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Short Communication

SHORT COMMUNICATION

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

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One sentence summary: The present findings constitute the first demonstration of the influence of concomitant tuberculosis on Chagas disease through stimulation of a networks of immunological interactions.
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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 *Trypanosoma 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. Non-vaccinated 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. The present findings constitute the first demonstration of 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 microorganisms (Monack, Mueller and Falkow 2004; Oxford *et al*. 2015). This fact has been observed by several authors in cases of infections by microbes such as *Trypanosoma cruzi* and *Mycobacterium tuberculosis* (Bottasso *et al*. 2007; Kleinnijenhuis *et al*. 2012, 2014).

Trypanosoma cruzi 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

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lymphocytes or antigen-presenting cells (Majumder 1995; De Arruda Hinds, Alexandre-Moreira and Decoté-Ricardo 2001; Morrot *et al*. 2012). Also, individuals with chronic *T. cruzi* infection were found to have depressed delayed-type hypersensitivity reactions when skin was tested with classical or new tuberculins (Bottasso *et al*. 1994).

Trypanosoma cruzi is also able to induce autoimmune responses that 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*. 2012, 2014). 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 (Roth *et al*. 2004; Krone *et al*. 2005; 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 were included for comparison purposes. We included 186 individuals with chronic Chagas heart disease (CCHD), attending the Clinical Service of the 'J. B. Iturraspe' Hospital (Santa Fe, Argentina), aged 51.6 $\pm$ 11.9 years, who were subjected to a complete clinical evaluation. None of the patients referred to gastrointestinal symptoms or showed pathological signs in complementary studies upon abdominal echography. Diagnosis of TB was made by bacteriological examination involving staining for acid-resistant *M. tuberculosis* bacilli and/or culture of the bacteria in secretions or tissues.

According to the study purposes individuals were grouped as follows: Group A, nine patients with Chagas disease, not vaccinated with BCG, and with concomitant TB; Group B, 119 individuals with Chagas disease and the antecedent of BCG vaccination; and Group C, 58 individuals with Chagas disease lacking the BCG scar or presence of TB disease. There were no age- 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 extent of pulmonary TB involvement was established according to del Rey *et al*. (2007).

Of the nine individuals with Chagas disease and concomitant pulmonary TB, six had mild and three had moderate disease on the basis of chest X-ray criteria. All were treated with the 6-month anti-bacillary schedule experiencing a favorable evolution. With regard to Chagas heart disease stages, five of the 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.

With reference 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 ($n = 18$). Regarding ECG tracings in patients with Chagas disease and concomitant TB, four patients had complete right bundle branch block associated with atrial fibrillation whereas the remaining five 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-parasite homogenate (-PH) and anti-flagellar repetitive antigen (-FRA)) 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, while anti-p2β and anti-B13 were positive in most individuals, 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 a relationship with the clinical manifestation of Chagas disease (ANOVA test). Concerning patients with concomitant pulmonary TB there were no differences in auto-antibody levels when comparing according to the presence or absence of Chagas disease symptoms (Independent T-test).

Previously we have reported that BCG vaccination was related to a 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 Groups A and B. However, 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 than Group C (ANOVA test). Figure 1 shows the levels of auto-antibodies for each group.

The 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. 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 their life.

Several studies demonstrated that BCG vaccination protects against some cancers and infectious diseases in children reducing infant mortality (Roth *et al*. 2004; Krone *et al*. 2005; Stensballe *et al*. 2005; Hanekom 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, Alcantara and Brener (1981). In addition, we have previously reported that individuals who have been vaccinated with BCG presented lower levels of antibodies induced by *T. cruzi*, including those with a pathological role such as anti-p2β and anti-B13, which cross-react to host antigens (Vicco *et al*. 2014). Confirming and extending former observations, the 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 a consequence of a phenomenon denominated 'trained immunity', which confers a long-term non-specific immune memory of innate immunity improving its capacity to respond upon reinfections.

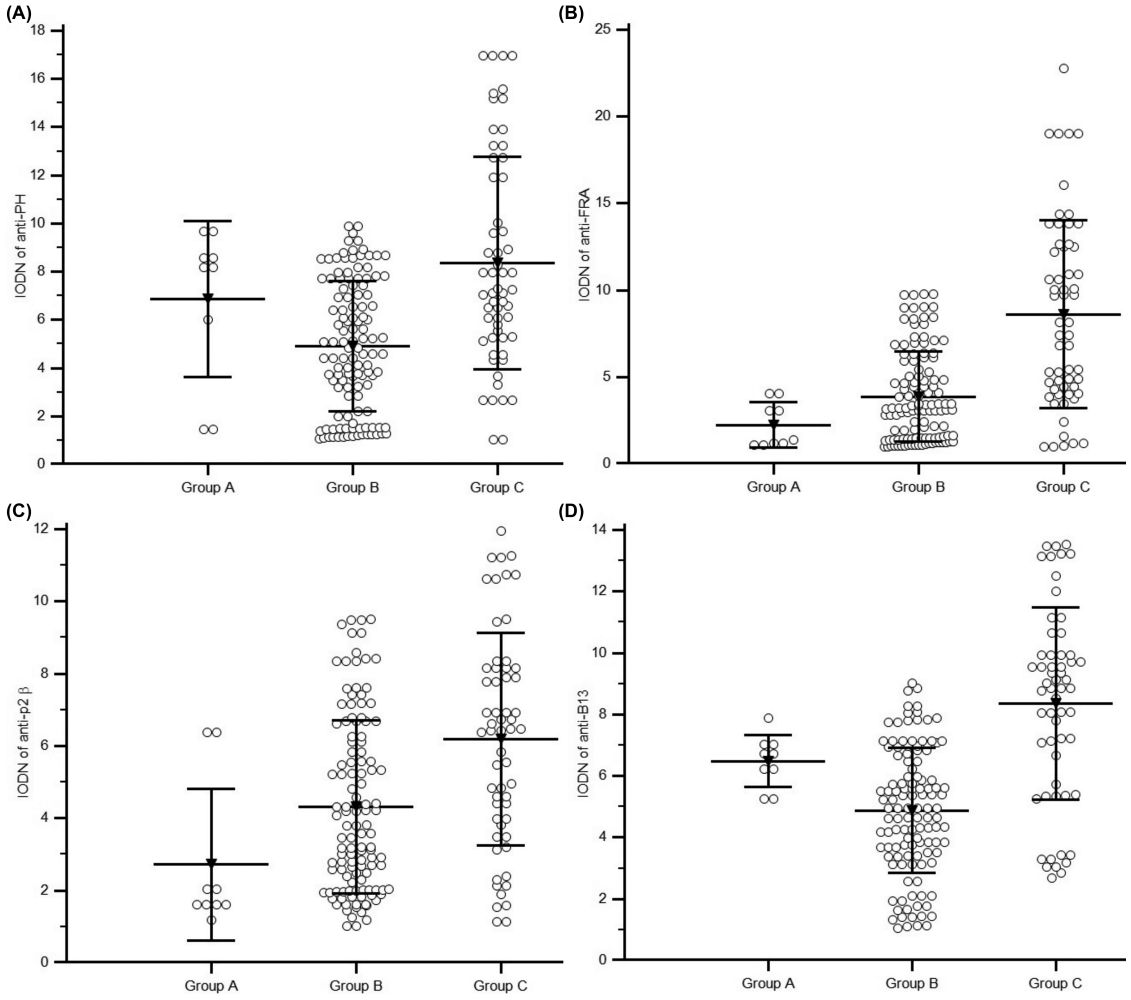

Figure 1. Levels of IODN of anti-PH (**A**), anti-FRA (**B**), anti-p2β (**C**) and anti-B13 (**D**). Patients were classified as follows. Group A: patients with Chagas disease co-infected with *M. tuberculosis*; Group B: patients with Chagas disease who were vaccinated with BCG; Group C: individuals with Chagas disease without BCG vaccine or co-infection with *M. tuberculosis*. Patients in Group C had the highest levels of the antibodies evaluated. Lines represent means ± standard deviation.

To the best of our knowledge, the present findings constitute the first demonstration of the potential influence of concomitant TB on Chagas disease, which promotes 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 continue showing lower levels of auto-antibodies and a more favorable clinical course of their trypanosomiasis.

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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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