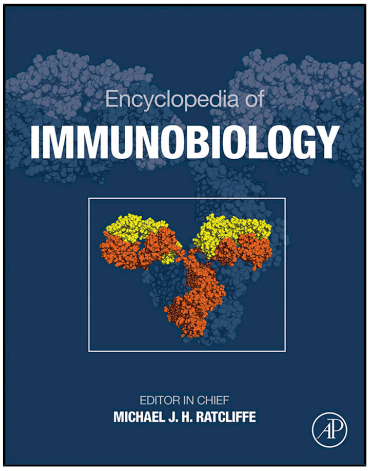

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Immunity to *Trypanosoma cruzi*

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Abstract

The protozoan *Trypanosoma cruzi* infects many different hosts and host cell types within those hosts. Chronic infection in humans can culminate in Chagas disease, a result of parasite-dependent damage to the heart and other tissues wherein *T. cruzi* persists. Immune control of *T. cruzi* is quite good, involving highly potent and effect humoral and cellular immune responses that contain, but usually fail to eliminate this systemic infection. Our understanding of how *T. cruzi* evades immune control is still evolving, but likely includes among other mechanisms, the modest triggering of pathogen sensors in and on host cells during the invasion process and the expansion of multigene families that present a confusing set of targets for the immune response.

Introduction

Trypanosoma cruzi is a protozoan parasite and the agent of human Chagas disease. Chagas disease is the highest impact infectious disease in Latin America and the most common cause of infectious myocarditis in the world (Feldman and McNamara, 2000). Although human Chagas disease is a huge public health problem, humans are in fact only incidental hosts for *T. cruzi*. *Trypanosoma cruzi* is very widely distributed in many wild and domestic mammals and thus will never be eradicated. However, there is one major benefit to zoonotic infections like *T. cruzi*: the many hosts that *T. cruzi* infects besides humans, including rodents, canines, and nonhuman primates, make outstanding models for studying the immunology of *T. cruzi* infection, providing highly reliable information relevant to the human infections. Descriptions of the immune response to *T. cruzi* are all over the map, from suppressed and ineffective to overexuberant and disease promoting. However, the wealth of evidence shows that immunity to *T. cruzi* is generally highly effective, resulting in excellent control of parasite load and, occasionally, complete pathogen clearance. Unfortunately the consequence of the more common 'control without elimination' outcome is the cumulative tissue damage and increasing chances of clinical disease that become more severe with increasing length of infection. The mechanisms associated with effective control of *T. cruzi* infection are discussed more fully below.

Infection and Life Cycle

Trypanosoma cruzi is predominantly transmitted to mammals by triatomine insects commonly inhabiting nests and burrows of animals living in a region from approximately the middle of North America to the southern aspects of South America (**Figure 1**). These solely blood-feeding insects become important vectors of human infection due to their colonization of poor quality houses (Cohen and Gurtler, 2001). The insects acquire *T. cruzi* infection by feeding on a parasitemic host (host with parasites circulating in the bloodstream) and transmit to new hosts, not via blood bites as in the case of many vector-borne infections but by defecating after feeding, depositing *T. cruzi* on potential victims. These parasites may then enter hosts through breaks in skin or mucous membranes. Oral infection in humans and other animals via accidental or intentional ingestion of infected insects, parasite-contaminated insect feces, or insects crushed in foods or beverages is becoming appreciated as a major infection mechanism (Benchimol Barbosa, 2006). Infections in humans can also occur congenitally or by blood or tissue transplantation, making the spread of *T. cruzi* a threat even in areas where the insect vectors are absent (Gascon et al., 2010; Schmunis, 2007). There are more than a dozen triatomine species thought to transmit *T. cruzi*, and past programs to control vector populations within houses have been highly effective in reducing transmission in several countries (Dias et al., 2002). Nevertheless, vector-borne transmission of *T. cruzi* still persists in much of Latin America (Gurtler et al., 2007; Schofield et al., 2006) and development of insecticidal resistance (Picollo et al., 2005), an unsurprising outcome of the long-term and widespread use of insecticides, potentially compromises the future effectiveness of this control method.

In mammals, flagellated trypomastigotes circulate briefly in the blood but are nonreplicative; expansion of the parasite population is dependent on the invasion of and replication within host cells. Trypomastigotes enter a range of different host cell types initially within a phagocytic vacuole, but quickly escape from this compartment, lose their flagella via a process of asymmetric cell division (Kurup and Tarleton, 2014), and replicate in the cytoplasm as amastigotes. During a 4- to 5-day period the cytoplasmic amastigotes go through eight or more rounds of binary fission, producing hundreds of progeny. As the host cell fills, becoming essentially a 'bag' of parasites, an unknown trigger stimulates the conversion of amastigotes back into trypomastigotes, which mechanically lyse the host cell, spilling the parasites into the extracellular space. The free trypomastigotes then either reinvade other host cells close by, or spread to more distant sites via the bloodstream or other interstitial fluids. Parasites in the blood may also be ingested by insect vectors during the course of a blood meal. There is no evidence of an arrested or dormant stage in either mammals or insects; so parasites are not *either* intracellular in tissues or free in the bloodstream but are constantly cycling between these compartments.

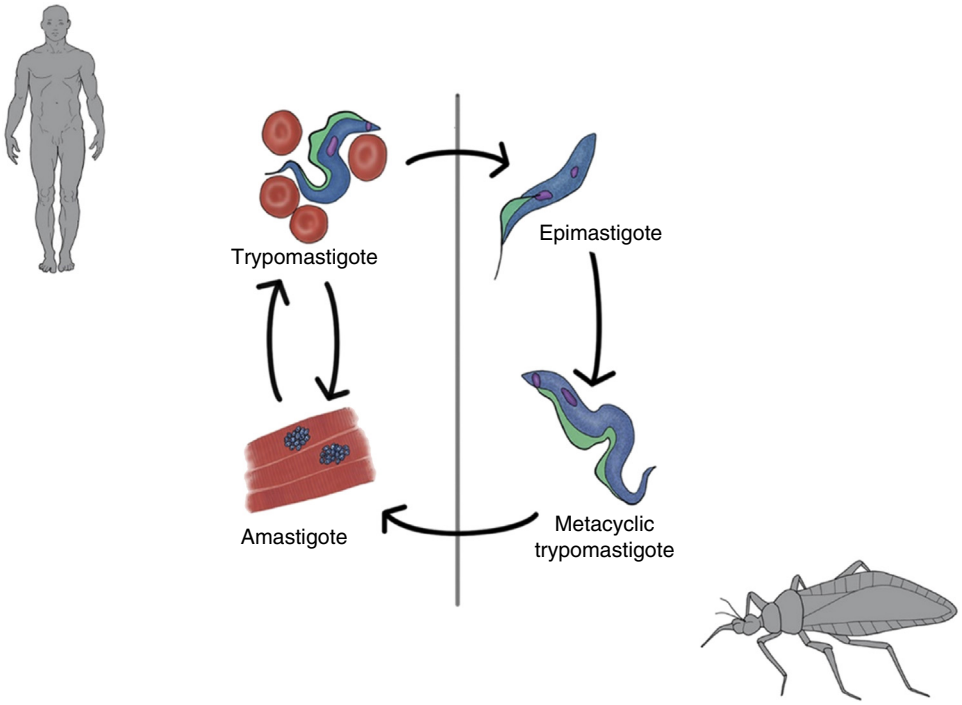

Figure 1 *Trypanosoma cruzi* life cycle.

The full impact of *T. cruzi* infection on humans is difficult to estimate since large scale diagnostic screens have not been performed in most endemic countries. However, it is clear that *T. cruzi* infection is the highest impact infectious disease in Latin America with a probable 10–20 million infected people in the region. Importantly, humans are only one of the >100 mammalian species in which *T. cruzi* naturally circulates, making it virtually impossible that the parasite will ever be eradicated as a potential human health threat.

An additional potentially important aspect of the biology of *T. cruzi* with respect to immune responses is its genetic complexity as a species. Isolates of *T. cruzi* have been classified into multiple distinct genetic types, and sexual recombination between these lineages appears to be extremely rare. However, there is increasing evidence that genetic diversity both between and within these broad genetic types is very high, likely the result of frequent gene rearrangement and recombination within and the movement of genetic information between these lineages (Minning et al., 2011). This genetic complexity – with each isolate being a distinct genotype – will also make it challenging to identify specific parasite traits that consistently associate with infection and disease outcomes.

Immune Control and Disease

As detailed below, effective immune control of *T. cruzi* infection requires both cell-mediated immunity targeting intracellular amastigotes and humoral responses directed at the extracellular trypomastigotes. And, in general, such responses are generated in infected hosts and are highly effective in controlling the infection. Exceptions to this are in cases of very high infecting doses (Aguilar et al., 2007; Shikanai-Yasuda et al., 1991; Benchimol Barbosa, 2006) or of immunosuppression (Ferreira and Borges, 2002; Andrade et al., 1997; Almeida et al., 1996; Jardim and Takayanagui, 1994). However, despite the ability of most hosts to control *T. cruzi* infection, the infection is rarely completely cleared but instead is persistent at low levels for the life of the host. In the acute infection period, parasite load is relatively high, and parasite-infected cells are broadly distributed throughout the body. As immune control is established in the weeks and months following infection, parasite numbers drop substantially and parasites become restricted to particular tissues. Indeed, one of the best demonstrations of the effectiveness of the immune system in controlling *T. cruzi* infection is the difficulty in detecting parasites in chronically infected hosts (reviewed in Cooley et al., 2008). One study that extensively and serially sampled a set of long-term chronically infected subjects for up to 21 time points over several years showed that only 20% of subjects were consistently parasite positive (Cerisola et al., 1974).

Persistent infections of any type are a significant challenge for the immune system – to maintain pathogen control over a long period of time without peripheral host tissue damage. In some hosts, including most humans, this balance appears to be relatively well maintained, and the life-long infection with *T. cruzi* has few major clinical consequences. However, in a significant proportion of subjects, persistent *T. cruzi* infection is accompanied by life-threatening heart and/or gut pathology that is variable in its severity and rate of progression and is generally focal in presentation (Rassi et al., 2010). Although the specific origins of clinical disease in human

T. cruzi infection are still sometimes debated, there is strong experimental evidence and emerging consensus that it is the persistence of parasites in muscle and the continuous immune assaults on these apparently intractable parasites that is the primary cause of tissue damage in chronic *T. cruzi* infection (Tarleton, 2003). The experimental support for the often cited alternative view that Chagas disease pathogenesis is primarily autoimmune in nature is modest and indirect.

It is likely that there is an association between parasite load, immune responses, and the course and severity of chagasic pathology, but the details of this complex interaction have not been sufficiently defined as of yet. Therefore, it is not possible to predict, based upon parasite numbers or genetic types, or the intensity or characteristics of immune responses, what the likelihood is that severe pathology will ultimately occur. Clearly, immune control is not equally effective in all cases and one can imagine that even subtle shortcomings in the generation of immune responses could result in inefficient control of the infection and, when magnified by the decades long infection, the generation of more severe chronic-phase disease.

As *T. cruzi* naturally infects a broad range of hosts in addition to man, this offers unrivaled opportunity to study the infection in experimental models that are also natural hosts, including rodents, dogs, and nonhuman primates. Although the length of infection in some of these shorter-lived species is not comparable to humans, in general, immunity to *T. cruzi* in these hosts is very similar to that in humans, making these experimental models highly informative for the study of immunity to *T. cruzi*.

Innate Recognition of *Trypanosoma cruzi*

A variety of tissues and cell types support the replication of *T. cruzi*, including muscle, adipose, neuronal, endothelial, and epithelial, in addition to macrophages/monocytes and dendritic cells. Recognition of pathogens by immune and nonimmune cells, including those that are the targets of pathogen infection, is mediated by germ line–encoded pattern recognition receptors (PRRs) that sense conserved pathogen structures known as pathogen-associated molecular patterns (PAMPs). Collectively, these PRRs provide the ability to detect pathogens and other stress signals that are outside, within vacuoles, or in the cytoplasm of host cells. Triggering of PRRs results in endogenous activation of cells through a number of signaling pathways leading to cytokine and chemokine production that may directly regulate pathogen development or indirectly impact pathogen success by driving and regulating adaptive immune responses.

PAMPs are generally molecules that are unique to classes of pathogens and are crucial for their survival. A number of components from *T. cruzi*, including specific antigens, lipid anchors, and DNA, have been implicated in the interaction of *T. cruzi* with the best characterized class of PRR, the cell surface, and vacuolar Toll-like receptors (TLRs) (reviewed in Kayama and Takeda, 2010). Once the infection is established, these parasite PAMPs, predominantly from killed parasites, contribute to the activation of PRR and the subsequent directing of acquired immune responses (see below). However, these putative *T. cruzi* PAMPs are likely not readily available on live/intact *T. cruzi* and, as a result, *T. cruzi* is a surprisingly weak activator of pathogen-sensing pathways in both immune and nonimmune cells (reviewed in Tarleton, 2007). This latter conclusion is best exemplified by the modest level of gene expression changes in cells recently infected by *T. cruzi*. The most palpable alteration in host cells upon infection is the stimulation of type I interferons and the subsequent induction of a set of interferon-response genes (Vaena de Avalos et al., 2002; Zhang et al., 2010). *In vivo*, this apparent weak sensing of parasite invasion is accompanied by a sluggish inflammatory response at the site of initial infection and a relatively delayed induction of adaptive immune responses (Padilla et al., 2009). Collectively, these data indicate that viable *T. cruzi* entering a host is relatively poorly immunogenic and thus may 'fly under the radar' of innate immune sensing mechanisms both at the beginning of the infection and when invading new tissue sites in a persistently infected host.

Natural killer (NK) and NKT cells appear to contribute marginally to the control of *T. cruzi* infection; the specific receptors and/or parasite ligands involved in the recognition of *T. cruzi* or *T. cruzi*–infected cells by NK cells are not known (Lieke et al., 2004, 2006; Duthie and Kahn, 2005).

Adaptive Immunity to *Trypanosoma cruzi*

Trypanosoma cruzi induces and is controlled by a combination of specific, highly potent, multifunctional, and largely successful humoral and cell-mediated immune responses dominated by type I cytokines, lytic and opsonic antibodies, and $CD8^+$ cytolytic T cells (Tarleton, 2007). When effectively induced, as happens in most hosts, these combined adaptive immune mechanisms tightly control parasite replication, prevent severe and life-threatening pathology in the acute infection phase, and maintain a low parasite burden in chronically infected hosts. The absence of any one of these mechanisms results in the inability to regulate parasite growth and subsequent death of the host (Kumar and Tarleton, 1998; Tarleton et al., 1996, 1992). Further, various induced and acquired immunosuppression events often lead to overwhelming exacerbation of the chronic infection with high parasite burden (Vaidian et al., 2004; Silva et al., 1999).

Humoral Immune Responses

Antibodies induced by active infection or by vaccination have been shown to facilitate the killing of *T. cruzi in vitro* and to provide substantial protection from *T. cruzi* infection *in vivo* (Almeida et al., 1994; Umekita et al., 1988). This is to be expected since *T. cruzi* trypomastigotes must circulate outside of host cells in order to invade new cells and to be transmitted to its blood-feeding insect vectors, and thus would be exposed to antibodies in the blood and interstitial spaces. Animals lacking B cells are highly susceptible to infection. Mice with a knockout in the μ immunoglobulin heavy chain are able to control acute infection somewhat longer than do mice lacking T cells, although both immune deficiencies result in fatal infection with very high parasite burden (Kumar and Tarleton, 1998). The mechanism of antibody-mediated control of *T. cruzi* infection *in vivo* is not fully understood but

anti-*T. cruzi* antibodies have been demonstrated *in vitro* to partially block host cell invasion, to facilitate *T. cruzi* uptake by phagocytic cells and to induce complement-mediated and complement-independent lysis of *T. cruzi* (reviewed in Tarleton, 1997). Two rather unique reported activities in the pool of lytic antibodies induced by *T. cruzi* infection are anti-galactosyl antibodies that lyse *T. cruzi* without the participation of the classical or alternative complement pathway (Gazzinelli et al., 1991) and antibodies against a complement regulatory protein (CRP) that act by preventing the normal decay-accelerating factor-like activity of this parasite CRP (Norris et al., 1991). These lytic antibodies have been suggested as good markers of spontaneous or drug-induced parasitological cure in *T. cruzi* infection (Norris et al., 1994).

Some of the major targets of anti-*T. cruzi* antibodies are encoded in the very large and highly variable families of surface proteins, the trans-sialidases, mucins, and mucin-associated surface proteins (MASPs). Collectively, these account for over 4000 genes and gene pieces in the *T. cruzi* genome and presumably the extensive size of these families and their variation, aided by frequent recombination, serve an immune evasion function for *T. cruzi*, although this has not been definitively demonstrated.

T Helper Cell Responses

Trypanosoma cruzi elicits a highly polarized type 1 T helper cell response accompanied by significant production of IFN-gamma (IFNγ) and tumor necrosis factor. Although low levels of Th2 and Th17 cytokines are sometimes observed, blocking or inhibition of type 2 cytokine responses has little impact on parasite numbers or the inflammatory response (Tarleton et al., 2000). Blocking Th1 responses at the level of IL-12 production (Silva et al., 1998), the signaling molecules involved in the generation of type 1 responses (Tarleton et al., 2000) or IFNγ production itself (Silva et al., 1992) prevent control of the acute infection.

$CD4^+$ T cells contribute to the control of *T. cruzi* infection by a number of mechanisms, including the activation of killing by macrophages, promotion of antibody production, and the potentiation of inflammatory responses and cell recruitment to sites of parasite replication. Indeed, macrophages are a preferred host cell for *T. cruzi* replication early in infection, until the increasing levels of IFNγ appear to make them inhospitable.

$CD8^+$ T Cell Responses

As noted above, *T. cruzi* invades and replicates in a relatively wide range of host cell types. It is the job of $CD8^+$ T cells to recognize and kill these pathogen-infected cells, and the cytoplasmic localization of *T. cruzi* in host cells places proteins released by the parasite in the perfect position to be processed and presented in association with host class I major histocompatiblity complex (MHC) on the cell surface, and thus recognized by *T. cruzi*–specific $CD8^+$ T cells (reviewed in Tarleton, 2015). The ability of *T. cruzi*–infected cells to be specifically recognized by $CD8^+$ T cells was initially demonstrated using transgenic expression of the model antigen chicken ovalbumin (OVA) by *T. cruzi* and OVA-specific T cells (Garg et al., 1997), and was followed quickly by the identification of trans-sialidase (ts) family proteins as dominant targets of *T. cruzi*–specific $CD8^+$ T cells (Wizel et al., 1997, 1998; Low et al., 1998; Martin et al., 2006). The impressive immunodominance of specific ts peptides in certain mouse strains – in some cases representing >30% of all $CD8^+$ T cells in the animal at the peak of the infection – made them among the first such $CD8^+$ T cell responses in parasite models that could be easily tracked using MHC-peptide tetramers (Martin et al., 2006). This finding provided a useful tool for monitoring the development of $CD8^+$ T cell responses over time – from the initiation of infection to years into the chronic infection. Among other findings, the subsequent studies of *T. cruzi* ts-specific $CD8^+$ T cell responses revealed that these cells remained highly active and potent effector cells hundreds of days into the infection (Bixby and Tarleton, 2008) and upon infection cure, continued to persist as memory T cells with potent recall potential (Bustamante and Tarleton, 2015; Bustamante et al., 2008). These findings contrasted with the results of many studies mostly in viral systems, indicating that persistent infections drive pathogen-specific $CD8^+$ T cells toward an exhausted and less effective phenotype (Shin and Wherry, 2007). The continued competence of $CD8^+$ T cells in *T. cruzi* infection likely results in and is due to the low antigen load that is maintained in *T. cruzi* infection. However, even low parasite levels can eventually compromise T cell function when extended over decades, as is the case in chronic human *T. cruzi* infection (Albareda et al., 2009; Arguello et al., 2014; Albareda et al., 2013; Arguello et al., 2012; Albareda et al., 2006; Alvarez et al., 2008).

Immune Evasion

Trypanosoma cruzi infection is, in most cases, persistent for the life of a host, although evidence for spontaneous cure in experimental animal and natural human infection is increasing (Tarleton, 2013; Francolino et al., 2003; Pinto Dias et al., 2008). This persistence prompts the obvious question of how the infection can be so well controlled immunologically but remain resistant to complete clearance in most cases. In natural human infections or nonlethal experimental infections, it is difficult to find convincing evidence for the impact of a number of typical immunoregulatory mechanisms that might diminish the effectiveness of anti-*T. cruzi* immunity (e.g., regulatory T cells or high level production of regulatory cytokines) (Kotner and Tarleton, 2007; Martin et al., 2007) on parasite persistence. Additionally, the fact that the anti-*T. cruzi* $CD8^+$ T cell response is incredibly strong is another indication that immunosuppression in general is not central to parasite persistence in natural hosts. So what accounts for the ability of *T. cruzi* to persist and continue to invade and replicate within host cells?

In keeping with the observation that *T. cruzi* infection of host cells is relatively 'quiet,' it was observed that *T. cruzi*–specific $CD8^+$ T cell responses are extremely slow to develop, with the detection of *T. cruzi*–specific $CD8^+$ T cells not evident until 8–9 days postinfection (Martin et al., 2006; Padilla et al., 2009). This delay suggests a model in which development of T cell immunity in an animal and the subsequent detection of

sites of infection in a host by these developing T cells occur only after host cell death and the consequent release of parasite 4–5 days later. Thus, because *T. cruzi* fails to trigger innate immune sensors at the time of host cell invasion, it eludes host detection for a period of time. Repetition of this delayed-detection scenario at each newly infected tissue site in a host throughout the infection may provide the advantage needed for establishing a persistent infection. Indeed, increasing the immunogenicity of *T. cruzi* by transgenic expression of bacterial molecular patterns enhances antiparasite immune responses and reduces the ability of *T. cruzi* to persist (Kurup and Tarleton, 2013).

A possible second layer of immune evasion by *T. cruzi* may be provided by the simultaneous expression of substantial numbers of variants of the major targets of adaptive immune responses, the ts family proteins. Trans-sialidase activity is required for the survival of *T. cruzi* in mammals (Dc-Rubin and Schenkman, 2012). However, and in addition to producing these critical molecules, trypomastigotes and amastigotes of *T. cruzi* also simultaneously express enzymatically dead ts protein variants encoded by thousands of diverse and recombining genes (Weatherly et al., in preparation). A reasonable hypothesis for this massive expansion and high variability of ts family genes of *T. cruzi* is its potential to provide a complex and constantly changing target for immune effectors, including $CD8^+$ T cells. However, this hypothesis still awaits formal confirmation. Although the ts molecules are strongly immunodominant in mice (Martin et al., 2006) and humans (Alvarez et al., 2008), they are not the only targets of *T. cruzi*–specific $CD8^+$ T cells. Redirecting the immune response to recognition of nonvariant targets, such as those in the parasite flagellum, provides more effective infection control (Kurup and Tarleton, 2014).

Conclusions

The immune mechanisms important for control of *T. cruzi* infection are slightly complex but reasonably well understood. This is in part due to the quality of the animal models available for such studies. However, translating that information into applications such as immunotherapies or vaccines is proving to be very challenging. To date, most vaccine approaches reduce the severity of infection but none actually prevent the establishment and persistence of *T. cruzi* infection (Bustamante and Tarleton, 2015). This result is likely due to parasite immune evasion, particularly the ability of *T. cruzi* to infect without triggering pathogen sensors. Overcoming this obstacle may be difficult using existing knowledge and technologies.

See also: **Immunity to Bacterial, Parasitic and Fungal Infections:** Immunology of Bacterial and Parasitic Diseases: An Overview; Subverting Immunity from the Inside: Strategies of Intracellular Survival – Protozoans. **T Cell Activation:** Recirculating and Resident Memory $CD8^+$ T Cells.

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