobacterial killing (43). Direct antimycobacterial effects of antibody were described as well, namely, agglutination, neutralization, bacteriolysis, and bacteriostasis (44–46). In some of the studies, these antimycobacterial effects were associated with the use of gamma-globulin fraction of immune sera (37, 45, 46). An indirect effect of antibodies directed to mycobacterial polysaccharide was described in one study; the antibodies appeared to bind free polysaccharide and inhibit its immunoregulatory effect (31). Despite these results, overall in vitro studies demonstrated supportive and non-supportive evidence for the role of antibodies in modifying the course of MTB infection to benefit the host [reviewed in (1)].

Tuberculosis in Antibody-Deficient Hosts

Assessing the outcome of infection in antibody production-deficient hosts constitutes an additional approach for evaluating the role of antibodies in protection against MTB. Although defects in humoral immunity have not been associated with TB in humans, studies looking systematically for such associations have not been reported, to the best of our knowledge. Several studies looked at the course of experimental TB in B cell deficient mice. One study demonstrated higher organ CFUs in B cell deficient (μ chain knock-out) mice, challenged intravenously with MTB H37Rv as compared to controls that consisted of non-B cell-deficient mice (47). Despite the higher CFUs in the B cell-deficient animals, mortality rate did not increase (47). In contrast, another study showed no CFU or histopathological differences between aerosol infected (100–1000 bacilli per animal) B cell-deficient-mice and controls (48). A third group of investigators studied the outcome of B cell-deficient mice infected with aerosolized MTB CDC1551 (50–100 bacilli per animal) and reported

reduced CFU in the spleen and liver, and less severe lung granulomatous formation as compared to controls. Naïve B cell reconstitution of these mice before infection prevented these changes, and liver and spleen CFU as well as lung granuloma were similar to those of wild-type mice (49). Administration of immune serum containing antibodies to MTB did not lead to the same effect. The inconsistency between studies using B cell-deficient mice can be explained by some of the shortcomings of the model. If the control wild-type mice make primarily non-protective antibodies, differences between B cell-deficient mice and controls may not be evident. In addition, B cells have several immunological functions aside from antibody production, among them cytokine production and antigen presentation (50). Furthermore, production of small amounts of IgA were reported in some B cell-deficient mice (51).

A recent study examined the specific role of secretory IgA in host-defense against mycobacterial infection, utilizing polymeric IgR (pIgR)-deficient mice. pIgR is expressed at the basolateral surface of epithelial cells and it mediates the active transport of dimeric IgA to exocrine secretions (52), as well as leaving the Secretory Component (SC) bound to the dimeric IgA. The antibody response induced after intranasal immunization with PstS-1, a mycobacterial antigen, demonstrated loss of antigen-specific IgA response in the saliva of the pIgR knock-out mice. These mice were also shown to be more susceptible to BCG and MTB infection than immunized control wild-type mice, as manifested by higher lung CFUs and reduced production of proinflammatory cytokine response such as IFN-γ and TNF-α in the lungs (52).

In conclusion, mouse models with deficiency in antibody production or secretion can be useful in understanding certain roles of the antibody in protection against mycobacterial infection, but study results need to be interpreted with caution. In this regard it has been suggested that variation in experimental results of different studies with antibody/B cell-deficient mice may be due to overlap in the function of immune components and potential differences in host responses (50). Furthermore, these studies do not offer much information regarding the potential effects of augmenting antibody immune responses through active or passive immunization (50).

Monoclonal Antibodies against Tuberculosis: An Overview

Further advances in antibody technology, marked by the development of the mAb selection method in the 1970s (53), permitted the identification of particular homogeneous populations of antibodies that are specific in terms of their target epitope, isotype, affinity, and function and can be produced in large quantities. The study of mAb function against MTB was initiated in the 1990s, promoted by the rise in the incidence of TB around the world (54) and by the controversial efficacy of the BCG vaccine (5). Investigators hoped

to overcome the variable results previously obtained with polyclonal antibody preparations against TB. The working hypothesis was that both protective and non-protective antibodies to MTB could be found (1).

Effect of Monoclonal Antibodies on Various Aspects of MTB Infection

Survival

MAb 9d8, an IgG3 generated against MTB arabinomannan (AM), a surface capsular polysaccharide, (55,56) was the first mAb studied (Table 6.1). Mice infected with MTB Erdman strain coated with mAb 9d8, via the intratracheal route, survived longer as compared to controls receiving an isotype-specific irrelevant mAb (56). The effect was observed in several mouse strains, including mice deficient in MHC class II and IFN-γ (56). Although no reduction in CFU accompanied the prolongation of survival, an enhanced granulomatous response was observed in the lung histology specimens of mice receiving mAb treatment (56). In contrast to the effect of mAb 9d8 on survival, mAb 5c11, an IgM subclass antibody, recognizing both AM and LAM, did not affect the course of infection (56). These data provided evidence, for the first time, that both protective and non-protective antibodies directed to MTB can be identified.

MAb SMITB14 to the AM portion of LAM was also shown to prolong survival (Table 6.1). Prolongation of survival was associated with reduced organ CFU and prevention of weight loss in mice infected intravenously with MTB (57). The mAb was administered intravenously prior to or simultaneously with the infectious inoculum and its effect was dose dependent (57). F(ab')2 fragment of mAb SMITB14 conferred a similar protective effect, indicating that protection was independent of the antibody Fc portion, and that the Fc phagocytic receptor was not involved in the process (57).

Another study demonstrated prolongation of the survival of mice that had been infected intravenously with *M. bovis* precoated with mAb MBS43, an IgG2b directed to MPB83, a surface lipoglycoprotein (58) (Table 6.1). Similarly to the study conducted with mAb 9d8, prolongation of survival was not associated with CFU reduction, but was associated with more preservation of normal lung parenchyma in histopathological specimens of mice treated with mAb MBS43 (58).

The experimental models using mycobacteria precoated with mAb and administered through the intratracheal or intravenous routes were criticized as non-physiological. However, similar methodology was used in the past to establish the benefit of antibodies against *Neisseria meningitidis* and *Streptococcus*

Table 6.1 Effect of mAbs and Polyclonal Antibody Preparation on the Course of Mycobacterial Infection

MAbs	Isotype	Target Antigen	Challenge	Host	Main Biological Effect	Interface with Cell-mediated Immunity	References
MAbs to Polysaccharides							
9d8	IgG3	AM	MTB	Mouse	Prolonged survival	Enhanced granulomatous formation	(56)
5c11	IgM	LAM	LAM	Mouse	Enhanced serum clearance; modified pharmacokinetics		(66)
SMITB14	IgG1	LAM	MTB	Mouse	Prolonged survival Reduced CFU Weight loss prevention		(57)
MAbs to Proteins							
TBA61	IgA	16 kDa α-crystallin	MTB	Mouse	1. CFU reduction – day 9 2. CFU reduction – day 21	Enhanced mAb effect by IFN-γ and IL-4 depletion Enhanced granulomatous formation	(61,62,97)
4057D2	IgG3	HBHA	MTB BCG	Mouse	Reduced dissemination		(69)
3921E4	IgG2a	HBHA	MTB BCG	Mouse	Reduced dissemination		(69)
MBS43	IgG2b	MPB83	M. bovis	Mouse	Prolonged survival	Enhanced granulomatous formation	(58)
Polyclonal Antibodies							
Immune serum (mouse)	poly-clonal	RUTI	MTB	SCID Mouse	CFU reduction		(72)
Immune serum (rabbit)	poly-clonal	Cord factor	MTB	Mouse	Prolonged survival CFU reduction		(73)
IVIG	poly-clonal	various	MTB BCG	Mouse	CFU reduction	No protection in athymic mice	(77–79)

pneumonia (2, 59). Criticism regarding the possibility that antibody-mediated aggregation was responsible for the reduction in CFU was raised as well. However, the lack of differences in CFU between the experimental and the control groups, 24 hours after infection (56), did not support the occurrence of aggregation.

Early Stages of Mycobacterial Infection

In contrast to CMI, which is thought to protect the host after infection had already established by controlling mycobacterial replication, antibodies could potentially have a role in earlier stages of infection.

MAb TBA61, an IgA subclass antibody targeted to the 16 kDa α-crystalline antigen of MTB (60), was studied in a mouse model. Intranasal administration of this mAb resulted in lung CFU reduction nine days after intranasal or aerosol challenge with the MTB (61) (Table 6.1). Although the antibody effect was short-lived (no differences in lung CFU were noted between the antibody-treated and the control mice on day 28 post-infection), (61) this experiment suggests that IgA could provide a protective effect against MTB. Of particular importance is the finding that the antibody can affect the early stages of infection. Furthermore, the study indicated that antibody administration time with respect to infection can be of great importance. Pre- as well as post-challenge mAb doses were required to reach a statistically significant CFU reduction (61).

Progressive Stages of Mycobacterial Infection

A recent study evaluated the role of mAb TBA61 administered 30 minutes prior to intratracheal infection with MTB H37RV on the course of progressive MTB infection in mice (62). CFU reduction and better organized granulomata were observed 21 days after infection, as compared to mice receiving a control antimycobacterial IgA mAb or PBS (Table 6.1) (62).

These results are particularly interesting in that they demonstrate that the same mAb can have a protective effect on different aspects of MTB infection using different models of infection and mAb administration, thus indicating the strong protective qualities of this mAb.

Antigen Clearance

Studies carried out in rabbits in the middle of the twentieth century led Seibert and her colleagues to propose in 1956 that antibodies directed towards mycobacterial polysaccharide may confer protection against mycobacteria through an indirect mechanism (32). Their hypothesis was that antibodies bound to free mycobacterial polysaccharide allowed other natural antimycobacterial 'agents' to apply protective effect against mycobacteria (32). Polysaccharides are important surface components of many pathogens and have been described as

virulence factors (63). In this regard, the surface of MTB was found to contain polysaccharides and polysaccharide-containing fractions (64) and it has been suggested that Mannose-capped Lipoarabinommanan (ManLAM) has a role in the immunopathogenesis of TB (65).

Seibert's theory was examined by using purified ManLAM and mAb 5c11 (an IgM recognizing ManLAM) in a mouse model. Intravenously administered purified ManLAM without mAb 5c11 was found mostly in the spleen marginal-zone macrophages with some detected in the liver. In mice receiving mAb 5c11 intravenously prior to ManLAM administration, significantly faster serum clearance of ManLAM and a modified tissue deposition were observed (66), with redirection of ManLAM to the hepatobiliary system, where bile and bile salts decreased its immunoreactivity (66) (Table 6.1). Thus, this study supports the concept that the antibody can have an effect on mycobacterial polysaccharides and suggests that both the liver and bile salts have a role in the defense against MTB by inactivating mycobacterial polysaccharides.

Although this effect of mAb 5c11 did not correlate with a modification of the overall course of infection (56), the demonstrated effect of the antibody on the antigen clearance and distribution strongly supports the possibility of an indirect contributory effect of certain antibodies on the overall course of mycobacterial infection. In this regard, it is important to remember the immunomodulation effects exerted by mycobacterial fractions such as LAM (65, 67) and the trehalose-6,6'-dimycolate (known also as cord factor) (68). Altering the pharmacokinetics and thus the immunomodulatory effect of one or several mycobacterial fractions by antibody binding could thus contribute to the protection against TB.

Mycobacterial Dissemination

MTB has the capacity to spread to distal organs, primarily the spleen, liver, and central nervous system as well as to lymph nodes. A study by Costello et al., reported in the early 1990s, found a relationship between low serum titre of IgG to LAM and an increase in disseminated TB in young children (27). Two routes of mycobacterial dissemination were described: one through entry into alveolar macrophages and the other through interaction with respiratory epithelial cells (69). Mycobacterial binding to epithelial cells involves the Heparin Binding Hemagglutinin Adhesin (HBHA), a surface-exposed glycoprotein (69). In mice infected with MTB and *M. bovis* disrupted for the *hbha* gene, CFUs were reduced in the spleen but not in the lungs when compared to the wild-type mycobacterial strains (69). These findings thus suggest that HBHA is involved in mycobacerial dissemination. Two mAbs to HBHA, mAb 3921E4—an IgG2a and mAb 4057D2—an IgG (70), were used to assess antibody effect on mycobacterial dissemination. In mice receiving mycobacteria precoated with

either mAb, spleen CFUs were reduced while lung CFUs were comparable to those of controls (69). Hence these results suggest that certain anti-HBHA antibodies impede mycobacterial dissemination (52).

Polyclonal Antibody Studies Revisited

While mAb studies provided proof of principle that protective antibodies against MTB can be found, the question of how to elicit a consistently protective serum antibody response remains unsolved. A recent study re-examined the usefulness of immune serum as part of an effort to develop an effective therapeutic vaccine against TB (71, 72). Immune serum was generated by immunizing mice with RUTI—a vaccine generated for therapeutic purposes (71). RUTI consists of detoxified MTB extracts delivered in liposomes (72). Severe Combined Immunodeficiency (SCID) mice were infected with MTB and treated with chemotherapy for 3–8 weeks, following which they were treated for up to 10 weeks with intraperitoneal injections of immune serum. Mice treated with immune serum demonstrated significant decreases in lung CFU (and smaller decreases in spleen CFU), as well as reduction in the extent of granulomatous response and abscess formation as compared to controls (71). These results suggest that protective serum antibodies can be elicited by vaccine, and that antibodies may be useful as adjunct to chemotherapy.

Another immune serum was generated by injecting trehalose-6, 6'-dimycolate (cord factor)-methylated bovine serum albumin complex (cord factor-MBSA complex) to rabbits (73). Pooled rabbit immune serum containing anti-cord factor antibodies was injected subcutaneously to mice. This passive immunization regimen included daily administration, starting one week prior to challenge, and continued every other day for 30 days after intravenous challenge with high inoculum of MTB H37Rv. Mice receiving immune sera survived significantly longer than controls and had a lower lung CFU (73).

An additional approach attempted by scientists involved the use of intravenous immunoglobulin (IVIG) in animals infected with MTB. IVIG is indicated for use in humans with primary and secondary antibody deficiencies (74). It is also used in a higher dose to treat a variety of autoimmune and inflammatory conditions (75) as well as infectious diseases (76). To evaluate the effect of IVIG on the course of MTB infection, high dose IVIG was administered intraperitoneally to C57BL/6 and BALB/c mice, after intravenous infection (77). Statistically significant CFU reduction was observed in the lungs and spleens of mice receiving IVIG either within 24 hours of infection or within 3–5 days of infection. The difference in CFU increased over time and lasted up to 133 days post-challenge (which is substantially longer than the half-life of

IVIG). This protective effect was dose dependent and was observed also in mice receiving IVIG 18 or 108 days after infection (77). Another group examined the usefulness of IVIG pre-administered intranasally, intraperitoneally, or after pre-incubation, against intranasal inoculation with *M. bovis* BCG, and found significant decrease in lung CFU in all mice groups receiving IVIG as compared to untreated controls (78). Protective effect of IVIG was also found in mice infected intratracheally with MTB. Mice receiving IVIG intranasally 2 hours prior to infection demonstrated a significant decrease in lung CFU as compared to controls (79). CFU differences lasted as long as two months after infection. Results were similar when MTB was pre-incubated with the IVIG before infection (79). The protective effect of IVIG formulation disappeared after absorption with MTB, suggesting that specific antibodies had a role in this protective effect (79).

These studies provide a consistent support for the usefulness of IVIG against TB. Although one of these studies indicated that the protective effect was due to specific anti-tuberculous antibodies (79), a beneficial immunomodulatory effect of IVIG cannot be ruled out.

Antibody-Mediated Mechanisms against MTB

Despite the support provided by studies conducted thus far, for the antibody's role in defense against MTB, the mechanisms by which antibodies mediate protection remain unknown. Antibodies can have multiple functions in promoting host defense against MTB. Based on existing literature, potential antimycobacterial mechanisms appear to be exerted directly or through immunoregulatory changes.

Direct Antimycobacterial Mechanisms

Interference with Mycobacterial Adhesion

Pathogen adhesion to host cells and tissues is a crucial step for establishing an infection. This is a stage where antibodies could potentially interfere and affect the course of infection to benefit the host. In this regard one study demonstrated that ManLAM from *M. bovis* BCG binds to macrophages and granulocytes through serum mannan-binding proteins (80). This binding facilitated a selective granulocyte uptake of mannosylated bacteria. Another study showed the binding of MTB ManLAM to human macrophages through mannose receptors (81), and the inhibition of the mycobacterial adherence by mAb CS-40 to LAM. Additional studies demonstrated that specific antibodies could prevent the attachment of *M. leprae* and *Mycobacterium w* to granulocytes and macrophages (82, 83).

Another intriguing example is provided by the study utilizing mAbs generated to HBHA, a surface glycoprotein that is involved in the binding of MTB to epithelial cells, discussed in the previous section (69). That study, which shows that intranasal administration of mycobacteria coated with these mAbs was associated with reduced dissemination, suggested that the mAbs interfered with mycobacterial dissemination and with the outcome of infection by potentially preventing the adhesion of mycobacteria to epithelial cells.

Toxin Neutralization

Neutralization of damaging 'microbial substances' is a well-described antibody function. 'Mycobacterial substances' that are released during infection could potentially affect the host immune response. Studies with immune sera published in the pre-antibiotic era showed that immune sera protected animals from the toxic effects of mycobacterial antigens (8, 84–87). This protection was most probably due to antibodies contained in the serum, binding to and neutralizing mycobacterial toxins.

The concept of antibody effect on mycobacterial products was supported by the use of mAb 5c11 (an IgM to ManLAM) and ManLAM (66), a surface mycobacterial lipopolysaccharide involved in the immunopathogenesis of TB (65). MAb 5c11 was found to enhance serum clearance of LAM and alter its organ distribution (66). Antibody was also reported to neutralize the toxic effects of cord factor (88, 89), which is known to have immunoregulatory effects on the innate and the adaptive immune systems (68).

Promotion of Phagosome-Lysosome Fusion

Interference with phagosome-lysosome fusion is considered a hallmark of MTB infection (42). A study by D'Arcy Hart showed that phagosome-lysosome fusion was enhanced by the antibody (42). Such a process, if indeed it was to occur in vivo, could potentially enhance the phagocytic-microbicidal function.

Additional Direct Mechanisms

Additional possible direct antimycobacterial mechanism is opsonization. It has not been demonstrated in the case of MTB infection thus far, but multiple examples have been reported with regard to other pathogens (90).

Agglutination of pathogens has been mostly discussed in the context of affecting the accurate evaluation of CFUs; however, since agglutination of mycobacteria could potentially affect the outcome of infection, it should be taken into account as a potential antimycobacterial mechanism. In this regard, mAbs to the MPT51 antigen of MTB were shown to lead to mycobacterial agglutination at certain concentrations (91).

Immunoregulatory Mechanisms

In addition to the direct effect of antibodies on MTB, antibodies may exert immunoregulatory effects. Through such mechanisms, antibodies may be able to bring about changes in the equilibrium of the regulatory elements that control protective immunity, and lead to a new balance, one that is more effective against the mycobacteria.

Effect on Cytokine Expression

Enhancement of cytokine release through Fc receptor cross-linking is a possible antibody-mediated mechanism. TNF-α is an important proinflammatory cytokine. It is involved in the host response to MTB as well as in the immunopathological process of TB. The effect of antibodies directed to PPD on expression and modulation of TNF-α by monocytes was studied systematically (92). TNF-α secretion by PPD-stimulated monocytes from donors with negative PPD test was promoted by heat-inactivated serum obtained from patients with pulmonary TB. (92) The secretion of TNF-α was in direct relationship to the serum concentration of IgG1 to PPD. IgG1 adsorption from the serum was associated with a reduction of TNF-α secretion (92). The same group of investigators also studied monocytes stimulated by MTB-secreted protein fractions. The presence of antigen-specific IgG1 promoted the production of the proinflammatory cytokines TNF-α and IL-6 and the reduced production of the down-regulatory cytokine IL-10 (93). These reports suggest a potential role for antibodies in affecting cytokine release and thus a biological effect. In addition, they imply that antibody subclass and antigen specificity may be important with regard to the effect on cytokine release.

Activation of Complement

Despite the fact that lysis of MTB does not occur by complement, the complement system can be used by this pathogen to enter cells. MTB was reported to enter mononuclear phagocytes through complement receptors CR1, CR3, and CR4, and the complement system has an important role in mycobacterial opsonization prior to entry into cells [reviewed in (94)]. The phagocytosis of MTB is promoted as a result of complement activation, which then leads to bacterial opsonization with C3b and 3bi. In this regard, natural antibody was reported to mediate C3 fixation to phenolic glycolipid-I (PGL-1) of *M. leprae* via the classical complement pathway (95). Another study demonstrated that IgG antibodies directed to LAM, found in humans or rabbits with TB, mediated classical complement activation that was induced by mycobacteria (96). These studies suggest that antibodies can potentially modify the extent and outcome of MTB phagocytosis through the complement system, and as a result alter the fate of MTB infection in a beneficial or detrimental direction.

Additional Immunoregulatory Mechanisms

Additional possible immunoregulatory mechanisms to consider are mediation of antibody-dependent cellular cytotoxicity, as well as enhancement of antigen presentation. The antibodies' effect against MTB may be exerted independently or via promotion and redirection of CMI functions. In this regard, promotion of cellular immunity against MTB by the antibody was previously shown, where antibody-mediated survival prolongation was associated with enhanced granulomatous formation (56).

Although none of the mechanisms mentioned here were proven thus far to have an effect against MTB infection, they were discussed here and are suggested as subjects for future studies.

Interaction between Antibody-Mediated and Cell-Mediated Immunity

The classical scientific thinking has been that antibodies provide protection against extracellular pathogens and CMI provides protection against intracellular pathogens (3). However, several examples indicate that antibodies can work against intracellular pathogens [reviewed in (3)]. Furthermore, accumulating data suggests that the two arms of the immune system can and do interact to affect the course of MTB infection. For example, mAb 9d8 to MTB arabinomannan was shown to exert its protective effect by enhancing granulomatous formation (56), the ultimate expression of orchestrated CMI. MAb TBA61, an IgA to mycobacterial α-crystallin, was recently shown to promote granuloma formation in mice infected intratracheally with MTB (62). In a different model of infection, the effect of mAb TBA61 was extended by the addition of IFN-γ (both administered intranasally) (97). In that study, treatment with IFN-γ three days before, at the time of infection, as well as two and seven days after aerosol challenge with MTB, resulted in the extension of an IgA mAb effect in terms of CFU reduction (lasting four weeks as compared to nine days) and a decrease in granulomatous infiltration into the lungs of mice (97).

Depletion of the Th2 cytokine IL-4 by gene knockout or by neutralizing antibodies was recently shown to reduce the degree of MTB infection as manifested by organ CFU reduction (98). Administration of mAb TBA61 (the IgA to mycobacterial α-crystallin mentioned above) and recombinant IFN-γ intranasally led to a more profound decrease in lung CFU. IL-4 reconstitution reversed the effect of IL-4, both in terms of CFU reduction and in terms of the beneficial effects of the IgA mAb TBA61 and IFN-γ (98). Furthermore, a combined immunotherapy, consisting of intranasal recombinant IFN-γ, intranasal mAb TBA61, and intravenous anti-IL-4 polyclonal antibody, prevented

relapse of disease in mice infected with MTB and treated with isonoazid and rifampin for four weeks (starting two weeks after infection). This protective effect was demonstrated in several strains of mice (99).

Although the beneficial effects of human IVIG against mycobacterial infections (77–79) are thought to be at least in part due to the presence of MTB specific antibodies (79), IVIG antibodies may also affect the balance of cell-mediated regulatory mechanisms controlling the protective immunity against mycobacteria.

Cumulatively, these studies demonstrate that antibody-mediated immunity and CMI can affect each other and thus the overall course of MTB infection. These findings are of paramount importance for the design of novel vaccines and new therapeutic protocols against MTB.

Antibody-Based Vaccines: Possibilities and Obstacles

Intensive discussions about the need for a new vaccine against TB started in the mid-1980s. At that time it became evident that the rise in the incidence of TB worldwide, particularly in the context of HIV coinfection and MDR-TB, called for new control measures. The only vaccine currently licensed against TB is the BCG vaccine, which has been utilized since the beginning of the 1920s. It was shown to prevent disseminated TB in young children, but not pulmonary TB (5), the most prevalent and contagious form of the disease. Efforts made in recent years towards developing a new candidate vaccine against TB explored several possibilities, namely, live attenuated vaccines, subunit vaccines, and DNA vaccines [reviewed in (15, 100)]. The common purpose of these vaccination strategies has been to augment CMI and control the multiplication of MTB after infection is established. This approach is based on the fact that MTB is a facultative intracellular pathogen against which CMI has been thought of as the sole protective immune response. The studies reviewed above suggest that some antibodies can provide protection against MTB. These advances could potentially be utilized for the development of a new vaccine approach which will promote a protective antibody response. Although this concept is contrary to the one that has been accepted for many years with regard to how protection is conferred against MTB, it is important to consider that most licensed vaccines are thought to elicit protective antibody responses against the target pathogen (101). Polysaccharide conjugate vaccines are important examples in this regard because of their efficacy, predictable immune responses, safety profile, lack of virulence in the context of host immunosuppression or revertant vaccine strains, and production consistency. Thus far, licensed polysaccharide conjugate

vaccines include vaccines against *Haemophilus influenzae* type B, *Streptococcus pneumoniae*, *Salmonella enterica* serovar Typhi, and *N. meningitidis* (102, 103). Although polysaccharides are considered poor immunogens that by themselves promote T cell-independent antibody responses, their conjugation to protein carriers results in effective vaccines that promote T cell-dependent antibody responses toward the polysaccharide antigens. The later allows their effective use in young children who do not develop effective T cell-independent antibody responses (104). The polysaccharide conjugate vaccine against the facultative intracellular pathogen, *Salmonella enterica serovar Typhi,* provides an example that this class of vaccines can be potentially effective against other intracellular pathogens.

Studies demonstrating the protective effects of mAbs to AM (and to the polysaccharide portion of LAM) (56, 66), provide evidence that mycobacterial polysaccharide antigens have the potential of eliciting protective antibody responses. Based on these studies, several polysaccharide conjugate candidate vaccines were studied. The vaccine candidates reported thus far contain LAM-derived oligosaccharides (105), the capsular polysaccharide AM, (106) and the capsular polysaccharide glucan (107). Their protein carriers included tetanus toxoid, cross-reactive mutant (CRM197) diphtheria toxoid (105, 108), and *Pseudomonas aeruginosa* Exoprotein A (rEPA) (107, 109). Interesting examples of protein carriers are the MTB antigens 85B or the 75-kDa proteins used in some studies (108), because of the possibility that in addition to their function as protein carriers, these proteins can potentially elicit specific protective immune responses against MTB. The vaccine candidates described here were demonstrated to elicit IgG responses in experimental animals (105–108). Furthermore, the vaccine candidates composed of LAM-derived oligosaccharides were demonstrated to promote survival and prevent weight loss in mice and guinea pigs infected with MTB (mice were infected intranasally or intravenously and guinea pigs were infected by aerosol challenge) (108). Immunization of mice with the AM-rEpA vaccine resulted in CFU reduction seven days following the challenge with MTB or *M. bovis* BCG (106). This CFU reduction was modest and did not sustain beyond the early stages of infection (106). However, it is worth noting that in terms of timing, this effect on CFU comes earlier than the development of CMI (106), suggesting that this type of vaccines deserves further investigation.

The studies with mAbs to HBHA showing mycobacterial CFU reduction in the spleen constitute an example for the potential importance of antibodies to mycobacterial protein antigens (69). Therefore, it may be worth considering also the use of proteins to induce a protective antibody response against MTB. In fact, an HBHA vaccine candidate elicited high antigen-specific antibody response and was associated with lung and spleen CFU reduction

in mice challenged with MTB (69a), an effect that was similar to that of BCG immunization. Thus, the results of these studies suggest that the effect of the HBHA vaccine candidate may be due, at least partially, to the induction of beneficial antibody response.

Another interesting example of a vaccine reported to induce protective antibody response, is the cord factor–Methylated Bovine Serum Albumin (MBSA) complex vaccine (73). Cord factor is a glycolipid virulence factor with toxic activity (89). Mice immunized with cord factor–MBSA vaccine prior to intravenous MTB challenge survived significantly longer than unvaccinated controls, and the protective effect was similar to that of BCG (73). Immune serum generated with this vaccine protected mice against lethal infection with MTB (73). This study suggests that a lipid based vaccine can also induce protective antibodies.

The main goal for generating a vaccine against MTB, that will rely on promoting a protective antibody response, is to prevent disease development, potentially by elimination or containment of the pathogen's inoculums (101) rather than containing mycobacterial multiplication after infection had already established.

Although the classical scientific view has been that a dichotomy exists between the two arms of immune response, with antibody-mediated immunity targeted towards extracellular pathogens and CMI targeted against intracellular pathogens [reviewed in (3)], scientific advances reviewed here suggest that interactions between the two arms of immune response do exist. Thus, it is unclear whether vaccine-induced antibodies work independently or in conjunction with CMI. In this regard, the potential for enhancement of CMI by antibodies was suggested by the improvement of granulomatous formation in mice treated with mAb (56). It is conceivable that CMI may be important in killing those mycobacteria that escape neutralization by antibodies, or that it is responsible for modifying antibody activity. In this context, antibody activity was demonstrated to depend on T cell function (110). Specifically, the addition of the Th1 cytokine IFN-γ (97) and the depletion of the Th2 cytokine IL-4 (111) were both demonstrated to enhance the protective effect of an IgA mAb TBA61 to MTB (97).

In addition to polysaccharide antigens, it is worth considering proteins as immunogens. In this regard, the proteins MBP83 (58) HBHA (69) and 16 kDa α-crystalline antigen (61, 62), against which mAbs were found to have protective effects, are important. The incorporation of immunogenic proteins into a new vaccine preparation, as part of a multi-subunit vaccine which contains both poly- or oligo-saccharides and proteins, is worth considering. The addition of other classes of mycobacterial fractions such as cord factor, which was found to induce protective antibodies (73, 89), should be considered as well.

It is interesting to note that both the proteins and the lipid, discussed here as potential vaccine components, are glycosylated.

In addition to the search of a new vaccine that will prevent TB, the studies demonstrating the efficacy of the therapeutic vaccine RUTI, and the protective antibodies it elicits, suggest that antibody-inducing vaccines may have an important role also after infection had already established (71, 72). These findings highlight additional options worth considering in the fight against TB.

Assessment of Antibody Activity against MTB: Measures and Variables

Multiple variables can affect the function of antibodies [reviewed in (112)] and examining these variables is important in order to gain full understanding of how to utilize antibodies against MTB.

The timing of specific antibody administration may be paramount for the outcome of infection. Co-administration model was used by several studies (56, 58), where mycobacteria were coated with mAbs prior to administration. In other studies beneficial effects were obtained when mAbs were administered 1–3 hours prior to infection (or antigen administration) or 3 hours prior and 3 days following infection (61, 62, 66). Administration of antibodies after an infection had been established was effective thus far in the cases of polyclonal antibody use: specifically the immune serum generated by the therapeutic vaccine RUTI (72) and high-dose IVIG (77).

The presence of antibodies at the time of infection and their delivery methods may be of particular importance. In this regard, a study by Falero-Diaz et al. showed an effective transmission of mAbs to MTB of the IgG1 and IgG2a isotypes from the serum or from a back pack hybridoma cell tumour to the lung (60). In the same study, IgA mAb was not transmitted (60).

Investigators used several parameters for assessing the effect of mAbs on the outcome of mycobacterial infection. Careful examination of the studies done with antibodies reveal important findings worth considering. CFU determination has been classically considered a surrogate marker for the success of an intervention and a correlate for the ultimate prolongation of survival. However, two of the studies reviewed here demonstrated that the use of specific mAbs was associated with prolongation of survival without CFU changes (56, 58). These findings suggest that using CFU as the sole measure for antibody efficacy against mycobacterial infection may be insufficient.

Timing of CFU determination is another key issue in assessing antibody effect. The classical time used for CFU determination has been three to four

weeks after challenge. This time has been used due to the slow growth of MTB and the presumption that CMI is the sole mechanism of protection. However, three studies using mAbs or conjugate vaccines showed reduction in CFUs at earlier time points after infection. In the case of mAb TBA61, an IgA to the MTB 16 kDa α-crystallin, CFU reduction in the lung was detected as early as nine days after challenge (61); the use of mAb SMITB14 to LAM led to *M. bovis* CFU reduction as early as two days postinfection (58); following AM-rEpA immunization, CFU reduction was detected seven days after infection (106). Although prolongation of survival was reported only in one of these studies (58), they demonstrate the need to determine mycobacterial burden at early time points after infection when assessing antibody effect.

Other variables that can be important for the overall effect of antibodies such as isotype, antigen specificity, and antibody dose were extensively explored for *Cryptococcus neoformans* (113–115). With regard to the later, it is intriguing to note that a prozone-like effect was demonstrated where higher antibody dose was not shown to be necessarily more effective (115).

In conclusion, the above studies indicate that multiple variables may be involved in the overall effect of antibodies on the course of infection, all of which deserve careful consideration in future studies.

Effect of Antibodies on Other Intracellular Pathogens

The studies demonstrating beneficial effects of antibodies to the host against MTB occurred over a period of time in which antibodies were also shown to have beneficial effects against a variety of intracellular pathogens in animal models; these include: *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Listeria monocytogenes*, and *Lishmania mexicana* [reviewed in (112)]. In addition, antibodies elicited by vaccines were demonstrated to prevent infections caused by viruses (which are intracellular pathogens) as well as certain intracellular bacteria such as *Salmonella enterica serovar Typhi* (116, 117). Antibodies administered passively, such as Hepatitis B, Varicella zoster, and Rabies immunoglobulins were shown to prevent the development of disease in exposed humans [reviewed in (3)]. PalivizumAb, a humanized IgG mAb to Respiratory Syncytial Virus, was licensed for the purpose of preventing severe respiratory illness in infants born prematurely (118). In the process of re-evaluating the traditional dogma, it is important to realize that as part of their life cycle, intracellular pathogens can be located in the extracellular space, specifically following the host cell death or prior to entering new cells. During this extracellular phase, pathogens are easily accessible to antibodies.

Furthermore, certain antibodies were described to enter cells. These include IgA mAbs directed to the influenza and Sendai viruses (119, 120). In summary, the above mentioned studies provide additional support to the potential importance of antibodies in host defense against MTB.

Conclusion

The data reviewed here demonstrates the progress made in the attempt to understand how antibodies can beneficially affect the course of mycobacterial infections. Despite the fact that the role of natural antibody response on the fate of MTB infection and disease is still unclear, the data indicates that it is possible to generate protective antibodies. More information is required to understand the importance of antibody isotype, affinity, and antigen specificity. In addition, issues of antibody delivery, concentration, as well as the use of more than one antibody need to be examined. The presence of specific antibodies at the appropriate stages of infection, either before the entry of MTB into host cells, or prior to dissemination, may be of particular importance. In this regard, induction of mucosal antibodies or the passage of antibodies from serum to the respiratory mucosa may be imperative as well. The studies demonstrating the role of IgA (52, 61, 97) and the studies detecting IgG mAbs given intravenously in mouse lung fluid shortly thereafter support these concepts (60).

The progress of the past decade supports the quest to develop a vaccine that will induce a protective antibody response. A novel vaccine strategy to combat TB is particularly important considering the ongoing rise of MDR and recently XDR strains of MTB (4), and the continued high prevalence of TB around the world.

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