Published in final edited form as:

Curr Opin Immunol. 2009 August ; 21(4): 385–390. doi:10.1016/j.coi.2009.07.006.

CD8⁺ T cells in *Trypanosoma cruzi* infection

Angel M Padilla¹, Juan M Bustamante¹, and Rick L Tarleton^{1,2}

¹ Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, 500 D.W Brooks Dr. S310 Coverdell Center. GA, 30602, USA

² Department of Cellular Biology, University of Georgia, Athens, 500 D.W Brooks Dr. S310 Coverdell Center. GA, 30602, USA

Summary

CD8⁺ T cells have emerged as crucial players in the control of a number of protozoan pathogens, including *Trypanosoma cruzi*, the agent of human Chagas disease. The recent identification of the dominant targets of *T. cruzi*-specific T cells has allowed investigators to follow the generation of and document the functionality of T cell responses in both mice and humans. Although slow to develop in the early stages of the infection, *T. cruzi*-specific CD8⁺ T cells reach prodigious levels and remain highly functional throughout chronic infections in mice. Following drug-induced cure during either the acute or chronic stage, these immunodominant T cells persist as stable, antigen-independent memory populations. *T. cruzi*-specific CD8⁺ T cells in humans are less-well studied but appear to lose functionality and decline in numbers in these decades long infections. Changes in the frequency of parasite-specific T cell upon therapeutic treatment in humans may provide a new metric for determining treatment efficacy.

Introduction

CD8⁺ T cells are well known for their importance in the control of many viral and bacterial infections, but it is rarely mentioned that the control of eukaryotic pathogens such as intracellular protozoans is also subject to detection and destruction by CD8⁺ T cells. Infection by *Trypanosoma cruzi*, the agent of human Chagas disease, is perhaps the best studied example of the significance of CD8⁺ T cells in immunity to a protozoan pathogen. In mammals, *T. cruzi* continuously cycles between extracellular trypomastigote forms and intracytoplasmic amastigotes. The range of host cell types invaded by *T. cruzi* is broad, including myocytes, adipocytes, epithelial cells, fibroblasts, and macrophages, among others. In its cytoplasmic niche, *T. cruzi* releases proteins that are processed for presentation by class I MHC molecules [1] and each intracellular round of replication likely lasts up to 5 days, thus providing substantial time for detection of these infected cells by parasite-specific CD8⁺ T cells.

In addition to humans, *T. cruzi* naturally infects a wide range of other mammals, including mice. Thus the role of CD8⁺ T cells in immunity can be studied experimentally in both short and medium term (several years) infections in mice and other hosts as well as in decades-long infections in humans, where curative treatment is often not fully available or is scantily used. *T. cruzi* can also be manipulated genetically, for knockout of individual parasite genes and for the introduction of foreign genes, including model antigens such as ovalbumin [1]. Thus *T. cruzi* is an excellent model for studying the generation and maintenance of CD8⁺ T cell

responses during antigen persistence and is one of the few infections in which T cell memory following cure of a chronic infection can be studied [2].

Targets of the *T. cruzi* –specific CD8⁺ T cell response

The crucial role of CD8⁺ T cells in control of *T. cruzi* infection is evident from multiple studies demonstrating the inability of mice lacking CD8⁺ T cell to survive infection and the ability to raise the level of resistance to infection by boosting CD8⁺ T cell responses (reviewed in [3]). A breakthrough in the understanding CD8⁺ T cells in *T. cruzi* infection came with the identification of the highly immunodominant epitopes encoded in the trans-sialidase (ts) family of genes [4]. In C57Bl/6 mice, up to 30% of the total CD8⁺ T population at the peak of the infection is specific for a single ts peptide (ANYKFTLV; TSKb20). This degree of immunodominance is remarkable for any pathogen but is even more so for *T. cruzi*, considering its genome complexity (with >12,000 genes in the haploid genome). The ts gene family that encodes these strongly immunodominant epitopes contains more than 1400 annotated [5] and another ~1800 unannotated (Weatherly, unpublished observation) full and partial ts variants in the reference CL Brener strain *T. cruzi* genome. Kinetoplastid relatives of *T. cruzi* have none (*Leishmania sp.*) or few (6 in the case of *Trypanosoma brucei*) ts genes, suggesting a massive expansion of this gene family specifically in *T. cruzi*. Given the fact that the vast majority of these ts family proteins in *T. cruzi* apparently lack the trans-sialidase enzymatic activity that defines the family and that is necessary for parasite survival [6], it seems most likely that this expansion was driven by processes unrelated to enzymatic function – perhaps by immunological pressure. However it has yet to be determined precisely how the simultaneous expression of many variant ts-encoded epitopes – some of which become very strong and apparently highly effective immune targets – might facilitate immune evasion and thus contribute to parasite persistence. The simple competition for MHC class I presentation on APCs by this huge diversity of epitopes could make the activation threshold of naïve T cells difficult to reach. The potential for inhibitory effects of altered peptide ligands [7] is also an interesting hypothesis but is difficult to demonstrate in this system where so many variants exist [4]. Also appealing are the possibilities that the production of new variants by recombination of the existing genes and gene fragments during the course of an infection could contribute to parasite persistence and/or that strain-specific variations in ts gene repertoires could facilitate the superinfection of hosts by distinct *T. cruzi* isolates.

Rodrigues and co-workers [8,9] have proposed that the strong immunodominance evident in the mouse response to *T. cruzi* infection may itself contribute to immune evasion and persistence of the infection, by inhibiting the development of “broader” immune responses and permitting escape variants. However neither of these possibilities seems highly likely. Tolerizing C57Bl/6 mice to the strongly dominant TSKb20 epitope has a modest, but significant negative impact on host control of parasite load and inflammation, but does not influence the overall size of the CD8⁺ T cell response to *T. cruzi* nor the ultimate outcome of infection (Rosenberg, et al., unpublished). Furthermore, given that the cross-reactive TSKb20 and TSKb21 epitopes are encoded by > 200 ts genes in the CL Brener reference strain [4], it is hard to imagine how all of these genes could be silenced and or all epitopes mutated as would be required for an effective escape from these TSKb20-specific T cell responses. Finally, the incredible focusing of the CD8⁺ T cell response in some inbred mouse strains, to a very few epitopes, (from among the hundreds of thousands of potential epitopes encoded by the ts gene family in addition to other secreted/released proteins produced by *T. cruzi*; [4] may not be the rule for all hosts. Indeed CD8⁺ T cell responses to *T. cruzi* appear to have more breadth in other mouse strains [9] Martin, et al. unpublished) and in humans [10,11]. One explanation for the particularly strong immunodominance pattern in C57Bl/6 mice is the overlap between the motif for peptide binding to H-2K^b and a region of constrained variation in the ts sequences – which may specifically restrict indiscriminate epitope variation in this region (Figure 1).

Generation of CD8⁺ T cell responses in *T. cruzi* infection

After recognition of their cognate antigen presented in the context of MHC class I molecules on APCs, naïve CD8⁺ T cells undergo a relatively fixed program of expansion that allows the infrequent pathogen-specific CD8⁺ T cells to increase up to 10,000-fold within 4 to 5 days. A contraction phase then follows as antigen/pathogen is cleared or controlled. This general pattern and timing of T cell activation is characteristic of acute viral and bacterial infections that are rapidly controlled and ultimately results in the establishment of a stable memory T cell population within a few weeks after infection. However in *T. cruzi* infection, the pattern of induction and contraction is significantly delayed in time, with initial CD8⁺ T cell activation not evident until more than one week after parasite introduction [4,12,13]. Increasing the infective dose of *T. cruzi* to unnatural levels can hasten this response, but only marginally ([12], Padilla, et al. unpublished) and the delay does not appear to be the result of the absolute failure of parasites to traffic to lymph nodes draining the sites of infection [14] and Padilla, unpublished) nor to an active suppression of the anti-*T. cruzi* T cell responses [15,16]. We favor the hypothesis that the initial infection by *T. cruzi* is “silent” and that triggering of innate, and subsequently, adaptive immune responses does not occur until the first round of parasite replication and reinvasion – at 4–5 days post infection – is complete. This hypothesis is supported by the finding of a rather slow and subtle host cell response to *T. cruzi* invasion [17], and by the fact that no ligands for host pattern recognition receptors – the molecules responsible for induction of innate immune responses - have been shown to be exposed on *T. cruzi* during or soon after host cell invasion (reviewed in [18]). This inconspicuous invasion and expansion strategy is not unique to *T. cruzi*, but rather may be characteristic of a variety of pathogens that establish chronic infections, despite the eventual activation of potent cellular immune responses [19].

Once the CD8⁺ T cell response to *T. cruzi* is triggered, it displays similar kinetics as described for many viral and bacterial infections [2,4,20]. *T. cruzi*-specific CD8⁺ T cells develop in the absence of CD4⁺ T cells and display similar effector functions as their “fully helped” counterparts, but fail, on their own, to control the parasite load [21]. A strong anti-*T. cruzi* CD8⁺ T cell response as evidenced by the development of TSKb20 specific cells and in vivo CTL activity is also generated in IFN γ deficient mice infected by *T. cruzi*, although also in this case the response is unable to control the parasite level and mice succumb during the acute phase ([2] and unpublished data). Thus, although CD8⁺ T cells are crucial for the control of *T. cruzi* infection – they are clearly not the entire story.

CD8⁺ T cell function in chronic *T. cruzi* infection

Even with a fully competent immune system and the generation of robust CD8⁺ T cell responses, *T. cruzi* infection appears to be rarely, if ever, completely resolved in either experimentally infected animals or in humans. In all cases studied to date, persistence of *T. cruzi* is most evident in muscle and adipose [22] tissues. The observation of CD8⁺ T cells at sites of parasites persistence (and thus disease) has been variable interpreted – on the one hand as a sign of an appropriate response to contain a potentially lethal infection and on the other, as perpetrators of tissue damage and disease. Although there is likely some truth in both points of view, it seems clear that the CD8⁺ T cell response in *T. cruzi* infection is a “necessary evil” in infection control and that its contribution to disease is secondary to this containment function. Additionally, when the CD8⁺ T cell response to *T. cruzi* is abrogated, inflammatory disease actually worsens [23]; Collins, et al., unpublished).

The fact that parasites persist in tissues despite the presence of CD8⁺ T cells at these sites calls into question the functional capabilities of these tissue-homing T cells. The relative failure to restimulate cytokine production in T cells extracted from sites of parasite persistence suggested

that these T cells may be dysfunctional [24]. However more recent studies hint that this “dysfunction” is more likely a reflection of the prior and recent activation of these cells by persistent parasite antigen in these sites rather than a true inactivity (Collins et al., unpublished). In addition, various possible mechanisms for the active regulation of CD8⁺ T cell function at this or other sites, including CD4⁺CD25⁺ T regulatory cells [16] and the action of TGF- β [15] have been ruled out as contributing to the failed CD8⁺ (or other effectors) clearance of the infection. Indeed, despite the characterization of *T. cruzi* as an inducer of a strong immunosuppressive state, there is little evidence for either a specific or general restriction on CD8⁺ T cell responses. Thus it appears that *T. cruzi*-specific CD8⁺ T cell are generated in abundance, traffic to sites of parasite persistence where they encounter antigen, carry out effector activity, and then acquire the phenotype of recently activated cells that are resistant to further reactivation ex vivo. The importance of these tissue-infiltrating T cells in infection control at sites of persistence is supported by the rise in parasite load that is seen when the CD8⁺ T cell presence or function in these sites is abrogated [23] and Collins et al, unpublished). The question still remains as to why *T. cruzi* is controlled at most tissues sites but is able to persist, albeit at very low levels, specifically in muscle and adipose tissue. Interestingly, muscle and adipose tissue are similar in that they preferentially store and utilize fatty acids as energy sources and it is possible that this metabolic condition provides a slight growth or survival advantage for *T. cruzi* in these sites.

CD8⁺ T cell memory in *T. cruzi* infection

As parasite numbers are substantially controlled in the acute phase of *T. cruzi* infection, parasite-specific CD8⁺ T cells contract to form a memory population. Similar to other persistent infections [25,26] the CD8⁺ T cells evident in *T. cruzi* infection are maintained primarily by the presence of antigen, and thus have the phenotype of T_{EM} cells (CD62L^{lo}, CCR7^{lo}, CD127^{lo}, CD44^{hi}; [2,4,20]. However in contrast to classic examples of chronic viral infections (e.g. LCMV clone 13, HIV, hepatitis) which are characterized by varying degrees of functional impairment of pathogen-specific T cell responses [25–30] *T. cruzi* infections in mice lasting > 2 years do not result in detectable exhaustion of parasite-specific cells ([2,4,20]. In this respect, *T. cruzi* infection is more similar to gamma and beta herpesvirus infections which are characterized by low and or intermittent antigen exposure and pathogen persistence without immune exhaustion [31–33]. Low antigen load in chronic *T. cruzi* infection is further supported by the presence of a subpopulation of T_{CM} cells (CD127^{hi}) within the parasite-specific CD8⁺ T cells that is detectable late in the acute phase of the infection and increases in frequency as the infection becomes more chronic [20]. These T_{CM} CD8⁺ cells expressing CD62L^{hi} phenotype are maintained in the absence of antigen, suggesting a self renewal maintenance driven by IL-7 and IL-15, and have low expression of KLRG1, a marker of repetitive antigen stimulation, again supporting the hypothesis of infrequent reactivation through TCR stimulation despite parasite persistence [20].

Notwithstanding the apparent low antigen exposure in chronic *T. cruzi* infection, when infections last for decades as is the case in most human *T. cruzi* infections, they appear to eventually take their toll on *T. cruzi*-specific CD8⁺ T cell responses. Humans with chronic *T. cruzi* infections of >20 years have low and sometimes undetectable levels of *T. cruzi*-specific T cell responses and the frequency of these responses tends to correlate with the severity of clinical disease – with largely undetectable responses in those subjects with the most severe disease [34]. Additionally, chronically infected subjects exhibit a highly differentiated T cell compartment overall with terminally differentiated cells prone to apoptosis, suggestive of antigen-driven exhaustion [35]. Lastly, therapeutic treatment of these chronically infected subjects with a protocol that is believed to result in parasite clearance in at least a subset of subjects results in a loss of detectable *T. cruzi*-specific T cells, again indicating their antigen-dependence and tendency for deletion (Lauella, et al., unpublished). Collectively these studies

in mice and humans suggest that *T. cruzi* infection is controlled sufficiently well in shorter term infections (up to 2 years) to allow for the maintenance of a highly effective CD8⁺ T cell response that is crucial to pathogen control, but that when extended to decades, even a low level pathogen persistence may compromise preservation of CD8⁺ T cell responses.

Protective CD8⁺ T cell responses after complete *T. cruzi* clearance

Benznidazole (BZ), the principal drug available for the treatment of *T. cruzi* infection has been recently shown to provide complete parasite clearance in mice with acute or chronic *T. cruzi* infections [2]. This drug-mediated cure is accompanied by a shift in the *T. cruzi*-specific CD8⁺ T cells from the predominantly T_{EM} phenotype (CD62L^{lo}, CCR7^{lo}, CD127^{lo}, CD122^{lo} and Bcl-2^{lo}) characteristic of the persistent infection to a majority T_{CM} cell population (CD62L^{hi}, CCR7^{hi}, CD127^{hi}, CD122^{hi} and Bcl-2^{hi}) [2]. *T. cruzi*-specific CD8⁺ T cells in these drug-cured mice have additional characteristics of T_{CM} cells—they undergo low-level, antigen-independent proliferation in vivo, rapidly expand in response to either a homologous or heterologous strain *T. cruzi* challenge and nearly completely inhibited the response of endogenous naïve T cells, suggesting a greater ability of the T_{CM} to compete for antigen and other resources, relative to naïve T cells or *T. cruzi*-specific T_{EM}. These T_{CM} from cured mice also exhibited increased protective capacity as compared to the predominantly T_E/T_{EM} population from chronically infected mice. Thus, CD8⁺ T cells from treated-cured mice, upon pathogen clearance, develop all the traits of stable and highly protective, memory T cells [2] and Bustamante, et al., unpublished).

This T_{CM} population likely has its origins in the small subset of T_{CM} cells within the parasite-specific CD8⁺ T cells that is detectable throughout the acute and chronic infections [20] although the conversion from *T. cruzi*-specific T_{EM} has not been ruled out. The change in the dominant phenotype of the *T. cruzi*-specific CD8⁺ T cells is also observed when cure was achieved with compounds other than benznidazole, suggesting that these memory markers could be used as surrogates for the assessment of drug treatment efficacy and cure in *T. cruzi* infection [2] and Bustamante, et al., unpublished). Unfortunately, in human infections where such surrogates of treatment efficacy are badly needed, the ability to track and phenotype *T. cruzi*-specific T cells is lacking due to their low frequency and largely unknown antigen specificity [10,34].

Conclusions

T. cruzi is one of a number of pathogens that establish persistent infections that are controlled, but not eliminated by the action of pathogen-specific CD8⁺ T cells. The long-term maintenance of *T. cruzi*-specific CD8⁺ T cell as fully functional T_{EM} and T_{CM} populations, despite pathogen persistence, adds an additional option for how CD8⁺ T cells can function in an infection, in addition to the better studied “infection → cure → stable memory” and “infection → persistence → T cell exhaustion” models. *T. cruzi*-specific T cell responses should also be useful as surrogates for determining infection clearance. However the impact of the massive variation in the gene family encoding the primary targets of *T. cruzi*-specific CD8⁺ T cells remains enigmatic and may blunt efforts to use these as prophylactic or therapeutic targets of vaccination.

Acknowledgments

The authors thank other past and present members of the Tarleton lab as well as our collaborators in Argentina for their contributions to the research and ideas discussed in this review. Special thanks to D. Brent Weatherly for production of Figure 1. Work in the author's lab is supported by grants R01AI-22070, R01AI-33106 and P01AI-44979 from the U.S. National Institutes of Health.

References

1. Garg N, Nunes MP, Tarleton RL. Delivery by *Trypanosoma cruzi* of proteins into the MHC class I antigen processing and presentation pathway. *J Immunol* 1997;158:3293–3302. [PubMed: 9120286]
- 2••. Bustamante JM, Bixby LM, Tarleton RL. Drug-induced cure drives conversion to a stable and protective CD8+ T central memory response in chronic Chagas disease. *Nat Med* 2008;14:542–550. [PubMed: 18425131] The authors demonstrate that parasite-specific CD8+ T cells remain highly functional throughout chronic *T. cruzi* infection, despite parasite persistence, and can develop into a stable T_{CM} population upon parasitologic cure. Also the first study to conclusively demonstrate that treatment with Beznidazole completely cures *T. cruzi* infection in mice
3. Martin D, Tarleton R. Generation, specificity, and function of CD8+ T cells in *Trypanosoma cruzi* infection. *Immunol Rev* 2004;201:304–317. [PubMed: 15361249]
4. Martin DL, Weatherly DB, Laucella SA, Cabinian MA, Crim MT, Sullivan S, Heiges M, Craven SH, Rosenberg CS, Collins MH, et al. CD8+ T-Cell responses to *Trypanosoma cruzi* are highly focused on strain-variant trans-sialidase epitopes. *PLoS Pathog* 2006;2:e77. [PubMed: 16879036]
5. El-Sayed NM, Myler PJ, Bartholomeu DC, Nilsson D, Aggarwal G, Tran AN, Ghedin E, Worthey EA, Delcher AL, Blandin G, et al. The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. *Science* 2005;309:409–415. [PubMed: 16020725]
6. Frasch AC. Functional diversity in the trans-sialidase and mucin families in *Trypanosoma cruzi*. *Parasitol Today* 2000;16:282–286. [PubMed: 10858646]
7. Lau LL, Jiang J, Shen H. In vivo modulation of T cell responses and protective immunity by TCR antagonism during infection. *J Immunol* 2005;174:7970–7976. [PubMed: 15944303]
8. Rodrigues MM, Alencar BC, Claser C, Tzelepis F. Immunodominance: a new hypothesis to explain parasite escape and host/parasite equilibrium leading to the chronic phase of Chagas' disease? *Braz J Med Biol Res* 2009;42:220–223. [PubMed: 19287899]
- 9•. Tzelepis F, de Alencar BC, Penido ML, Claser C, Machado AV, Bruna-Romero O, Gazzinelli RT, Rodrigues MM. Infection with *Trypanosoma cruzi* restricts the repertoire of parasite-specific CD8 + T cells leading to immunodominance. *J Immunol* 2008;180:1737–1748. [PubMed: 18209071] The authors examine CD8+ T cell responses in a number of *T. cruzi* strain: mouse strain combinations and suggest that competition for antigen presentation may restrict responses to some epitopes.
- 10•. Alvarez MG, Postan M, Weatherly DB, Albareda MC, Sidney J, Sette A, Olivera C, Armenti AH, Tarleton RL, Laucella SA. HLA Class I-T Cell Epitopes from trans-Sialidase Proteins Reveal Functionally Distinct Subsets of CD8 T Cells in Chronic Chagas Disease. *PLoS Negl Trop Dis* 2008;2:e288. [PubMed: 18846233] The authors conducted a broad screen of potential *T. cruzi* epitopes recognized by humans with chronic *T. cruzi* infection. In addition to identifying a number of promising targets recognized by different subjects, this study shows that most CD8+ T cells in these subjects are monofunctional, secreting only IFN-gamma, and thus “exhausting”.
11. Diez H, Lopez MC, Del Carmen Thomas M, Guzman F, Rosas F, Velazco V, Gonzalez JM, Puerta C. Evaluation of IFN-gamma production by CD8 T lymphocytes in response to the K1 peptide from KMP-11 protein in patients infected with *Trypanosoma cruzi*. *Parasite Immunol* 2006;28:101–105. [PubMed: 16441508]
12. Tzelepis F, Persechini PM, Rodrigues MM. Modulation of CD4 T cell-dependent specific cytotoxic CD8 T cells differentiation and proliferation by the timing of increase in the pathogen load. *PLoS ONE* 2007;2:e393. [PubMed: 17460760]
13. Tzelepis F, de Alencar BC, Penido ML, Gazzinelli RT, Persechini PM, Rodrigues MM. Distinct kinetics of effector CD8+ cytotoxic T cells after infection with *Trypanosoma cruzi* in naive or vaccinated mice. *Infect Immun* 2006;74:2477–2481. [PubMed: 16552083]
14. Giddings OK, Eickhoff CS, Smith TJ, Bryant LA, Hoft DF. Anatomical route of invasion and protective mucosal immunity in *Trypanosoma cruzi* conjunctival infection. *Infect Immun* 2006;74:5549–5560. [PubMed: 16988230]
15. Martin DL, Postan M, Lucas P, Gress R, Tarleton RL. TGF-beta regulates pathology but not tissue CD8+ T cell dysfunction during experimental *Trypanosoma cruzi* infection. *Eur J Immunol* 2007;37:2764–2771. [PubMed: 17823982]

16. Kotner J, Tarleton R. Endogenous CD4(+) CD25(+) regulatory T cells have a limited role in the control of *Trypanosoma cruzi* infection in mice. *Infect Immun* 2007;75:861–869. [PubMed: 17101658]
17. Vaena de Avalos S, Blader IJ, Fisher M, Boothroyd JC, Burleigh BA. Immediate/early response to *Trypanosoma cruzi* infection involves minimal modulation of host cell transcription. *J Biol Chem* 2002;277:639–644. [PubMed: 11668183]
18. Tarleton RL. Immune system recognition of *Trypanosoma cruzi*. *Curr Opin Immunol*. 2007
19. Davenport MP, Belz GT, Ribeiro RM. The race between infection and immunity: how do pathogens set the pace? *Trends Immunol* 2009;30:61–66. [PubMed: 19138564]
- 20••. Bixby LM, Tarleton RL. Stable CD8+ T cell memory during persistent *Trypanosoma cruzi* infection. *J Immunol* 2008;181:2644–2650. [PubMed: 18684955] Demonstration that a proportion of *T. cruzi*-specific CD8⁺ T cells throughout *T. cruzi* infection in mice have the phenotype of stable, central memory T cells - despite the persistence of parasites and antigen in this chronic infection. In addition to expressing Tcm phenotype markers, these T cells are antigen independent and respond robustly to re-exposure to parasites, showing that stable T cell memory can develop in the presence of antigen persistence.
21. Padilla A, Xu D, Martin D, Tarleton R. Limited role for CD4+ T-cell help in the initial priming of *Trypanosoma cruzi*-specific CD8+ T cells. *Infect Immun* 2007;75:231–235. [PubMed: 17043105]
22. Combs TP, Nagajyothi, Mukherjee S, de Almeida CJ, Jelicks LA, Schubert W, Lin Y, Jayabalan DS, Zhao D, Braunstein VL, et al. The adipocyte as an important target cell for *Trypanosoma cruzi* infection. *J Biol Chem* 2005;280:24085–24094. [PubMed: 15843370]
23. Tarleton RL, Sun J, Zhang L, Postan M. Depletion of T-cell subpopulations results in exacerbation of myocarditis and parasitism in experimental Chagas' disease. *Infect Immun* 1994;62:1820–1829. [PubMed: 8168945]
24. Leavey JK, Tarleton RL. Cutting Edge: Dysfunctional CD8(+) T Cells Reside in Nonlymphoid Tissues During Chronic *Trypanosoma cruzi* Infection. *J Immunol* 2003;170:2264–2268. [PubMed: 12594245]
25. Wherry EJ, Barber DL, Kaech SM, Blattman JN, Ahmed R. Antigen-independent memory CD8 T cells do not develop during chronic viral infection. *Proc Natl Acad Sci U S A* 2004;101:16004–16009. [PubMed: 15505208]
26. Shin H, Blackburn SD, Blattman JN, Wherry EJ. Viral antigen and extensive division maintain virus-specific CD8 T cells during chronic infection. *J Exp Med* 2007;204:941–949. [PubMed: 17420267]
27. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 2006;443:350–354. [PubMed: 16921384]
28. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2006;439:682–687. [PubMed: 16382236]
29. Shin H, Wherry EJ. CD8 T cell dysfunction during chronic viral infection. *Curr Opin Immunol* 2007;19:408–415. [PubMed: 17656078]
30. Rehermann B. Chronic infections with hepatotropic viruses: mechanisms of impairment of cellular immune responses. *Semin Liver Dis* 2007;27:152–160. [PubMed: 17520515]
31. Cush SS, Anderson KM, Ravneberg DH, Weslow-Schmidt JL, Flano E. Memory generation and maintenance of CD8+ T cell function during viral persistence. *J Immunol* 2007;179:141–153. [PubMed: 17579032]
32. Klenerman P, Hill A. T cells and viral persistence: lessons from diverse infections. *Nat Immunol* 2005;6:873–879. [PubMed: 16116467]
33. Cush SS, Flano E. Protective antigen-independent CD8 T cell memory is maintained during {gamma}-herpesvirus persistence. *J Immunol* 2009;182:3995–4004. [PubMed: 19299697]
34. Laucella SA, Postan M, Martin D, Fralish BH, Albareda MC, Alvarez MG, Lococo B, Barbeieri G, Viotti RJ, Tarleton RL. Frequency of Interferon-gamma-producing T cells specific for *Trypanosoma cruzi* inversely correlates with disease severity in chronic human Chagas disease. *J Infectious Diseases* 2004;189:909–918. [PubMed: 14976609]

35. Albareda MC, Laucella SA, Alvarez MG, Armenti AH, Bertochi G, Tarleton RL, Postan M. Trypanosoma cruzi modulates the profile of memory CD8+ T cells in chronic Chagas' disease patients. *Int Immunol*. 2006

Tb927.2.22	----	MVNST	-----	WVST	-----	GLAGAGIT	FTFGTARGG	SVGS	-----	QVGH	-----	THV	-----	LTIE	THGAPPA	VLVTRYL	YNSYID	IFETD	-----	-DKMHM	QVAGYSGTSGQMAL	MQHIAF	124		
Tb94bm.2.52	----	AVST	-----	WVST	-----	GLAGAGIT	FTFGTARGG	SVGS	-----	QVGH	-----	THV	-----	LTIE	THGAPPA	VLVTRYL	YNSYID	IFETD	-----	-DKMHM	QVAGYSGTSGQMAL	MQHIAF	124		
Tb94bm.206	----	PAVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb927.7.3	----	PAVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb927.7.3	----	PAVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb927.5.64	----	PAVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb927.5.64	----	PAVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb94bm.169	----	PAVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb927.7.4	----	PAVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb927.7.4	----	PAVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
cong1530c	----	PAQSN	-----	WVST	-----	VTSD	ADSL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125
Tb927.7.68	----	PAVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb94bm.363	----	PAVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
cong94150	----	ELTET	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb94bm.1080c	----	GHAST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb844	8----	AVST	-----	WVST	-----	AAVDSGL	GLQKND	SVGS	-----	VBSE	-----	GOOQ	-----	YH	-----	QLAET	QNTSIV	SVL	-----	-DVCRA	GAQVSGA	VEDCSQ	NTGIVA	125	
Tb11.01.32	----	DLGDS	-----	WVST	-----	AAKTLING	FTSGN	RAV	-----	YH	-----	NTG	-----	YH	-----	VDPR	DSKTE	VLV	-----	-DDMW	VTGGRHV	FTGS	SHINTV	123	
Tb94bm.400	----	DLGDS	-----	WVST	-----	AAKTLING	FTSGN	RAV	-----	YH	-----	NTG	-----	YH	-----	VDPR	DSKTE	VLV	-----	-DDMW	VTGGRHV	FTGS	SHINTV	123	
Tc10275.35	----	NPSEN	-----	WVST	-----	KGGA	AAK	EDG	QV	-----	QV	-----	QV	-----	QV	-----	QV	-----	QV	-----	-DDMW	VTGGRHV	FTGS	SHINTV	123

Figure 1.

Alignment of regions of selected *trans*-sialidase genes from related Trypanosomatids showing homologies to the H2K^b binding motif XX(Y,P)X(F,Y)XX(V,L,M,I): A set of *trans*-sialidase protein sequences from *T. brucei* (prefix “Tb”), *T. brucei gambiense* (“Tbgamb”), *T. congolense* (“congo”), *T. vivax* (“Tviv”), *T. rangelli* (“Tr”) were aligned with a TSKb-20-containing region from a single *T. cruzi* (“Tc”) *trans*-sialidase family gene (Tc10275.35). The TSKb20 peptide (ANYKFTLV) is underlined. The gray-scale shading indicates the degree of homology at that particular residue (black=perfect identity, gray=conserved, white=non-conserved).