

Invited Review

# Parasite polyclonal activators: new targets for vaccination approaches?

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## Abstract

Taking into consideration that the immune response following infection promotes the expansion of lymphocyte clones that are essentially non-specific, ensuring both parasite evasion and persistence inside the host, what would be the major consequences of this polyclonal response to the development of immunopathology? We favor the hypothesis that the polyclonal B cell responses triggered by the infection is responsible of the host susceptibility and is a major contributor to the maintenance of a progressive disease. In particular, the activation of B cells by parasite mitogens would contribute to the class determination of T cell responses and to the inhibition of macrophages - target cells for parasite multiplication and also responsible for parasite clearance. We also envisage that the activation of T cells by parasite 'super-antigens', and the ensuing energy and deletion of these cells, processes that are frequently observed, would contribute for the immunosuppression as well as to parasite escape and persistence in the host. We had concentrated our efforts on the study of the non-specific aspects of the immune response following *Trypanosoma cruzi* infection. We aimed at finding new strategies to modulate and control the mechanisms leading to both the immunosuppression and the development of chronic auto-immunity leading to rational vaccine approaches against parasite infection and immunopathology. © 2001 Australian Society for Parasitology Inc. Published by Elsevier Science Ltd. All rights reserved.

**Keywords:** Polyclonal lymphocyte activation; Mitogens; *Trypanosoma cruzi*

## 1. Rationale

We had previously shown that the main characteristic of the mouse and human infections with *T. cruzi* is the induction of a massive lymphocyte polyclonal activation involving all major and minor lymphocyte B and T cell subsets (Minoprio et al., 1986a,b). We had confirmed that B- and T-cell activation is indeed polyclonal by the molecular analysis of B (BCR) and T (TCR) cell repertoires (Leite de Moraes et al., 1994; Minoprio et al., 1989a). An important feature of this response is the preferential activation of CD5 + B and  $\gamma\delta$  + T cells (Minoprio et al., 1989b,c). These cells, closely related in ontogeny are associated with auto-immune disorders (Marcos et al., 1988).

A major and dramatic aspect of Chagas disease is the development of chronic active myocarditis after a prolonged asymptomatic infection. Studies in human and experimental models highlight the importance of auto-immune mechanisms in the pathogenesis of Chagas disease. Additionally, the 'panclonal' activation described above is responsible for the hypergammaglobulinemia observed during the acute and chronic phases of the disease. We showed that the B

cells activated by the infection are in their great majority (98%) responsible for the production of antibodies that lack parasite specificity, in agreement with the polyclonal nature of the B cell response (Minoprio et al., 1988). It would also seem that B and T cell polyclonal activation following *T. cruzi* infection are at the origin of the humoral and cellular immunosuppression towards homologous and heterologous antigens (Minoprio et al., 1989c). Furthermore, the B cell polyclonal activation following the infection includes the expansion of auto-reactive B cell clones that may play a key role in the physiopathology of the infection and can be involved in late developing auto-immunity (Minoprio et al., 1988; Spinella et al., 1990). Thus it is frequently hypothesised that auto-immunity arises from cross-reactivity between host and parasite antigens or from the expansion of anti-self clones generated during non-specific polyclonal activation of the immune system (Eisen and Kahn 1991; Ribeiro-dos-Santos and Pirmez, 1991). However, the development of auto-immune tissue damage in late phases of Chagas disease may well result from a combination of polyclonal (non-specific) and parasite-directed (specific) immune responses (Reina-San-Martin et al., 2000a; Tarleton et al., 1997).

We believe that the non-specific polyclonal activation of

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the immune system induced by parasite mitogens and superantigens may play an important role in the precocious phases of infection by enlarging the repertoire of B and T cells reacting, for instance, to ‘self’ molecules and thus contributing to tissue damage. We cannot rule out the expansion of lymphocyte clones that are specifically directed against parasite antigens that are also able to cross react with host molecules, i.e. ‘self’ (molecular mimicry). This hypothesis, although not excluding the polyclonality of the immune response and its consequences, would have a distinct and significant additional effect on the breakdown of tolerance to self antigens and allow the development of organ-specific auto-immunity in the chronic phases of infection. In Chagas disease, heart auto-immune pathology results from a progressive and time-dependent destruction of cardiocytes culminating in myocardium failure, thus the analysis of the immune mechanisms involved with the triggering of auto-immunity is very attractive and deserves attention.

## 2. Polyclonal activation is a general immunological feature following the infectious process

Mitogens and superantigens have been described to explain the strategy used by micro-organisms to avoid the host specific immune responses and to ensure persistence. These moieties are responsible for the initiation of non-specific (polyclonal) immune responses. The importance of polyclonal responses following infections can be evaluated by the degree of disturbances presented by the infected host, namely splenomegaly, adenopathy, immunosuppression, toxic shock syndromes, granuloma formation, progressive auto-immunity.

The complex antigenic composition of micro-organisms might be thought as the main cause of the hyperstimulation of the immune system thus leading to the expansion of lymphocyte clones specifically directed to the multitude of challenging antigens. However, a better control of infection should then be expected in the initial phases of infection. Instead, the molecular and functional analysis of B- and T-cell repertoires have indeed shown that the indiscriminate utilisation of B- and T- cell genes encoding BCR and TCR is incompatible with oligoclonal responses. We have then proposed that the difficulties faced by the immune system to eliminate micro-organisms may rely on the fact that parasites, viruses, bacteria and fungi trigger non-specific polyclonal B- and T- lymphocyte responses and ensuring evasion through avoiding efficient control of the beginning of the infection. We have summarised the major alterations of the immune system functions following the infectious process and considered new alternatives for vaccination approaches (Reina-San-Martin et al., 2000a). We took into account that classical vaccination approaches have focused on the study of ‘immunodominant’ or ‘immunopathological’ epitopes, and that the great majority of the immunolo-

gically relevant interactions occurring after infection are not limited to specific immune responses to these epitopes. We have then proposed that a better goal to provoke immunity should necessarily include: (a) Studies to elucidate of the mechanisms used by micro-organisms to trigger polyclonal responses, (b) Isolation of the moieties responsible for this triggering and (c) Neutralisation of these molecules and activities in the host or their inactivation in the micro-organism.

## 3. A B-cell mitogen of *T. cruzi* is the first eukaryotic proline-racemase

We have previously shown that the abrogation or reduction of polyclonal lymphocyte activation leads to an increased resistance to *T. cruzi* infection (Minoprio et al., 1991, 1987; Santos-Lima and Minoprio 1996; Santos-Lima et al., 2001). Given that the parasite can release B cell mitogenic proteins (Cordeiro da Silva et al., 1998; Montes et al., 1999), we looked for new molecules secreted by metacyclic infective forms that could be responsible for such a mechanism of immune evasion.

Using biochemical and molecular approaches we isolated from culture supernatants of *T. cruzi* cultures, a parasite protein (TcPA45) involved in the polyclonal activation of B lymphocytes (Reina-San-Martin et al., 2000b). We obtained the full sequence of the gene encoding TcPA45 and its flanking regions. By the analysis of the genomic organisation and transcription of the Tc45 gene we showed the presence of two gene copies per haploid genome and that Tc45 mRNA is present in the different parasite forms. By immunofluorescence and western blotting we showed that the parasite can differentially express intracellular and secreted forms of the TcPA45 protein. The analysis of the Tc45 gene revealed the presence of a signal peptide indicating active secretion by *T. cruzi*. Interestingly, we found an alternative trans-splicing signal about 170 bp upstream of a second ATG codon within the coding region that suggests that, if used, would allow for the expression of a truncated protein lacking 69 amino acids that would not be secreted. In fact, we detected a isoform of the TcPA45 protein of around 39 kDa in epimastigote (non-infective) form of the parasite and a 41.5 kDa protein in infective metacyclic forms. The computer analyses of the TcPA45 sequence indeed predicted Mr of 43.4 and 39 kDa for a secreted and a non-secreted form of the protein.

The comparison of the nucleotide and peptide sequences of TcPA45 with several data bases revealed a striking homology with the only proline racemase described, an homodimeric protein isolated from *Clostridium sticklandii*. This enzyme catalyses the interconversion of L- and D-proline enantiomers. The active site of the bacterial proline racemase was previously identified and is conserved in the TcPA45 protein. We then produced a recombinant TcPA45 protein by overexpressing the gene in *E. coli*. By in vitro and

in vivo proliferation assays we showed that rTcPA45 is indeed a T- cell independent B- cell mitogen. By biochemical assays we also showed that TcPA45 is indeed a proline racemase that racemises both L- and D- proline and not any other amino acid. Furthermore, the TcPA45 mitogenic activity is abolished, or severely compromised whenever enzymatic activity is inhibited by specific inhibitors or substrates (L- or D- proline). We presented evidences for the linkage of both activities of TcPA45 and showed that the integrity of the enzyme active site is necessary to allow mitogenicity.

To our knowledge, this is the first report of a eukaryotic amino acid racemase gene. The implications of our findings to *T. cruzi* biology and pathogenicity, as well as to the possible role of D- amino acids in immune phenomena were discussed in our paper (Reina-San-Martin et al., 2000b).

#### 4. Towards new vaccination approaches

There is no effective treatment or vaccine against *T. cruzi* infection and its consequent pathology. In order to validate our hypothesis that the reduction of immune activities could lead to a re-orientation of the immune system and consequently to a better control of infection we tried to use TcPA45 for immune intervention. Our aim was to induce specific responses against TcPA45 and neutralise its mitogenic activity.

In one of the protocols we used intramuscular DNA ‘vaccination’ containing the TcPA45 gene. We showed that ‘immunoprotection’ is certainly possible, as we obtained 85% decrease in parasitaemia levels after challenge with infective forms of the parasite. Moreover, even higher levels of parasitaemia control have been observed when sub-mitogenic doses of the protein were injected. Thus, specific B cell responses against mitogens is possible if low (‘antigenic’) doses of mitogens are used (Coutinho et al., 1974). We observed that the i.p. injection of low doses of rTcPA45 protein was able to decrease up to 95% the parasitaemia of mice after challenge with a lethal inoculum of *T. cruzi*. We showed that both protocols of immunisation were able to trigger specific B cells and high levels of antibodies anti-rTcPA45 were detected in sera.

These preliminary results suggest that mitogenic proteins can be used as ‘vaccines’ or ‘drug targets’: neutralisation of these proteins could abort this particular parasite strategy that seeks to deviate immune responses into a non-specific activation on the immune system and immunosuppression. A better and systematic comprehension of common mechanisms used by microorganisms to induce polyclonal activation, such that microbes can evade and persist in the host may open the way to new vaccination and therapeutics strategies. Our work may stimulate further research on the biological role of mitogenic molecules and their implications in immune phenomena.

#### 5. Summary

Our goal is to elucidate the events that trigger polyclonal activation of lymphocytes and contribute to autoimmune chronic pathology which is characteristic of Chagas disease. In so doing we may reveal general principles involved in the development of autoimmunity following infections. We will determine if both polyclonal and oligoclonal parasite-induced immune responses of specific lymphocyte populations are needed for the establishment of pathology. We aim to identify the parasite molecules responsible for this process. This study will improve our knowledge of the interactions of the host’s immune system with the parasite and may lead to a rational strategy to prevent the development of autoimmunity in general.

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