

A B-cell activator in Chagas disease

Identification of a secreted proline racemase as a *Trypanosoma cruzi* B-cell mitogen provides mechanistic insight into chronic Chagas disease as well as a potential new drug target (890–897).

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CHAGAS DISEASE IS a debilitating condition that has a considerable impact on public health in Latin America. Disease pathology seems to be predominantly a consequence of interactions between the causative agent, the protozoan *Trypanosoma cruzi*, and the host immune system¹. In infected individuals, *T. cruzi* elicits polyclonal lymphocyte activation². This type of response is a widespread feature of many viral, bacterial and parasitic diseases and is thought to constitute a non-specific immune evasion strategy. It has also been linked with the immunosuppression and pathology associated with the chronic form of Chagas disease. In the current issue of *Nature Medicine*, Reina-San-Martin *et al.* report the discovery of a *T. cruzi* antigen involved in polyclonal B-cell activation³.

In Latin America, 20 million people are infected with *T. cruzi*. This parasite is spread by Reduviid bugs that live in cracks and holes of substandard housing. Insects contract the parasite after biting an animal or person already infected (Fig. 1). The parasite is spread to humans when an infected bug deposits feces on the skin, usually while the person is sleeping at night. Individuals often accidentally rub the feces into the bite wound, an open cut, the eyes or mouth. Drugs currently used to treat Chagas disease have toxic side effects and limited efficacy.

T. cruzi parasites have a wide host cell range, including macrophages, muscle cells of the heart, and digestive tract. Cardiomyopathy is the most common clinical manifestation in chronic disease (see Box), appearing in 20–40% of diagnosed individuals. The prognosis for these patients is usually poor.

The mechanisms underlying the chronic disease pathology are not fully understood. *T. cruzi* infection induces non-specific polyclonal B-cell and T-cell responses as well as hypergammaglobulinemia. In an attempt to identify the precise stimuli that trigger the proliferative re-

sponse, Reina-San-Martin *et al.* expanded on previous work demonstrating polyclonal B-cell activation by *T. cruzi* antigens⁴. They first established the presence of B-cell mitogens in culture supernatants of differentiated metacyclic trypomastigotes⁵.

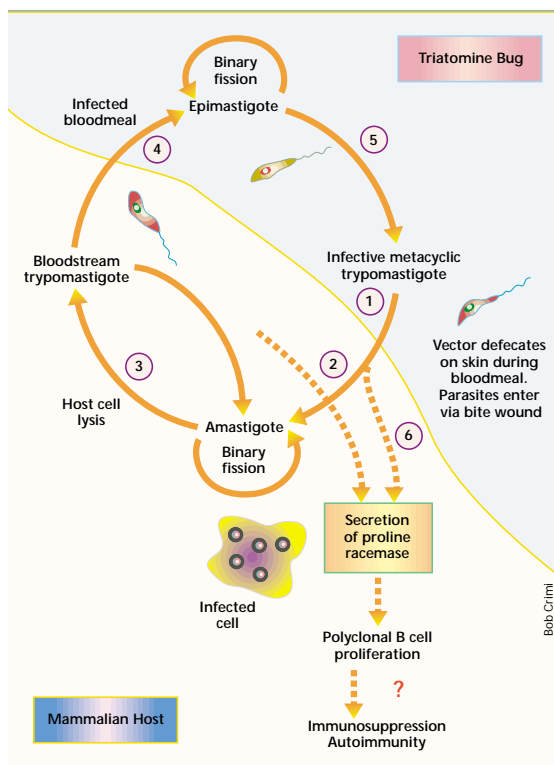


Fig. 1 Life cycle of *Trypanosoma cruzi*. *T. cruzi* can be transmitted by more than 100 species of insects of the family Reduviidae (subfamily Triatominae). Most commonly, parasites enter the bloodstream after contamination of the bite wound with insect feces that contain metacyclic trypomastigotes (red parasite, 1). These can then invade a wide range of cells including macrophages, heart muscle and nerve tissue, where they transform into ovoid amastigotes and undergo replication by binary fission (2). These differentiate into bloodstream trypomastigotes that are released after lysis of the host cell (3). Circulating trypomastigotes can invade other cells or be taken up by the triatomine vector during a bloodmeal (4). In the insect midgut, the parasites differentiate into epimastigotes (green parasite), which multiply and migrate to the insect's hindgut. Here, they transform back to metacyclic trypomastigotes (5). Reina-San-Martin *et al.* have now shown that a proline racemase, secreted by the trypomastigote forms, is capable of triggering polyclonal B-cell activation in the host (6). The extent to which this influences the immunosuppression and/or autoimmunity that characterises Chagas disease can now be tested experimentally using genetically modified parasites.

otes³, the form of the parasite that initiates infection (Fig. 1). The authors purified the mitogen, which they named TcPA45, for *T. cruzi* polyclonal activator 45-kDa³. Cloning and sequence analysis of TcPA45 showed that it encoded a secreted proline racemase – an enzyme that catalyses the interconversion of the L- and D- forms of this amino acid. *In vitro* proliferation assays showed that the proline racemase was capable of activating B-cell proliferation and polyclonal antibody production.

This is the first time that a proline racemase has been found in a eukaryote. Localization studies indicate that the enzyme is predominantly cytosolic in the non-infective epimastigote stage of the life cycle (Fig. 1), whereas in the bloodstream forms, it is readily detectable on the parasite membrane³. Most interestingly, in the context of drug design, the authors show that the mitogenic properties of TcPA45 require that the enzyme remains active. A second related gene is also present in the parasite genome, although detailed characterization is not presented in this report.

The ability of *T. cruzi* to stimulate uncontrolled proliferation of B- and T-cell clones, some of which may be autoreactive, may underlie chronic Chagas disease. There exists a *T. cruzi* epitope that mimics mammalian heart and neuronal tissue⁵, and events that suppress or inhibit polyclonal lymphocyte activation have been shown to limit disease-mediated tissue damage⁶. However, a direct link between molecular mimicry and chronic Chagas disease has not been established.

Several recent reports indicate that the continued presence of the parasite is associated with the chronic disease process. Most notably, studies in mice have shown that parasitic infection of the heart is both necessary and sufficient for the induction of cardiac tissue damage⁷. These observations indicate that drugs targeted directly at the parasite have potential to reduce disease severity

Symptoms of Chagas disease:**Acute:**

The most recognized symptom of acute Chagas infection is swelling of the eye on one side of the face, usually at the bite wound or where feces were rubbed into the eye. Other symptoms may include fatigue, fever, enlarged liver or spleen, and swollen lymph glands. Sometimes, a rash, loss of appetite, diarrhea and vomiting occur. In infants and in very young children with acute Chagas disease, swelling of the brain can develop, leading to death. In general, symptoms last for 4–8 weeks and then they go away, even without treatment. Acute symptoms only occur in about 1% of cases and most infected people do not seek medical attention.

Indeterminate:

Within 8–10 weeks after infection, people no longer show symptoms of infection.

Chronic:

People may develop the most serious symptoms of Chagas disease 10–20 years after infection. Cardiac problems such as enlarged heart, altered heart rate or rhythm, heart failure or cardiac arrest are symptoms of chronic disease. Chagas disease can also lead to severe constipation or problems with swallowing. In persons who are immune compromised, such as persons with HIV/AIDS, Chagas disease can be severe. Approximately 30% of infected people will develop the chronic symptoms of Chagas disease.

and/or progression.

The study also raises as many questions about the biological functions of the racemase. The enzyme may be involved in energy metabolism, as *T. cruzi* can utilize

L-proline as an energy source and it has been postulated that this may occur through a D-proline intermediate. Another possibility is that the racemase activity may facilitate the synthesis of parasite proteins containing D-proline. This has been shown to confer resistance to proteolytic cleavage and, in the case of surface proteins, this property may act as a protective mechanism against host proteinase activity.

How does the racemase initiate non-specific B-cell activation? The data presented indicate a requirement for enzymatic activity, although it is not yet clear whether the activity itself has a direct function in immune activation. Alternative mechanisms, not necessarily mutually exclusive, are that the racemase triggers activation by binding to B-cell surface receptors or that it acts indirectly to modify host proteins, which themselves function as mitogens. An obvious next step in the investigation of the mitogenic properties of TcPA45 will be to determine the phenotype associated with genetically transformed parasites that have been manipulated to overexpress racemase activity, or in which the *TcPA45* gene has been disrupted by targeted integration.

There is a strong case for supporting the development of interventions to alleviate the symptoms of chronic Chagas disease. Even if all transmission could be blocked today, the large reservoir of infected individuals would mean that the disease would remain as a major public health problem for the next 40–50 years. The proline racemase represents a prime candidate

for either drug or vaccine design. The mitogenic properties are dependent on an active enzyme, and the absence of an endogenous racemase activity in the animal host indicates that chemotherapeutic approaches may be a viable strategy. Alternatively, vaccination targeted at the secreted racemase may act to reduce non-specific polyclonal activation and immunosuppression.

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A rare find – cells that improve bone marrow transplantation

A rare population of bone marrow-derived 'facilitating cells' promote hematopoietic stem cell engraftment without causing graft-versus-host disease, but little is known about the identity of these cells. A new facilitating cell surface marker will make them easier to study and may lead to improvements in bone marrow and solid organ transplantation (904–909).

THE SUCCESS OF allogeneic hematopoietic stem cell engraftment across major histocompatibility complex barriers can be promoted by passenger cells that are carried along in the donor bone marrow¹. Determination of the precise identity of these facilitating cells is a central issue in bone marrow transplantation. In this issue, Schuchert *et al.* describe a new T-cell receptor (TCR)-associated protein on a rare population of facilitating cells². Expression of this protein correlates with enhanced allogeneic stem cell engraftment.

Facilitating cells in the bone marrow are traditionally identified by a set of cell surface markers and isolated through multipa-

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parameter flow cytometric cell sorting. The facilitating cells studied by Schuchert *et al.* express several conventional T-cell components (such as TCR β , CD3 ϵ and CD8 α) and require RAG1, the enzyme that mediates TCR gene rearrangement, for their development. Interestingly, these unusual cells also express a previously unknown 33-kDa cell surface glycoprotein (FCp33) that is directly associated with TCR β and CD3 ϵ . Expression of the TCR β /CD3 ϵ /FCp33 trimolecular structure is correlated with their facilitating function.

Several other types of facilitating cells have been previously identified. One type resides in the bone marrow and has phenotypic characteristics of lymphoid dendritic cells³. In contrast to the facilitating cells described by Schuchert *et al.*, these cells require CD8 α , but not CD3 ϵ or TCR β , for their development and facilitating function.

How can different cell types show such functional similarities in promoting stem cell engraftment? One simple explanation is that facilitating cells are highly heterogeneous. These cells are traditionally identified by the presence of various cell surface molecules, and some of these molecules are