Antibody profiles induced by *T. cruzi* in chagasic patients with previous or current exposure to mycobacteria

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Abstract

Since the immune response mounted by the host to a particular microorganism might be influenced by the acquired immunological experience due to previous contact with other microorganisms, we performed a cross-sectional study to explore the pattern of *T. cruzi*-infection related antibodies in *T. cruzi*-seropositive individuals presenting concomitant tuberculosis, or the antecedent of BCG vaccination. Sampled individuals were grouped as follows: patients with Chagas disease, not vaccinated with BCG, who further developed pulmonary tuberculosis; individuals with Chagas disease BCG-vaccinated; and subjects with Chagas disease, presenting neither BCG scar nor tuberculosis disease. Nonvaccinated individuals or without tuberculosis, presented the highest values of anti-PH (p<0.001), anti-FRA (p<0.001), anti-p2β (p=0.0023) and anti-B13 (p<0.001) antibodies. Present findings constitute the first demonstration on the potential influence of concomitant tuberculosis on Chagas disease.

**Keywords:** Chagas disease; tuberculosis; BCG vaccination
It has been suggested that the immune response mounted by the host to a particular microorganism might be influenced by the acquired immunological experience due to previous contact with other microorganism (Oxford et al. 2015; Monack et al. 2004). This fact has been observed by several authors in cases of infections by microbes like *Trypanosoma cruzi* and *Mycobacterium tuberculosis* (Bottasso et al. 2007; Kleinnijenhuis et al. 2014; Kleinnijenhuis et al. 2012).

The first one is the etiologic agent of Chagas disease, which is now considered a worldwide disease affecting approximately 8 to 10 million people. This protozoan parasite is known to exert several *in vitro* immunomodulatory effects either on T lymphocytes or antigen presenting cells (De Arruda Hinds et al. 2001; Morrot et al. 2012; Majumder 1995). Also, individuals with chronic *T. cruzi* infection were found to have depressed delayed-type hypersensitivity reactions when skin tested with classical or new tuberculins (Bottasso et al. 1994).

*T. cruzi* is also able to induce autoimmune responses which may participate in target organ damage. Among reported autoantibodies, anti-p2β and anti-B13 were shown to be associated with metabolic and cardiac disturbances in experimental models and in humans (Cunha-Neto et al. 2011).

On the other hand, infection with *Mycobacterium tuberculosis* or BCG vaccination were shown to promote a phenomenon characterized by epigenetic reprogramming of immune cells, conferring non-specific immune memory to innate immune responses, termed 'trained immunity' (Kleinnijenhuis et al. 2014; Kleinnijenhuis et al. 2012). Several studies indicate
that in addition to the protective effects of BCG on some forms of tuberculosis-TB- (Thuc et al. 1994), this vaccine also develops beneficial effects on some cancers and infectious disorders of children (Krone et al. 2005; Roth et al. 2004; Stensballe et al. 2005).

Given this finding the question arises as to whether the immunological response to a particular infectious insult may be influenced by additional immunological experiences able per se to imprint a distinct profile of the immune surrogates under analysis. As such we explored the pattern of $T. cruzi$-infection related antibodies in $T. cruzi$-seropositive individuals presenting concomitant TB. Cases with the antecedent of BCG vaccination or not, where included for comparison purposes. We included 186 individuals with chronic Chagas heart disease (CCHD), attending at the Clinical Service of the “J. B. Iturraspe” Hospital, Santa Fe (Argentina), aged 51.6±11.9 years who were subjected to a complete clinical evaluation. None of the patients referred gastrointestinal symptoms or showed pathological signs in complementary studies upon abdominal echography. Diagnosis of TB was made by bacteriological examination by staining for acid-resistant and/or culture for $M. tuberculosis$ bacilli in secretions or tissues.

According to the study purposes individuals were grouped as follows: A. 9 patients with Chagas disease not vaccinated with BCG, with concomitant TB; B. 119 individuals with Chagas disease with the antecedent of BCG vaccination, and C. 58 individuals with Chagas disease lacking the BCG scar or presence of TB disease. There were no ages-or sex-related differences among groups. Chagas disease severity was assessed as follows: asymptomatic, CCHD I (n=69); with electrocardiogram disturbances CCHD II (n=67); or with dilated
cardiomyopathy and/or heart failure, CCHD III (n=50). On the other hand, the extension of pulmonary TB involvement was established according del Rey A et al (2007).

Among the 9 individuals with Chagas disease and concomitant pulmonary TB, they had mild (n=6) or moderate (n=3) disease on the basis of chest Rx criteria. All of them were treated with the 6-month anti-bacillary schedule experiencing a favorable evolution. As regards to Chagas heart disease stages, 5 patients with mild pulmonary TB were asymptomatic and the remaining one belonged to the CCHD II group. The three cases with moderate pulmonary TB pertained to the CCDH II group.

Turning to electrocardiogram disturbances, the commonest conduction alterations were left anterior fascicular block with right bundle branch block (n=43), atrial fibrillation (n=21), and complete right bundle branch block (n=18). As regards to ECG tracings in patients with Chagas disease and concomitant TB, four of them had complete right bundle branch block associated with atrial fibrillation whereas the remaining 5 cases had a normal electrocardiogram.

In relation to the T. cruzi-induced antibodies, we assessed the levels of antibodies against constitutive antigens of the protozoan (anti-PH -parasite homogenate- and anti-FRA -flagellar repetitive antigen-) and antibodies able to cross react with human antigen (anti-p2β and anti-B13). The levels of antibodies were expressed as an index consisting of the ratio between the optical density (OD) of the sample and the OD of the negative standard cut off. This index is referred to as IODN (Index of the Optical Density of antibodies in relation to the Negative control). An IODN \leq 1 was considered negative.
All individuals showed positive IODN for anti-PH and anti-FRA; whereas anti-p2β and anti-B13 were positive in most of them, respectively. All the individuals with concomitant TB yielded positive values of auto-antibodies. There was no association between IODN values from any of the tested auto-antibodies with age (Pearson correlation coefficient) or sex distribution (Independent T-test). As seen previously (Vicco et al. 2013), IODN of anti-B13 antibodies was higher in individuals from CCHD group III and was associated with heart failure. Conversely, none of the remaining IODN for auto-antibodies showed relation with the clinical manifestation of Chagas disease (ANOVA test). Concerning patients with concomitant pulmonary TB there were no differences in auto-antibodies levels when comparing according to the presence of Chagas disease symptoms or not (Independent T-test).

Previously we have described that BCG vaccination was related to decreased level of antibodies induced by *T. cruzi* (Vicco et al. 2014). Comparisons of IODN for the four antibodies among the three patient groups revealed no significant difference in IODN of antibodies between group A and B. Although, the latter two groups showed lower levels of anti-PH (p<0.001), anti-FRA (p<0.001), anti-p2β (p<0.001) and anti-B13 (p<0.001) antibodies, respect with the C group (ANOVA test). Figure 1 shows the levels of auto-antibodies by groups.

Present observation is a major finding of this study. Contact with mycobacteria has been recognized as an influential factor in subsequent immune responses given their effects on T lymphocyte maturation and the further production of different cytokine combinations. As being part of the normal commensal bacterial flora and given their immunogenic action,
mycobacteria may play a significant role in the immunological response mounted by an individual throughout his life.

Several studies demonstrated that BCG vaccination protects against some cancers and infectious diseases in children reducing infant mortality (Krone et al. 2005; Roth et al. 2004; Stensballe et al. 2005; Fontanella et al. 2007; Hanekomet al. 2005; Weir et al. 2008). Studies in mice infected with *T. cruzi* subjected to BCG vaccination showed decreased parasitemia during the acute phase and lower heart lesions in comparison with non-vaccinated mice (Bertelli et al 1981). In addition, we have previously described that individuals who have been vaccinated with BCG presented lower levels of antibodies induced by *T. cruzi*, including those with pathological role such as anti-p2β and anti-B13 which cross react to host antigens (Vicco et al. 2014). Confirming and extending former observations, present results indicate that antibody levels against parasite homogenate, FRA, p2β and B13 were lower in patients with TB co-morbidity and BCG-vaccinated cases.

This may be consequence of a phenomenon denominated “trained immunity”, which confer a long-term non-specific immune memory of innate immunity improving its capacity to respond upon reinfections.

To best of our knowledge, present findings constitute the first demonstration on the potential influence of a concomitant TB on Chagas disease, which promote a stimulating background for elucidating the networks of interactions during co-infection with *T. cruzi* and mycobacteria. A long-term follow-up study will help to find out whether chagasic
patients with concomitant *M. tuberculosis* infection or BCG vaccination persist with lower levels of auto-antibodies and a more favorable clinical course of their trypanosomiasis.

5. Conflict of Interest

These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation. There were no conflicts of interests.

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8. References


Figure 1. Levels of IODN of anti-PH (1), anti-FRA (2), anti-p2β (3) and antiB13 (4). Patients were classified as follows: Group A. patients with Chagas disease co-infected with *M. tuberculosis*; Group B. patients with Chagas disease who were vaccinated with BCG; and Group C. individuals with Chagas disease without BCG vaccine or co-infection with *M. tuberculosis*. Patients of the Group C had the highest levels of the antibodies evaluated. Lines represent means ± SD.