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CD8+ T Cells in *Trypanosoma cruzi* Infection

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Abstract

Trypanosoma cruzi infection and Chagas disease remains among the most neglected of the neglected tropical diseases. Despite this, studies of the immune response to *T. cruzi* have provided new insights in immunology and guidance for approaches for prevention and treatment of the disease. *T. cruzi* represents one of the very best systems in which to study $CD8^+$ T cell biology; Mice, dogs, and primates (and many other mammals) are all natural hosts for this parasite, the robust T cell responses generated in these hosts can be readily monitored using the full range of cutting edge techniques, and the parasite can be easily modified to express (or not) a variety of tags, reporters, immune enhances and endogenous or model antigens. The infection in most hosts is characterized by vigorous and largely effective immune responses, including $CD8^+$ T cells capable of controlling *T. cruzi* at the level of the infected host cells. However this immune control is only partially effective and most hosts maintain a low level infection for life. This review addresses the interplay of highly effective $CD8^+$ T cell responses with elaborate pathogen immune evasion mechanisms, including the generation and simultaneous expression of highly variant $CD8^+$ T cell targets and a host cell invasion mechanisms that largely eludes innate immune detection.

Keywords

$CD8^+$ T cells; *Trypanosoma cruzi*; Chagas disease; immunodominance; PAMPs; DAMPs

Introduction

Trypanosoma cruzi is the agent of Chagas disease, the Americas' highest impact infectious disease and world's dominant cause of infectious myocarditis. In infected mammals, extracellular trypomastigotes of *T. cruzi* circulate in the bloodstream, potentially carrying the infection to all parts of the body and providing a mechanism for transmission of infection to appropriate blood-feeding insects. However *T. cruzi* parasites spend the vast majority of their time in mammals as amastigote forms, replicating in the cytoplasm of a range of host cell types. Consequently, $CD8^+$ T cells capable of recognizing *T. cruzi* – infected cells are absolutely essential for control of the infection; deleting or inhibiting $CD8^+$ T cells results in uncontrollable parasite load early in infection and an exacerbation of

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infection in chronically infected hosts (1–3). The host's ability to control *T. cruzi* infection is substantial but only partially effective: most hosts tightly limit parasite numbers but fail to completely clear *T. cruzi* infection. The available data suggest that this failure to achieve parasitological cure is not a result of a suppressed or disregulated immune response but instead reflects the success of *T. cruzi* in evading host immune responses. This review will focus primarily on recent advances in our understanding of the role of $CD8^+$ T cells in immunity during *T. cruzi* infection and the mechanisms utilized by *T. cruzi* to evade that response. There will be only brief mention of foundational data; Please refer to previous reviews for more detailed discussion of earlier data (4–6).

Generation and target specificity of *T. cruzi* –specific $CD8^+$ T cells

The ability of *T. cruzi*-infected cells to process and present parasite-encoded molecules for recognition 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 (7). Concurrent experiments examining known, amastigote-secreted proteins indicated that selected members of the trans-sialidase (ts) gene family were natural targets for *T. cruzi* infection-induced $CD8^+$ T cells (8–10). But it was not until completion of the first whole genome sequencing (11) and proteome analysis (12) of *T. cruzi* was obtained that a full evaluation of potential targets was possible, revealing an incredibly biased and potent response to a relatively small number epitopes encoded by multiple ts genes (13). The activity of enzyme-active ts family members is required for the survival of *T. cruzi* in mammals, since without the ability of the parasite to acquire sialic acid from host molecules, *T. cruzi* tyrpomastigotes invade host cells poorly and are highly sensitive to host complement-mediated lysis (14). In addition to the production of a small set of enzyme-active ts proteins (encoded by < 20 genes), the *T. cruzi* genome also contains 1000's of genes encoding full length and partial non-enzymatically active ts molecules, the exact function of which is not clear (11)(Weatherly, et all, unpublished). While ts molecules are not the only targets of *T. cruzi* – specific $CD8^+$ T cells, ts epitopes appear to be by far the most immunodominant, in some cases occupying >30% of the entire CD8 compartment at the peak of the response in mice (13) and an undetermined but significant proportion of the response in humans (15).

Such a potent and highly directed response is easy to track using MHC multimers, and these reagents have made possible very detailed studies of the *T. cruzi*-specific $CD8^+$ T cell responses. One of the first observations we made was that despite the numerical strength of the *T. cruzi*-specific T cell response, this response is relatively slow to develop, with the detection of *T. cruzi* –specific $CD8^+$ T cells not evident until 8–9 days post-infection (13, 16). These studies enumerating *T. cruzi*-specific $CD8^+$T cells using MHC-tetramers were complemented with measurements of cellularity, chemokine and cytokine production and cell proliferation at the infection site and the draining lymph nodes to document that the introduction of *T. cruzi* into the skin of mice failed to trigger any systemic recognition of infection until a minimum of 5–6 days post-infection (16). It is well-documented that in most host cell types in vitro, *T. cruzi* completes multiple rounds of replication, and emerges from host cells between 4 and 5 days after infection, each cell yielding 100's of newly converted trypomastigotes. We have recently confirmed via whole animal imaging that the

in vivo timing of parasite infection of and release from host cells in the skin is similar, with the emergence of motile trypomastigotes on ~day 5 post-infection (Padilla, unpublished). Thus, the first round of host cell death and parasite release coincides with the initiation of immune detection of *T. cruzi*, strongly suggesting that the stimulus for initiation of $CD8^+$T cell activation is not the initial infection of host cells soon after infection, but rather occurs 4–5 days *after* infection. In short, *T. cruzi* fails to trigger innate immune sensors at the time of host invasion, and thus gets a 'free pass' for the first 5 days of the infection. This short head start – time to expand up to 500-fold and then disperse throughout the body without restriction by the immune system - may be essential for the establishment of what will become a lifelong infection in most hosts.

The relative failure on the part of *T. cruzi* to strongly activate host cells upon initial host cell infection is evident from the rather paltry changes that occur in host gene expression upon in vivo or in vitro infection (17, 18). Essentially, infection seems to elicit production of type I IFN and subsequently, the activation of IFN-response genes, but little more. And this response is not sufficient to recruit and activate inflammatory cells to the infection site nor the movement of antigen-presenting cells carrying *T. cruzi* antigen to the draining lymph nodes (16).

We attribute the weak response of host cells to *T. cruzi* infection to the absence of triggers for innate immune sensors, the pathogen associated molecular patterns (PAMPs). This conclusion seems a bit paradoxical considering that *T. cruzi* has been a model among protozoans for the study of triggers of host pattern recognition receptors (PRR) (reviewed in (5)). However these previously studied *T. cruzi* PAMPs are not known to be exposed on intact parasites either during or after cell invasion and thus would not be capable of interacting with host PRR. Further, we recently demonstrated that immune detection and activation of $CD8^+$ T cell responses can be both accelerated and enhanced by the transgenic expression in *T. cruzi* of the well-characterized bacterial PAMPs *Salmonella typhimurium* flagellin and *Neisseria meningitidis* porin (19). Constitutive expression of these exogenous PAMPs by *T. cruzi* also allowed for the generation of a persistently potent adaptive immune response that extends for the full length of the infection and is associated with improved pathogen clearance and complete parasitological cure in some cases (19). Thus the lack of PAMPs in *T. cruzi* seems to have consequences not only with respect to the timing of the initial activation of immune responses, as is typically considered the role for innate immune signaling, but indeed throughout the infection. Notably, the ability to boost immunity to *T. cruzi* using transgenic expression of bacterial PAMPs argues that it is the absence of natural PAMPs in this parasite, rather than an active suppression of host PAMP signaling pathways, that is responsible for the generally poor innate and slow adaptive response to *T. cruzi* infection. This understanding may be useful for vaccine development (see below).

The contribution of ts proteins and PAMP recognition to immune evasion by *T. cruzi*

If *T. cruzi* is so deficient in endogenous PAMPs one might ask how the infection can elicit such a potent $CD8^+$ T cell response. As noted, the timing of the initiation of inflammation and immune activation (6, 16) suggests that the release of trypomastigotes at 4–5 days after

infection is the trigger for immune activation. This first round of parasite release appears to be nearly synchronous (although subsequent rounds are not) and includes the destruction of the cells formally hosting the parasites as well as the release of parasite byproducts from the intracellular period. Collectively the damage-associated molecular patterns (DAMPs) from the destroyed host cell and any released parasite PAMPs from dead parasites or degraded parasite products, presumably act as the triggers of inflammation and the initiators of adaptive immune responses. The result is an extremely robust, although significantly delayed induction of $CD8^+$ T cell immunity. The targets of this robust response are the parasite antigens exposed at the time of host cell rupture – most prominently the ts family proteins. Most ts-family proteins contain a GPI-anchor addition site that provides for their targeting to the parasite surface plasma membrane. However ts proteins are also secreted from both trypomastigotes and amastigotes when the ts fail to receive a GPI anchor – and this happens frequently (7, 20). Thus, with the lysis of host cells, ts proteins produced by the formally intracellular amastigotes and perhaps also by the newly released trypomastigotes, become the earliest and most abundantly presented *T. cruzi* proteins at the time of initiation of T cell responses, facilitating their immunodomiance.

Unfortunately for immune control of *T. cruzi*, the immunodominance of ts proteins focuses the $CD8^+$ T cell response on a set of variant epitopes that are also not normally presented until late in the host cell infection process. Many of the >3000 full and partial length ts genes appear to be expressed simultaneously, essentially flooding the immune system with a massively complex array of antigen variants. These genes are also undergoing constant rearrangement events to generate new variants within a lineage and a distinct set of ts genes in each parasite isolate (13, 21)(Weatherly et al, unpublished). Interestingly, despite this enormous repertoire of variant ts epitopes, the $CD8^+$ T cell response observed in H2-K^b mice to a very highly restricted set of ts epitopes (13). This results is probably a coincidence of an overlap between the H2-K^b binding motif with the most sequence-restricted region of the ts molecule (6). In other mouse strains and in humans, a much less focused ts-specific response occurs (13, 15). Further, even in the H2-K^b system, this focused response is not necessary for immune control since the induction of tolerance to these epitopes has almost no impact on infection dynamics (22).

In addition to its massive variation, the ts proteins are also suboptimal as $CD8^+$ T cell targets because of their timing of expression in infected host cells. In contrast to $CD8^+$ target epitopes encoded by paraflagellar rod proteins, which are detected in association with host cell surface MHC I within 6 hrs post-infection, the ts family epitopes are expressed relatively late in the infection cycle in host cells and are not visible to ts-specific T cells until between 24 and 48 hours post-infection (23). Thus, not only is the dominant $CD8^+$T cell response focused on a constantly changing and strain-variant array of targets but these targets are also expressed late in the host cell infection process, once again providing time for parasite expansion prior to target cell recognition. It is of little surprise that despite the enormous dominance of ts-specific responses in the *T. cruzi*-specific $CD8^+$ T cell response, the $CD8^+$ T cells with these particular specificities are totally dispensable for control of the infection (22).

Immune exhaustion during chronic *T. cruzi* infection

In many persistent infections, the constant presence of antigen results in exhaustion of pathogen-specific $CD8^+$ T cells. However this does not appear to be the case with respect to *T. cruzi*-specific T cells. In the blood and lymphoid tissue of chronically infected mice, most *T. cruzi* –specific $CD8^+$ T cells are $CD62L^{lo}$, indicative of an effector phenotype (24, 25). However up to 20% have the phenotype and function of a central memory population that has not recently seen antigen and is independent of antigen for long-term survival (24). Furthermore, the *T. cruzi*-specific $CD8^+$ T cell population in chronically infected mice generally does not express the markers of exhaustion such as PD-1 (Pack et al, unpublished) and when infections are cured in the long-term infected animals by drug treatment, a stable and persistent memory $CD8^+$T cell population is evident (26). Combined with the fact that depletion of $CD8^+$ T cells during the chronic stages of infection ((3) and Pack, et al, unpublished) results in increasing parasite load, these data support a conclusion that $CD8^+$ T cells in *T. cruzi* infection remain a highly functional and critical contributor to parasite control throughout the infection. In short, the long-term persistence of *T. cruzi* in infected hosts does not appear to be due to a deficit in or loss of $CD8^+$ T cell function.

Nevertheless, there are circumstances under which *T. cruzi* –specific $CD8^+$ T cell responses exhibit signs of exhaustion. In mice exposed to a highly virulent challenge, PD-1 expression and/or other signs of T cell exhaustion are evident (27, 28). Such high dose and acutely lethal infections are also associated with other immunological anomalies (29). However high dose infections are not the norm in humans and thus these particular experimental systems do not model well the majority of *T. cruzi* infections in humans. A more pertinent situation is that of very long-term infections in humans, where despite the apparently well-controlled and low –level parasite load decades into infection, a decay in T cell function becomes evident (15, 30–34). In contrast to shorter length experimental infections and *T. cruzi* infection in younger human subjects (with consequentially shorter infection length), long-term chronically infected individuals with have a substantially reduced number of *T. cruzi* –responsive T cells and a higher fraction of mono-functional T cells (32) that are dependent on antigen for persistence (35). Both in the peripheral blood and in cardiac tissue, *T. cruzi* -specific T cells in long-term infected adults also have a higher proportion of less differentiated cells, indicative of more recent recruitment into the response (30, 31, 34). Importantly, an antigen-independent, polyfunctional population of *T. cruzi*-specific T cells emerges in a significant proportion of these subjects after successful treatment (35) and Laucella, unpublished). Collectively these results show that either very high acute antigen load (as in experimental mouse infections) or very long term low level infection (in humans) can degrade the immune response to *T. cruzi* and suggest that a decaying T cell response to *T. cruzi* is more often the *result* of persistent or high level antigen rather than the *cause* of that persistence.

$CD8^+$ T cells in vaccine-induced protection

Just as $CD8^+$ T cells are crucial for immune protection in *T. cruzi* infection, they are also integral to the induction of protective immunity by anti-*T. cruzi* vaccines (reviewed in (36, 37). Unfortunately, no anti-*T. cruzi* vaccine developed to date generates immune protection

that is as good as, much less better than that achieved by the active infection. We have recently used an infection and cure model to study the development of immune protection in mice infected with fully virulent wild-type *T. cruzi* followed by drug-induced cure of that infection (38). Previous studies using benznidazole treatment to cure *T. cruzi* infection in mice demonstrated the development of central memory (Tcm) T cells in the cured mice that were capable of transferring a degree of early protection from *T. cruzi* infection to recipient mice (26). However, subsequent studies employing multiple rounds of infection and drug cure showed that the protection afforded by this "vaccination" protocol (where Tcm $CD8^+$ T cells dominate) was always inferior to that provided by an active chronic infection where T effector (Teff) cells dominate (38). One interpretation of these studies is that the Teff cells in actively infected mice (24, 25) are more protective than the exclusively Tcm $CD8^+$ T cells retained in mice cured of infection. If this hypothesis is true, then effective vaccination against *T. cruzi* infection may require methods that can maintain a stable level of Teff cells in the absence of active infection – a substantial challenge. A similar scenario indicating a requirement for Teff cells has been proposed and supported experimentally for $CD4^+$ -mediated immune protection in the related protozoan, *Leishmania major* (39).

Conclusions and Future Prospects

Briefly summarizing the evidence discussed above, $CD8^+$ T cells are highly effective in and crucial to control of *T. cruzi* infection, but these $CD8^+$T cell responses in concert with other immune effectors fail to clear the infection. This failure is attributed to the slow initial development of immune responses in the infected host and to the concentration of these responses on highly variant and late-expressing parasite epitopes. Can these limitations in the adaptive response to *T. cruzi* be overcome and in the process, this information used to enhance the control of *T. cruzi* infection in hosts or to develop preventatives such as vaccines?

One clearly addressable issue is that of the most advantageous parasite epitopes against which $CD8^+$ T cell responses should be directed. In addition to the "moving target" represented by constantly evolving but naturally immunodominant ts gene-encoded epitopes, these ts epitopes are also not presented by infected cells until relatively late in the infection cycle (23). The delay in ts epitope presentation may reflect the requirement for a sufficient number of ts-producing amastigotes to accumulate in the host cell cytoplasm and/or relate to huge number of ts variants being produced and thus competing for presentation by class I MHC molecules. A more favorable target for $CD8^+$ T cells would be the strain invariant epitopes derived from flagellar proteins that are available for presentation within hours after infection of host cells, a result of the release of the trypomastigotes flagellum by *T. cruzi* via an asymmetrical cellular division process soon after host cell invasion (23). Indeed enhanced induction of $CD8^+$ T cells specific for a flagellum-derived epitope is associated with significantly improved control of challenge infection (23). A similar enhanced protective capacity of $CD8^+$ T cells recognizing "early" antigens on infected cells has also been demonstrated in several viral systems, providing additional support for the hypothesis that recognition of a pathogen-infected cell early in the infectious cycle has significant benefits in terms of immune control (40–43).

The issue of the silent invasion process, due to the failure of *T. cruzi* to trigger host PRR, is a bit more difficult address. As we have shown, the infection can be made "louder" by forcing the expression by *T. cruzi* of strong PAMPs and resulting in stronger and more protective immune responses (19). This approach could significantly impact the efficacy of live attenuated vaccines. However it seems unlikely that the host innate immune system can be easily tuned to be more sensitive to *T. cruzi* invasion. This delayed detection of *T. cruzi* in newly infected hosts would seem to make it virtually impossible to prevent infection by prophylactic vaccination – unless host cell invasion itself can be prevented (by blocking antibodies, for example). Like the other data reviewed above, this information should be integrated into the design and proposed use of anti-*T. cruzi* vaccines.

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