Antibodies in the protection against mycobacterial infections: what have we learned?

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Abstract

Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis. Despite of the massive global use of BCG, there is a need for other TB vaccines. Newer animal models are needed to test candidate vaccine efficacy to protect animals against challenge with M. tuberculosis virulent strains in more realistic scenarios than currently done. Also, the elucidation of the importance of humoral immune defenses against intracellular pathogens constitutes a priority to improve the rational design of new vaccines. Our group has been actively testing the protective role of antibodies in different models of pulmonary TB infection through evaluation of bacterial loads and morphometric and histological changes in the lungs of infected mice. Results presented here suggest a protective role for antibodies and the humoral response against tuberculosis infection.

Keywords: tuberculosis, mice model, antibodies, 16 kDa protein

1. Introduction

Through all of history, tuberculosis (TB) has been a health problem for humanity. At the beginnings of civilization the disease was probably occasional, but with the increment of population densities, in the XVII-XIXth centuries, it took epidemic proportions [5,7,16].

In spite of the use of the live attenuated vaccine developed by Calmette and Guerin (BCG) and in spite of effective therapy with antibiotics like isoniazid, rifampicin and streptomycin, the number of TB cases has not ceased to increase until our days. The World Health Organization estimates that approximately a third of the world population

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is infected and that about eight million people acquire TB yearly. Without an adequate treatment, over 60% of the people with TB would be condemned to death [36].

In order to implement strategies to increase the effectiveness of BCG vaccine, or to replace it by a more effective vaccine, an important point is to try to elucidate the effector immune mechanisms at work in the fight against intracellular pathogens. For many years, cell-mediated immunity was attributed an exclusive role in the defense against intracellular pathogens. The Th1/Th2 paradigm prevailed for a long time and directed the development of most vaccines. In recent years, many experiments have however challenged this concept. Nowadays, it is well accepted that a combination of both arms of the immune system is optimal for fighting intracellular as well as extracellular pathogens [15].

2. A role for IgG antibodies

With the scientific development of hybridomas technology and the production of monoclonal antibodies, evidence that support a clear role of antibodies in the fight against intracellular pathogens such as fungi, viruses, parasites and bacteria has been accumulating [1]. The availability of new technologies for the study of antibody-mediated immunity and the need for new therapies for the control of re-emergent diseases had pushed forward the discovery of new functions of antibodies, such as their role as direct microbicide molecules [2,29,39] or as interactive and unique effector molecules [38].

In the specific case of \textit{M. tuberculosis}, initial work was developed using anti-arabinomanan (AM), anti-lipoarabinomannan (LAM), anti-heparin-binding haemaglutinin adhesin (HBHA) or anti- Mtb83 monoclonal antibodies [4,12,13,17,18,30]. These antibodies, which are usually able to opsonize mycobacteria, were administered to infected mice by different routes, yielding results such as prolonged host survival, increased serum clearance, diminished mycobacteria dissemination and reduction of colony forming units in the lungs and spleen. The interest of the scientific community in the elucidation of the real role of antibodies mediating protective immunity against TB began to increase, as illustrated in Figure 1.

![Figure 1: Antibodies and \textit{M. tuberculosis}. Data for this graphic were obtained by searches in Medline and PubMed and references from relevant articles.](image-url)
As reported by several authors, antibodies may be critical mainly during the extracellular phase of early disease stages caused by facultative intracellular pathogens. However, antibodies may also be able to penetrate recently infected cells, binding to the internalised pathogen and increasing antigenic processing. It is well accepted nowadays that antibodies can modulate the immune response, activate the secretion of cytokines that contribute to an efficient and rapid Th1 response [20,25], increase the efficacy of co-stimulatory signals, elicit antibody-dependent cellular cytotoxicity and the homing to the lungs after respiratory infection [3,19,24,34].

In 2005, de Valiere et al. reported for the first time that antimycobacterial antibodies stimulate the Th1 response instead of diminishing it, as believed previously [8]. In this study, serum samples obtained from volunteers vaccinated twice with BCG by the intradermal route, were shown to contain significant titers of specific antimycobacterial IgG antibodies against LAM. Moreover, these antibodies significantly increased BCG internalization into phagocytic cells as well as the inhibitory effect of neutrophils and macrophages on mycobacterial growth. Besides, they also induced a significant production of IFN-γ by CD4+ and CD8+ T cells.

3. IgA and Mucosal Immunity

Little is known of the possible role of IgAs in the defense against TB infection. Most studies on the protective effect of antibodies against tuberculosis are based on antibodies of IgG subclasses. During the last years several articles describing the importance of mucosal associated lymphoid tissue in the resistance to infections have however been published [10,14]. The mucosal immune response greatly contributes to the protection against pathogens entering the host at mucosal sites. Secretions found on mucosal surfaces contain significant levels of immunoglobulins, basically IgAs, whose function is to prevent infectious agents such as viruses or bacteria to cross the mucosal barrier. Experimental evidence suggests that the IgA polymeric receptor may also neutralize pathogens and antigens during their intracellular transport from the apical to the basolateral zone of epithelial cells [9,28]. Mechanisms that explain the protective role of IgAs involve microbes agglutination, motility inhibition, binding to bacterial adhesins that prevents binding of the bacteria to the mucosal epithelium, and microbial products elimination through activation of phagocytic cells. Immune exclusion and neutralization of viral infectivity are crucial mechanisms of IgA activity against viral respiratory pathogens [33]. Some IgA antibody isotypes have been associated with protection against M. leprae [6]. BCG vaccination also induces a substantial IgA response and IgA-deficient mice were found to less well control BCG infection [35].

These antibodies, if present at the site and the moment of infection, could modify the course and the outcome of the disease. Passive administration of IgA antibodies to high risk group individuals (such as immunocompromised individuals) could be a realistic immunotherapeutic strategy.

As M. tuberculosis specific antibodies must presumably act at the mucosal surfaces of the respiratory tract, a decisive point is the choice of the administration route selected to evaluate candidate antibodies. At the beginning, intravenous and subcutaneous routes were the more employed, but these routes do not resemble the natural way of infection. In this sense, the aerosol and intratracheal routes of administration are the most physiological routes to study the pathogenicity of the bacteria and the immune response induced in the host [27]. Our group reported for the first time the prophylactic effect of mucosal administration of human gammaglobulins in a model of progressive pulmonary infection with M. tuberculosis in mice. In addition, we demonstrated that incubation of M. tuberculosis with human antibodies could inhibit the bacteria’s infective potential [26]. The protective effect of monoclonal antibodies was also demonstrated in the same model of infection [23].

4. IgA monoclonal antibody against M. tuberculosis 16 kDa protein

M. tuberculosis 16 kDa protein (also called Acr antigen) has been identified as a major membrane-associated protein [22]. Its expression is increased in bacteria growing inside infected macrophages. Our group has been working for several years testing the possible role of IgA monoclonal antibodies directed against the 16 kDa protein in the defense against TB infection [11]. Several experiments have been carried out (Table 1), using different ways of
Table 1: Results from different experimental approaches involving a monoclonal antibody against *M. tuberculosis* 16 kDa protein (TBA61) as appeared in [23,31,3].

<table>
<thead>
<tr>
<th>MAb, delivery route and inoculation regime</th>
<th>Challenge</th>
<th>Days selected for lung CFU reduction</th>
<th>Parameter measured</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBA61 i.n (-3h, +3d, +6d)</td>
<td>H37Rv aerosol</td>
<td></td>
<td>Statistical reduction of CFU post-challenge</td>
<td></td>
</tr>
<tr>
<td>TBA61 i.n (-3h)</td>
<td>H37Rv aerosol</td>
<td>9 days</td>
<td>Non statistical reduction of CFU post-challenge</td>
<td>nd</td>
</tr>
<tr>
<td>TBA61 i.n (+3h)</td>
<td>H37Rv aerosol</td>
<td></td>
<td>Statistical reduction of CFU post-challenge</td>
<td></td>
</tr>
<tr>
<td>TBA61 i.n (-3h, +3d)</td>
<td>H37Rv aerosol</td>
<td></td>
<td>Statistical reduction of the granulomatous area in the lungs of treated as, compared to untreated mice</td>
<td></td>
</tr>
<tr>
<td>TBA61 + IFNγ (i.n) (-3h, -2h, +2d, +7d)</td>
<td>H37Rv aerosol</td>
<td>9, 21 and 28 days</td>
<td>Statistical reduction of CFU post-challenge</td>
<td></td>
</tr>
<tr>
<td>TBA61 i.t (-3h)</td>
<td>H37Rv i.t</td>
<td>24h, 72h, 21 days</td>
<td>Statistical reduction at 21 days post-challenge</td>
<td>Less interstitial and peribronchial inflammation. Well-organized granuloma</td>
</tr>
</tbody>
</table>

Note: i.n: intra-nasal; i.t: intra-tracheal; h: hours; d:days; nd: non determined.

Inoculation and challenge, in order to demonstrate the feasibility of using this IgA as a therapeutic strategy to fight the disease [23,31,37].

Possibly, blocking the Acr antigen with monoclonal antibody TBA61 may interfere with the intracellular growth of the bacteria and limit their cell-to-cell dissemination [32]. This could in part explain the protective character of the antibody. On the other hand, as demonstrated previously, IgAs may interact with Gal-3 (an intracellular binding β-galactosidase lectin), interfering with the interaction of mycobacteria with phagosomal membranes, finally resulting in the diminution of bacterial survival and replication in the phagosoma. In addition, the efficacy of the monoclonal antibody may be reinforced by IFN-γ, a potent pleiotropic stimulator of macrophage functions which is essential for resistance against TB.

Nowadays, vaccines that elicit antibody-based responses are much in favor [21]. These vaccines must induce IgA antibodies that inactivate bacterial components essential for survival in the host, activate complement for the direct lysis of bacteria, and/or opsonize bacteria to promote their capture by phagocytes, neutrophils, monocytes or macrophages.
5. Conclusion

Many questions still remain unanswered at the moment. It is important to try to understand the role of follicle-like B cells in the lungs of tuberculous patients and why, how and when do these B cells act to modulate the inflammatory and cytokine responses of the patients. Their likely relationship with the antibodies present in the mucosal secretions is also very important to elucidate. Better knowledge of these key phenomena could contribute to the rational and effective development of a new generation of effective TB vaccines.

References