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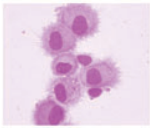
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An immunoinformatic approach for identification of *Trypanosoma cruzi* HLA-A2-restricted CD8⁺ T cell epitopes

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Keywords: Chagas disease, bioinformatics, CD8⁺ T cell, vaccine, epitope, protozoan parasite, immunity, Human leukocyte antigen (HLA)

Chagas disease is a major neglected tropical disease caused by persistent chronic infection with the protozoan parasite *Trypanosoma cruzi*. An estimated 8 million people are infected with *T. cruzi*, however only 2 drugs are approved for treatment and no vaccines are available. Thus there is an urgent need to develop vaccines and new drugs to prevent and treat Chagas disease. In this work, we identify T cell targets relevant for human infection with *T. cruzi*. The *trans*-sialidase (TS) gene family is a large family of homologous genes within the *T. cruzi* genome encoding over 1,400 members. There are 12 highly conserved TS gene family members which encode enzymatically active TS (functional TS; F-TS), while the remaining TS family genes are less conserved, enzymatically inactive and have been hypothesized to be involved in immune evasion (non-functional TS; NF-TS). We utilized immunoinformatic tools to identify HLA-A2-restricted CD8⁺ T cell epitopes conserved within F-TS family members and NF-TS gene family members. We also utilized a whole-genome approach to identify T cell epitopes present within genes which have previously been shown to be expressed in life stages relevant for human infection (Non-TS genes). Thirty immunogenic HLA-A2-restricted CD8⁺ T cell epitopes were identified using IFN- γ ELISPOT assays after vaccination of humanized HLA-A2 transgenic mice with mature dendritic cells pulsed with F-TS, NF-TS, and Non-TS peptide pools. The immunogenic HLA-A2-restricted T cell epitopes identified in this work may serve as potential components of an epitope-based T cell targeted vaccine for Chagas disease.

Over 1 billion people are infected with neglected tropical diseases (NTD), which predominantly affect poor people in developing countries.¹ Chagas disease is one such NTD, caused by chronic infection with the protozoan parasite *Trypanosoma cruzi*. An estimated 8 million people are infected with this tropical parasite resulting in thousands of deaths per year.² Due to emigration, Chagas disease is now a public health concern in many regions and countries throughout the world. For example, 300,000 people in the United States are estimated to be infected with *T. cruzi*.³ Up to 40% of infected individuals go on to develop clinical manifestations associated with chronic Chagas disease which include both cardiac (cardiomyopathy) and gastrointestinal abnormalities (megaesophagus and megacolon).² There are currently no vaccines available for prevention of *T. cruzi* infection. Drugs including nifurtimox and benznidazole have proven effective at treating *T. cruzi* infection, but both are associated with many adverse reactions and are not well tolerated in a large number of patients.⁴ However, the utilization of these drugs

has challenged and disproven the belief that the underlying cause of chronic Chagas disease is autoimmunity.^{5–9} In recent years there have been significant efforts to develop prophylactic and therapeutic vaccines as well as new drugs for the prevention and treatment of Chagas disease.

T. cruzi is primarily transmitted to humans by insect-derived metacyclic trypomastigotes (MT) present in the excreta of triatomine (reduviid) insects.² Epimastigotes present in the insect midgut differentiate into highly infectious MT with migration into the hindgut. Triatomines ingest blood from a variety of mammals, and quickly begin processing the blood meal. MT are flushed from the hindgut during the defecation process and are capable of initiating host infection through breaks in the skin (i.e., the insect bite site), or by entry through mucosal routes such as the eyes or mouth. Once inside the cell, MT differentiate into amastigotes (AMA) which are the only dividing parasite form present in humans. After several rounds of division, AMA differentiate into blood-form trypomastigotes (BFT) which are

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released upon cell lysis. These BFT can infect local cells, other cells in the body after dissemination through the circulation, or new insects. Proteins expressed in parasite life stages relevant for human infection (MT, AMA, and BFT) are worth consideration as potential targets of vaccine-induced immunity in humans and other susceptible mammals.

Immunity to *T. cruzi* infection is multifaceted involving a variety of cell types. *T. cruzi* infection induces robust B cell (antibody) responses in both mice and humans. However, antibody-secreting B cells may function more importantly to prevent CD8⁺ T cell exhaustion during chronic *T. cruzi* infection as we have recently described.¹⁰ Both CD4⁺ and CD8⁺ T cells are critical in the development of protective immunity.^{11–14} We recently demonstrated that dendritic cell vaccines pulsed with only a single CD4⁺ T cell epitope and a single CD8⁺ T cell epitope from *T. cruzi* *trans*-sialidase can protect mice against highly virulent *T. cruzi* challenge (manuscript submitted). CD4⁺ T cells are important in the priming of parasite-specific immunity, whereas CD8⁺ T cells are essential for effector function and parasite clearance. Once immunity is generated, CD8⁺ T cells alone can protect mice against parasite challenge as shown in adoptive transfer experiments.¹⁵ In humans infected with *T. cruzi*, vigorous CD8⁺ T cell responses have been reported.^{16–18} Furthermore, frequencies of parasite-specific CD8⁺ IFN- γ -producing T cells inversely correlate with severity of Chagas disease progression.¹⁶ Therefore, it is essential that vaccines target the induction of CD8⁺ T cell responses to offer optimal protective immunity.

The overwhelming majority of studies investigating immunity to *T. cruzi* infection are performed in mice. Murine models of *T. cruzi* infection offer clear cut answers to basic science questions, but may not be an effective proxy for studying human immune responses. T cell responses are not only species specific, but also MHC allele specific. Thus the specific peptides immunogenic in one mouse strain will likely not be immunogenic in other strains of mice or humans. Transgenic mice expressing human MHC (human leukocyte antigen; HLA) are useful tools for identifying T cell epitopes relevant for humans. Approximately 50% of humans express MHC class I alleles which share similar binding profiles and belong to the HLA-A2 supertype.¹⁹ T cell epitopes immunogenic in HLA-A2 transgenic mice therefore have a high likelihood of being immunogenic in many humans. The goal of our work here is to identify HLA-A2-restricted CD8⁺ T cell epitopes immunogenic in HLA-A2 transgenic mice that could help facilitate translational Chagas vaccine development efforts.

Multi-epitope vaccines are being investigated for numerous pathogens, including HIV, influenza, vaccinia, and others. While whole proteins and partial proteins have several vaccine development benefits in terms of antigen generation, testing, and scaling up of production for human use, there are certain disadvantages inherent in these types of vaccines. First, when choosing a single protein or multiple single proteins as vaccine candidates, the sheer number of T cell epitopes within these proteins is quite limited (in terms of absolute number of epitopes capable of stimulating T cells restricted by diverse HLA alleles expressed by all human populations at risk). Generating multi-epitope vaccines allows one to design and incorporate multiple highly immunogenic T cell

epitope that target known MHC alleles and supertypes. The resulting multi-epitope vaccines can thus achieve robust ‘population coverage’ ensuring that the vaccines can elicit immune responses in individuals with diverse HLA expression. Additionally, within any protein there are sequences present which share similarity between organisms (i.e., parasite and human). An immunoinformatic vaccine design approach with a strategy in place to identify and remove “self” homologous sequences allows vaccinologists to eliminate any potential cause of vaccine-induced autoimmunity or amplification of T regs involved in prevention of autoimmunity. This is particularly relevant when designing Chagas vaccines, as previous evidence suggests the disease is caused at least in part by autoimmunity.

As described above, the genome of *T. cruzi* contains multiple large gene families, and researchers have hypothesized that these gene families may have evolved as a mechanism of immune evasion.²⁰ The largest *T. cruzi* gene family is the *trans*-sialidase (TS) gene family. TS transfers sialic acid from host cells onto the parasite surface – a process known to be important in parasite infectivity.²¹ While some studies have suggested the dampening of immunity by TS family proteins, we and others have shown that TS antigens are highly immunogenic in both mice and humans.^{14,15,22,23} Immunization of multiple susceptible mouse strains with TS vaccines expressing enzymatically active (functional) TS antigens elicit robust T cell responses protective against highly lethal systemic *T. cruzi* challenges. TS gene family members are being pursued by multiple investigators as viable vaccine candidates, however, the most relevant T cell epitopes for human infection are unknown. One goal of our work described here is to identify T cell targets restricted by a major human MHC I supertype (HLA-A2) conserved within TS gene family members.

As shown in **Figure 1A**, nearly half of the 1,430 TS gene family members are pseudogenes, and of the remaining 735 TS genes, only 12 encode proteins with enzymatic activity.^{20,24,25} These 12 functional TS (F-TS) genes are highly homologous, sharing 75% to over 90% sequence identity to the consensus *T. cruzi* TS enzyme sequence (GenBank accession D50685). Conversely, the 723 non-functional TS (NF-TS) genes are much more diverse, and largely of unknown function. In order to identify T cell epitopes conserved within F-TS and NF-TS gene sets, we employed a 2-staged approach using immunoinformatic tools developed by EpiVax. First, the Conservatrix algorithm was used to identify 9-mer and 10-mer peptides that are conserved within F-TS and NF-TS gene families.²⁶ Briefly, input protein sequences were parsed into overlapping 9-mer or 10-mer frames where each frame overlaps the last by 8 or 9 amino acids, respectively. Identical 9-mer (or 10-mer) sequences were then grouped and sequences which were $\geq 50\%$ conserved among the 12 F-TS genes or expressed by $\geq 5\%$ of NF-TS genes were collected. Second, the EpiMatrix algorithm was used to screen the conserved sequences for predicted MHC binding affinity with respect to a panel of 6 MHC class I “supertype” alleles.^{26–28} For each amino acid in a given input sequence, the EpiMatrix algorithm retrieves a position specific binding coefficient. The individual coefficients are summed to produce a raw EpiMatrix score. Raw scores are normalized with respect to a large sample of randomly generated peptides.

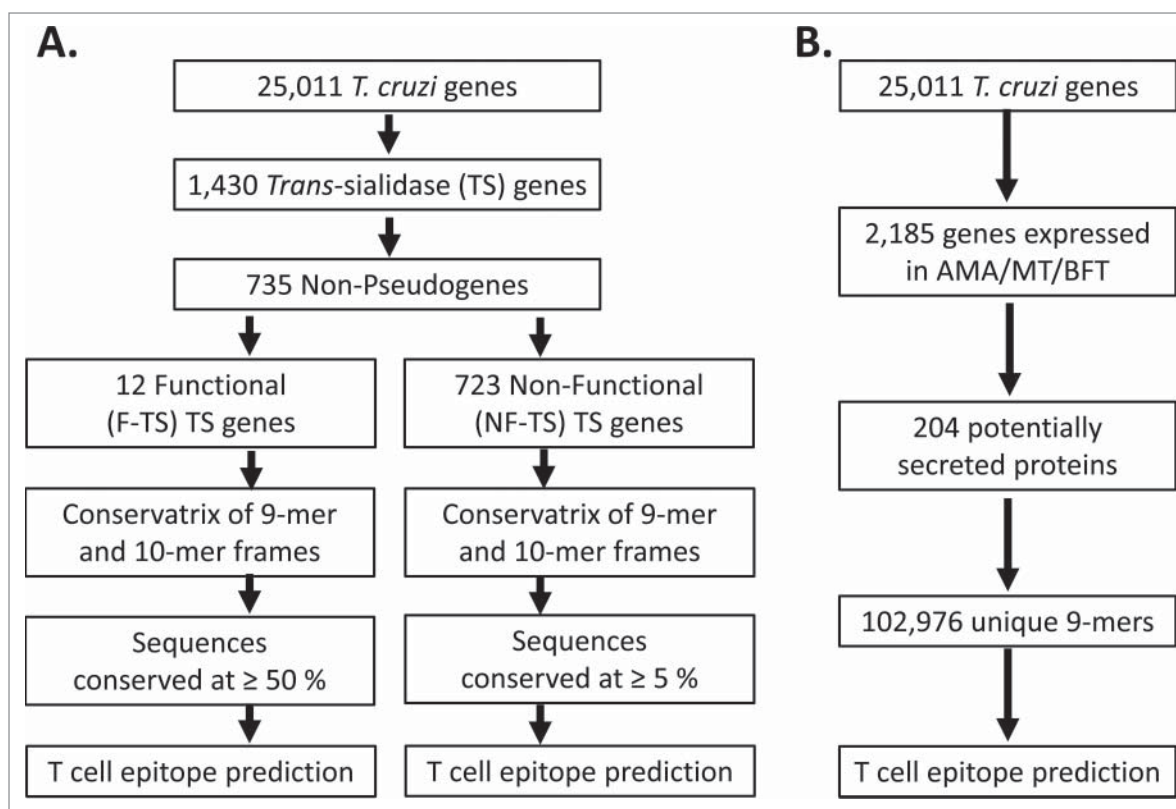


Figure 1. Immunoinformatic strategy for identification of HLA-A2-restricted CD8⁺ T cell epitopes from *T. cruzi*. Genomic and proteomic data from TriTrypDB²⁵ was mined in order to identify HLA-restricted T cell epitopes in the highly conserved functional (enzymatically active) TS family (F-TS) and the more diverse non-functional TS family (NF-TS), or within other antigens expressed in parasite life stages relevant for human immunity (Non-TS). Conservation and epitope prediction analyses were performed using Conservatrix and EpiMatrix, respectively, in order to identify relatively conserved HLA-A2 binders in F-TS and NF-TS gene family members (**A**). Potentially secreted *T. cruzi* proteins shown to be expressed by mass spectrometry in amastigote (AMA), insect-borne infectious metacyclic trypomastigote (MT) and blood form trypomastigote (BFT) equivalent life stages were identified and EpiMatrix utilized to predict HLA-A2 binding peptides (Non-TS genes; **B**).

EpiMatrix scores ≥ 1.64 , representing the top 5% of peptides, have a high likelihood to bind to the represented MHC-I super-type. Taken collectively the 6 HLA supertypes included in this analysis cover >95% of the human population. Peptides predicted to bind with HLA-A2 were checked for homology to known human sequences using BlastP. After screening out human homologues, the top 6 HLA-A2 binding peptides, for both the F-TS and NF-TS gene sets, were selected for further study.

In addition to identifying potential CD8⁺ T cell epitopes within F-TS and NF-TS gene family members, we utilized a 'whole genome approach' to identify a more diverse set of T cell epitopes as shown in **Figure 1B**. The genome and proteomes of all 4 major life stages of *T. cruzi* were analyzed in 2005, and data made available through the TriTrypDB.²⁵ The 2,185 genes expressed in life stages relevant for human infection (MT, AMA, and BFT), according to mass spectrometry evidence, were gathered for analysis utilizing tools on the TriTrypDB. Since secreted proteins are more likely to be recognized by the mammalian immune system because of delivery to the host cell cytoplasm, we narrowed our strategy to include genes with predicted signal sequences but without multiple predicted trans-membrane domains or potential lipid attachment sites utilizing publicly

available tools (TriTrypDB, LipoP, Phobius, SignalP). The amino acids sequences of 204 selected secreted proteins were parsed into 9-mer frames and then each frame was scored for predicted MHC I binding to 6 major supertypes using EpiMatrix as described above. We refer to epitopes predicted in this gene set as Non-TS epitopes (since TS sequences were included in the other gene set analyses and were thus excluded here). This diverse set of epitopes included sequences from mucin-associated surface proteins (MASP), gp63 surface proteases, cruzipain precursors, piti-lysin-like metalloproteases, and others.

We next developed a strategy involving peptide vaccination of HLA-A2 transgenic mice to identify immunogenic CD8⁺ T cell epitopes rather than performing MHC binding assays which do not confirm T cell reactivity. All animal studies were conducted in AAALAC accredited facilities and were approved by the Saint Louis University Institutional Animal Care and Use Committee. HLA-A2 HHDII transgenic mice were provided by Ted Hansen (Washington University) with permission from the Institut Pasteur and were bred and housed under pathogen-free conditions throughout our studies.²⁹ These mice are devoid of normal murine MHC I expression, and express a chimeric HLA-A2 molecule consisting of human $\beta 2m$ and the $\alpha 1$ - $\alpha 2$ domains of

HLA-A2.1 fused to the $\alpha 3$ and cytoplasmic domains of H-2D^b.²⁹ In order to prepare dendritic cells (DC) for use in peptide/DC vaccination experiments, 5×10^6 B16-Flt3L cells were injected into 3-month old female HLA-A2 transgenic mice to induce expansion of immature DC as previously described.³⁰ Two weeks later, DC were matured in vivo for 16–18 hours by intravenous injection of LPS (2 $\mu\text{g}/\text{mouse}$). Spleens were isolated and digested using collagenase and DNase, mechanically dispersed, processed to lyse red blood cells, and then CD11c⁺ cells were purified using magnetic beads following manufacturer guidelines (Miltenyi biotec). Mature DC (mDC) were suspended in complete DC media (2 parts fresh complete media plus 1 part B16-Flt3L conditioned media) supplemented with 1,000 U/ml GM-CSF, and pulsed with pools of F-TS, NF-TS, or Non-TS

predicted HLA-A2 binders (JPT Peptide Technologies) at 5 $\mu\text{g}/\text{ml}$ of each peptide for 2 hours. Mature DC were also pulsed with the known I-A^b-restricted CD4⁺ T cell epitope OVA₃₂₃₋₃₃₉ to provide CD4⁺ T cell help for the development of CD8⁺ T cell responses. Mice were vaccinated twice, 2 weeks apart with 1×10^6 peptide-pulsed mDC intravenously.

Four weeks following the final vaccination, individual peptide-specific CD8⁺ T cell responses were studied using IFN- γ ELISPOT assays.³¹ Briefly, splenic CD8⁺ T cells were isolated from control and peptide-pulsed DC vaccinated mice using Miltenyi CD8 microbeads and added to IFN- γ ELISPOT assays (1×10^5 CD8⁺ T cells/well; N = 2–3 mice/group). Naïve total spleen cells were pulsed with individual peptides (5 $\mu\text{g}/\text{ml}$ final concentration) and used as stimulator cells in these ELISPOT

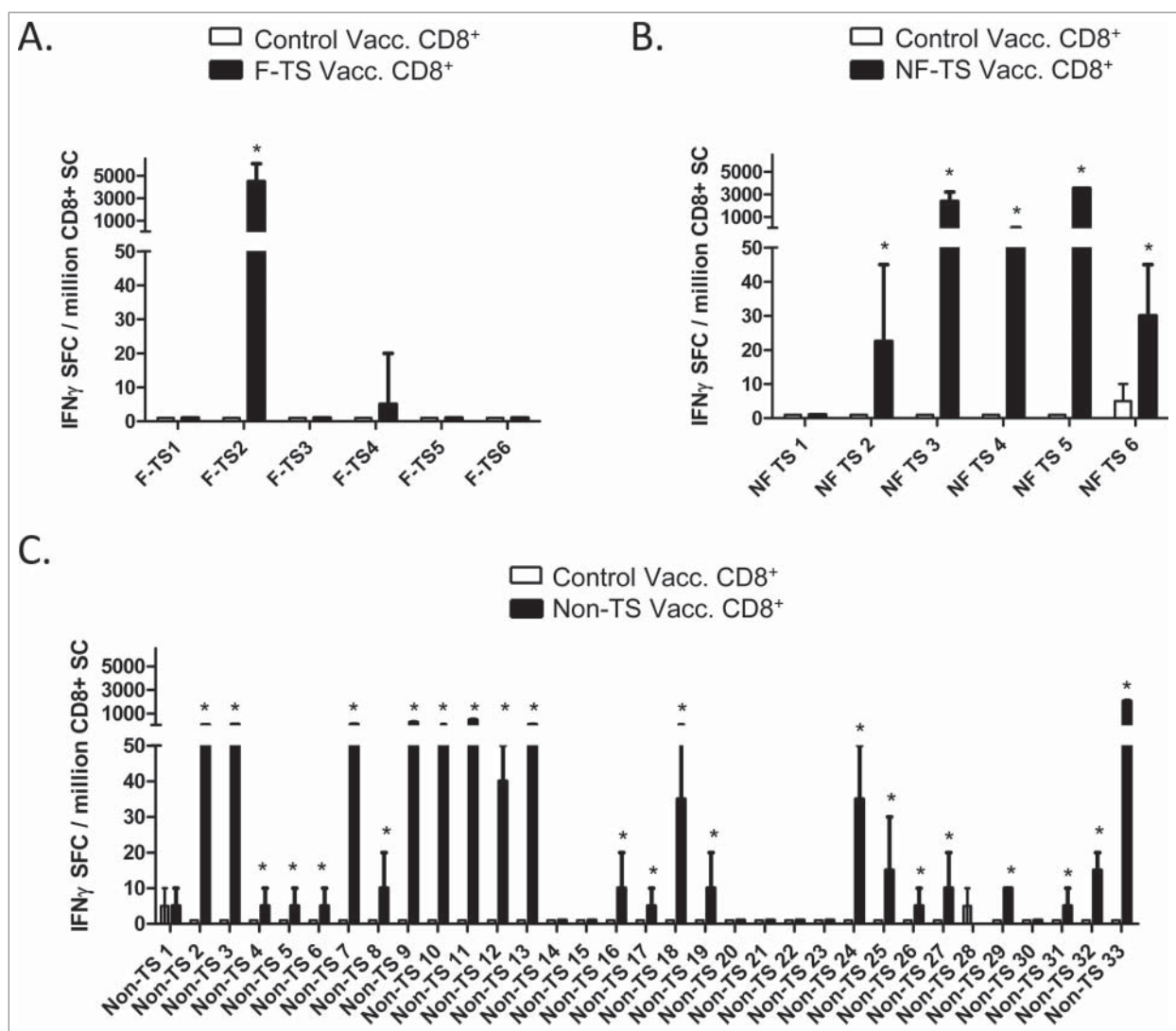


Figure 2. Immunogenicity of predicted HLA-A2-restricted *T. cruzi* epitopes. HLA-A2 transgenic mice were vaccinated twice, 2 weeks apart with mature dendritic cells pulsed with functional TS (F-TS; **A**), Non-functional TS (NF-TS; **B**), or whole genome derived Non-TS peptide pools (**C**). One month later, CD8⁺ T cells were isolated from control DC or peptide-pool pulsed DC vaccinated mice and stimulated overnight with APCs pulsed with individual F-TS, NF-TS, or Non-TS peptides in IFN- γ ELISPOT assays. Shown are mean \pm SE from representative experiments expressed as IFN- γ spot forming cells (SFC) per million CD8⁺ T cells above DMSO control. Asterisks denote immunogenic peptides defined as responses greater than the mean +2 SE of all control CD8⁺ T cell responses.

assays (3×10^5 per well). After overnight co-culture, IFN- γ ELISPOT plates were developed and spots enumerated as previously described.³¹ Shown in **Figure 2** are results from these experiments (background values subtracted). As expected, CD8⁺ T cells from mice vaccinated with control mDC did not respond to the predicted HLA-A2-restricted T cell epitopes. In contrast, many of the TS family and Non-TS predicted peptides elicited responses in mice vaccinated with the dendritic cells pulsed with peptides. The highly conserved functional TS family epitope F-TS-2 elicited the strongest response of any peptide studied in these assays (nearly 5,000 IFN- γ SFC/million CD8⁺ T cells; **Fig. 2A**). Several responses were elicited by stimulation of T cells from mice vaccinated with NF-TS peptides (5 of 6 NF-TS peptides were immunogenic; **Fig. 2B**). In addition, 24 Non-TS epitopes were found to be HLA-A2-restricted CD8⁺ T cell epitopes (**Fig. 1B**). In total, we identified 30 HLA-A2-restricted CD8⁺ T cell epitopes using a novel approach involving genomic, proteomic, and immunoinformatic analyses.

Previous studies aimed at CD8⁺ T cell epitope discovery in human-translatable models have focused on identifying T cell epitopes from either single *T. cruzi* proteins (i.e., paraflagellar rod proteins)³² or a small number of similar *T. cruzi* genes within the TS superfamily^{33,34}. Even though these proteins are immunogenic in mice (and to some extent in humans), there are concerns that developing vaccines targeting single proteins may not contain sufficient epitopes for induction of immunity in populations expressing diverse HLA alleles. Furthermore, it has been postulated that *T. cruzi* has developed complicated mechanisms to evade host cell immunity allowing for parasite persistence. The TS superfamily with 1,430 gene members is significantly overrepresented in the *T. cruzi* genome, however, the complexity of the TS superfamily and immunodominance patterns of specific TS CD8⁺ T cell epitopes are not well understood.^{20,24,35,36} The mechanism responsible for evolution and expansion of the TS gene superfamily is not known. It has been hypothesized that TS antigens may dampen overall immunity through generation of altered peptide ligands, possibly contributing to parasite persistence.³⁷ However, we and others have shown that TS is highly immunogenic in both mice and humans, and TS vaccines induce potent immunity protective against highly lethal parasite challenge in mice.^{14,15,22,23} While there is little evidence to support the belief that the large TS gene superfamily in *T. cruzi* evolved to evade host-cell mediated immunity, generating immunity to a diverse group of proteins using multi-epitope T cell targeted vaccines might prove to be a sensible tactic to circumvent these hypothesized parasite persistence strategies. We have utilized a novel approach to identify T cell epitopes expressed by very diverse *T. cruzi* proteins. The immunogenic epitope sequences from F-TS, NF-TS, and Non-TS gene sets (shown in **Fig. 2** and summarized in **Table 1**) are encoded by genes with diverse functions. In our limited study, we have identified T cell epitopes from enzymatically active as well as more diverse non-functional TS gene family members (6 total conserved immunogenic TS superfamily epitopes). In addition, we have identified 24 immunogenic HLA-A2-restricted epitopes from at least 13 different proteins using a modified

Table 1. Immunogenic HLA-A2 -restricted CD8⁺ T cell epitopes

Peptide ID	Sequence	Protein or Family
F-TS-2	EVYSLVFARL	F-TS family
NF-TS-2	NVLLYNRPL	NF-TS gene family
NF-TS-3	WLTDNTHIV	NF-TS gene family
NF-TS-4	NTWRDEYLGV	NF-TS gene family
NF-TS-5	KVGSDFVAV	NF-TS gene family
NF-TS-6	RVFTSAVLL	NF-TS gene family
Non-TS 2	VVMACLVPA	cruzipain precursor
Non-TS 3	TLLFQVLLL	surface protease GP63
Non-TS 4	RIGDVCAEV	surface protease GP63
Non-TS 5	LLFQVLLLC	surface protease GP63
Non-TS 6	YVVSLLADT	histone H3
Non-TS 7	ALHSLVFL	hypothetical protein
Non-TS 8	AVIPSTFPL	ATP synthase
Non-TS 9	SLFPFFFFV	hypothetical protein
Non-TS 10	FLVDTIYSI	citrate synthase
Non-TS 11	YLDAVFYPL	pitrilysin-like metalloprotease
Non-TS 12	SLLCVISFI	retrotransposon hot spot protein
Non-TS 13	ALLPWLLVL	hypothetical protein
Non-TS 16	LMNDVWFSL	heat shock protein 70
Non-TS 17	FLIFRFMVL	hypothetical protein
Non-TS 18	FMACHTNPI	hypothetical protein
Non-TS 19	MMHPFLCAL	surface protein ToIT
Non-TS 24	SMNAGLYLL	retrotransposon hot spot protein
Non-TS 25	AVFDSVYSV	surface protease GP63
Non-TS 26	FLNQFGTRL	hypothetical protein
Non-TS 27	SMQEYRHMV	dynein light chain
Non-TS 29	SVVSVFLL	histone H3
Non-TS 31	QMLHNVASL	pumilio/PUF RNA binding protein
Non-TS 32	MMMTGRVLL	mucin-associated surface protein
Non-TS 33	TLMDFCPYI	surface protease GP63

whole-genome epitope identification approach. Future studies should investigate the relative levels of protective immunity induced by vaccination with conserved F-TS, NF-TS, and diverse Non-TS epitopes described here.

The HLA-A2 transgenic mice utilized in the studies above are extremely susceptible to *T. cruzi* infection. The LD₅₀ in this mouse strain is less than 100 parasites, even when using relatively non-virulent culture-derived metacyclic trypomastigotes (CMT) challenges (not shown). Although we have been unable to induce long-term survival in naïve HLA-A2 transgenic animals after even low level parasite infection (preventing us from studying infection-induced T cell responses to the above epitopes), vaccines encoding multiple CD8⁺ T cell epitopes may be able to confer some degree of protection in this mouse strain. Our efforts to investigate protective immunity induced by multi-epitope CD8⁺ T cell targeted vaccines are ongoing. Liposomal-peptide formulations, naked DNA vaccines, adjuvanted recombinant protein, and attenuated viral vectors will be utilized to deliver multi-epitope antigens expressed in a single open reading frame (ORF). However, the optimal format, dose, and delivery routes are not well established. Future studies investigating protective immunity induced by multi-epitope T cell targeted *T. cruzi* vaccines should give special attention to these details.

An HLA supertype is a group of genetically distinct HLA alleles which share similar peptide binding properties. Nine MHC class I supertypes have been identified.³⁸ However, >95% of individuals carry at least one HLA allele classified

into one of 6 most common MHC I supertypes. An estimated 50% of individuals from wide-ranging backgrounds express the HLA-A2 supertype.¹⁹ In the current study we identified 30 diverse CD8⁺ T cell epitopes restricted by this major supertype (A2). The logical extension of our work described above is to develop multi-epitope vaccines to induce protective immunity in diverse human populations. Therefore, additional CD8⁺ T cell epitopes restricted by the other HLA supertypes need to be identified. Similar strategies to those described in this report utilizing EpiMatrix prediction of F-TS, NF-TS, and Non-TS gene sets restricted by additional HLA supertypes should be employed to identify additional T cell epitopes relevant for diverse human populations. Long-term strategies to develop either customized vaccines based on an individual's HLA type or a master vaccine encoding different T cell epitopes restricted by all 6 major supertypes should be investigated. The optimal number of epitopes needed to cover all populations, as well as the optimal vaccine formulations (DNA, viral vector, adjuvanted recombinant protein) must be carefully studied. Evidence from animal studies suggests that the number of epitopes required for full protection is a small and definable subset (less than 50) [^{39,40}]; thus, achieving a payload of 50–100 epitopes that provide broad coverage of human genetic backgrounds may be sufficient to provide protection.

It is well established that optimal memory CD8⁺ T cell responses are generated in the presence of robust CD4⁺ T cell help.¹⁴ CD4⁺ T cells are critical for the development of protective immunity against *T. cruzi* infection.^{12,14} When normally protective *T. cruzi* vaccines are depleted of CD4⁺ T cell epitope regions they lose their protective capacities.¹⁴ Thus, new T cell-based *T. cruzi* vaccines should include immunogenic CD4⁺ T cell epitopes to help drive effective CD8⁺ T cell responses. MHC class II epitope prediction algorithms have been shown to be accurate, and more recently tools have been developed to identify clusters of pan DR-restricted class II epitopes.^{27,28} For example, matrix-based epitope prediction algorithms have been utilized to identify conserved variola/vaccinia CD4⁺ T cell epitopes that when formulated as multi-epitope vaccines were able to confer protection against lethal vaccinia challenges in humanized mice.^{28,41} A similar strategy should be utilized to identify promiscuous CD4⁺ T cell epitopes (or T cell epitope clusters) within the F-TS, NF-TS, and Non-TS gene sets described in this report. Exclusion of epitopes that are highly cross-conserved at the human TCR face, and may be tolerogenic, should also be taken into consideration.⁴²

References

1. WHO. Neglected tropical diseases, hidden successes, emerging opportunities. Geneva, Switzerland: World Health Organization, 2009
2. Rassi A, Jr., Rassi A, Marin-Neto JA. Chagas disease. *Lancet* 2010; 375:1388–402; PMID:20399979; [http://dx.doi.org/10.1016/S0140-6736\(10\)60061-X](http://dx.doi.org/10.1016/S0140-6736(10)60061-X)
3. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. *Clin Infect Dis* 2009; 49:e52–e4; PMID:19640226; <http://dx.doi.org/10.1086/605091>
4. Jackson Y, Alirio E, Getaz L, Wolff H, Combescure C, Chappuis F. Tolerance and safety of nifurtimox in

- patients with chronic Chagas disease. *Clin Infect Dis* 2010; 51:e69–75; PMID:20932171; <http://dx.doi.org/10.1086/656917>
5. Brandariz S, Schijman A, Vigliano C, Arteman P, Viotti R, Beldjord C, Levin MJ. Detection of parasite DNA in Chagas' heart disease. *Lancet* 1995; 346:1370–1; PMID:7475804; [http://dx.doi.org/10.1016/S0140-6736\(95\)92388-8](http://dx.doi.org/10.1016/S0140-6736(95)92388-8)
 6. Schijman AG, Vigliano CA, Viotti RJ, Burgos JM, Brandariz S, Lococo BE, Leze MI, Armentis HA, Levin MJ. *Trypanosoma cruzi* DNA in cardiac lesions of Argentinean patients with end-stage chronic chagas heart disease. *Am J Trop Med Hyg* 2004; 70:210–20; PMID:14993635

Additionally, antigen-specific B cells have been shown to play a protective role in *T. cruzi* infection as both producers of lytic antibodies for extracellular parasites reducing overall parasite burden and as antigen presenting cells. Furthermore, we have recently shown that *T. cruzi* specific B cells producing potent antibody responses can help prevent CD8⁺ T cell exhaustion.¹⁰ Recent efforts by several groups utilizing overlapping peptide arrays or high-throughput bioinformatic approaches have resulted in the identification of specific antibody epitopes in several *T. cruzi* genes.^{43–45} A 3-tiered approach focused on the inclusion of CD4⁺ T helper epitopes, CD8⁺ T cell epitopes, and B cell epitopes should be investigated for use as both prophylactic and therapeutic vaccines for the prevention and treatment of Chagas disease.

The results presented here represent the first large-scale immunoinformatics driven-effort to identify CD8⁺ T cell epitopes from diverse *T. cruzi* gene sets relevant for human infection. Many previous attempts aimed at identifying immunogenic CD8⁺ T cell epitopes have utilized standard laboratory mouse strains expressing murine MHC.^{14,46} While results from these types of studies have helped elucidate much of what we know today about immunity to *T. cruzi* infection (including mechanisms of protection), many of the epitopes will not be presented by HLA to human T cells. Here, we have utilized a multifaceted approach to identify parasite proteins and their encoded T cell epitopes restricted by human MHC, specifically HLA-A2 which is expressed by nearly half of the human population. Thus, the CD8⁺ T cell targets identified in this work are highly relevant for the development of effective vaccines to protect humans at risk of Chagas disease.

Disclosure of Potential Conflicts of Interest

Daniel Hoft, Christopher Eickhoff, and Daniel Van Aartsen declare no conflicts of interest. Annie De Groot and William Martin are senior officers and majority shareholders at EpiVax, Inc. Leonard Moise and Frances Terry are also employed at EpiVax, Inc. These authors acknowledge a potential for conflict of interest and attest that the work contained in this report is free of any bias that might be associated with the commercial goals of the company.

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- SC. *Trypanosoma cruzi*: parasite persistence in tissues in chronic chagasic Brazilian patients. Mem Inst Oswaldo Cruz 2011; 106:85-91; PMID:21340361; <http://dx.doi.org/10.1590/S0074-02762011000100014>
10. Sullivan NL, Eickhoff CS, Sagartz J, Hoft DF. Deficiency of antigen-specific B cells results in decreased *Trypanosoma cruzi* systemic but not mucosal immunity due to CD8 T cell exhaustion. J Immunol 2015; 15:1806-18; <http://dx.doi.org/10.4049/jimmunol.1303163>
 11. Tarleton RL. Depletion of CD8⁺ T cells increases susceptibility and reverses vaccine-induced immunity in mice infected with *Trypanosoma cruzi*. J Immunol 1990; 144:717-24; PMID:2104903
 12. Hoft DF, Schnapp AR, Eickhoff CS, Roodman ST. Involvement of CD4⁺ Th1 cells in systemic immunity protective against primary and secondary challenges with *Trypanosoma cruzi*. Infect Immun 2000; 68:197-204; PMID:10603388; <http://dx.doi.org/10.1128/IAI.68.1.197-204.2000>
 13. Wizel B, Nunes M, Tarleton RL. Identification of *Trypanosoma cruzi* trans-sialidase family members as targets of protective CD8⁺ T cell responses. J Immunol 1997; 159:6120-30; PMID:9550413
 14. Fujimura AE, Kinoshita SS, Pereira-Chioccola VL, Rodrigues MM. DNA sequences encoding CD4⁺ and CD8⁺ T-cell epitopes are important for efficient protective immunity induced by DNA vaccination with a *Trypanosoma cruzi* gene. Infect Immun 2001; 69:5477-86; PMID:11500420; <http://dx.doi.org/10.1128/IAI.69.9.5477-5486.2001>
 15. Eickhoff CS, Vasconcelos JR, Sullivan NL, Blazevic A, Bruna-Romero O, Rodrigues MM, Hoft DF. Co-administration of a plasmid DNA encoding IL-15 improves long-term protection of a genetic vaccine against *Trypanosoma cruzi*. PLoS NTD 2011; 5:e983; PMID:21408124
 16. Laucella SA, Postan M, Martin D, Hubby Fralish B, Albareda MC, Alvarez MG, Lococo B, Barbieri G, Vioti 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 Infect Dis 2004; 189:909-18; PMID:14976609; <http://dx.doi.org/10.1086/381682>
 17. Laucella SA, Mazliah DP, Bertocchi G, Alvarez MG, Cooley G, Vioti R, Albareda MC, Lococo B, Postan M, Armenti A, et al. Changes in *Trypanosoma cruzi*-specific immune responses after treatment: surrogate markers of treatment efficacy. Clin Infect Dis 2009; 49:1675-84; PMID:19877967; <http://dx.doi.org/10.1086/648072>
 18. Lasso P, Mesa D, Cuellar A, Guzman F, Bolanos N, Rosas F, Velasco V, Thomas Mdcl C, Lopez MC, Gonzalez JM, et al. Frequency of specific CD8⁺ T cells for a promiscuous epitope derived from *Trypanosoma cruzi* KMP-11 protein in chagasic patients. Parasite Immunol 2010; 32:494-502; PMID:20591120; <http://dx.doi.org/10.1111/j.1365-3024.2010.01206.x>
 19. Reche PA, Reinherz EL. PEPVAC: a web server for multi-epitope vaccine development based on the prediction of supertypic MHC ligands. Nucleic Acids Res 2005; 33:W138-42; PMID:15980443; <http://dx.doi.org/10.1093/nar/gki357>
 20. 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; 309:409-15; PMID:16020725; <http://dx.doi.org/10.1126/science.1112631>
 21. Schenkman S, Eichinger D, Pereira ME, Nussenzweig V. Structural and functional properties of *Trypanosoma trans-sialidase*. Annual Rev Microbiol 1994; 48:499-523; PMID:7826016; <http://dx.doi.org/10.1146/annurev.mi.48.100194.002435>
 22. Ribeiro M, Pereira-Chioccola VL, Renia L, Augusto Fragata Filho A, Schenkman S, Rodrigues MM. Chagasic patients develop a type 1 immune response to *Trypanosoma cruzi* trans-sialidase. Parasite Immunol 2000; 22:49-53; PMID:10607290; <http://dx.doi.org/10.1046/j.1365-3024.2000.00260.x>
 23. Hoft DF, Eickhoff CS, Giddings OK, Vasconcelos JR, Rodrigues MM. *Trans-sialidase* recombinant protein mixed with CpG motif-containing oligodeoxynucleotide induces protective mucosal and systemic *Trypanosoma cruzi* immunity involving CD8⁺ CTL and B cell-mediated cross-priming. J Immunol 2007; 179:6889-900; PMID:17982080; <http://dx.doi.org/10.4049/jimmunol.179.10.6889>
 24. Atwood JA, III, Weatherly DB, Minning TA, Bundy B, Cavola C, Opperdoes FR, Orlando R, Tarleton RL. The *Trypanosoma cruzi* proteome. Science 2005; 309:473-6; PMID:16020736; <http://dx.doi.org/10.1126/science.1110289>
 25. Aslett M, Aurrecochea C, Berriman M, Brestelli J, Brunk BP, Carrington M, Depledge DP, Fischer S, Gajria B, Gao X, et al. TriTrypDB: a functional genomic resource for the Trypanosomatidae. Nucleic Acids Res 2010; 38:D457-62; PMID:19843604; <http://dx.doi.org/10.1093/nar/gkp851>
 26. De Groot AS, Ardito M, McClaine EM, Moise L, Martin WD. Immunoinformatic comparison of T-cell epitopes contained in novel swine-origin influenza A (H1N1) virus with epitopes in 2008–2009 conventional influenza vaccine. Vaccine 2009; 27:5740-7; PMID:19660593; <http://dx.doi.org/10.1016/j.vaccine.2009.07.040>
 27. De Groot AS, Martin W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. Clin Immunol 2009; 131:189-201; PMID:19269256; <http://dx.doi.org/10.1016/j.clim.2009.01.009>
 28. Moise L, McMurry JA, Buus S, Frey S, Martin WD, De Groot AS. In silico-accelerated identification of conserved and immunogenic variola/vaccinia T-cell epitopes. Vaccine 2009; 27:6471-9; PMID:19559119; <http://dx.doi.org/10.1016/j.vaccine.2009.06.018>
 29. Pascolo S, Bervas N, Ure JM, Smith AG, Lemonnier FA, Perarnau B. HLA-A2.1-restricted education and cytolytic activity of CD8(+) T lymphocytes from beta2 microglobulin (beta2m) HLA-A2.1 monochain transgenic H-2Db beta2m double knockout mice. J Exp Med 1997; 185:2043-51; PMID:9182675; <http://dx.doi.org/10.1084/jem.185.12.2043>
 30. Pham NL, Badovinac VP, Harty JT. A default pathway of memory CD8 T cell differentiation after dendritic cell immunization is deflected by encounter with inflammatory cytokines during antigen-driven proliferation. J Immunol 2009; 183:2337-48; PMID:19635915; <http://dx.doi.org/10.4049/jimmunol.0901203>
 31. Sullivan NL, Eickhoff CS, Zhang X, Giddings OK, Lane TE, Hoft DF. Importance of the CCR5-CCL5 axis for mucosal *Trypanosoma cruzi* protection and B cell activation. J Immunol 2011; 187:1358-68; PMID:21715689; <http://dx.doi.org/10.4049/jimmunol.1100033>
 32. Egui A, Thomas MC, Morell M, Maranon C, Carrilero B, Segovia M, Puerta CJ, Pinazo MJ, Rosas F, Gascón J, et al. *Trypanosoma cruzi* parafagellar rod proteins 2 and 3 contain immunodominant CD8(+) T-cell epitopes that are recognized by cytotoxic T cells from Chagas disease patients. Mol Immunol 2012; 52:289-98; PMID:22750229; <http://dx.doi.org/10.1016/j.molimm.2012.05.021>
 33. 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; PMID:18846233; <http://dx.doi.org/10.1371/journal.pntd.0000288>
 34. Wizel B, Palmieri M, Mendoza C, Arana B, Sidney J, Sette A, Tarleton R. Human infection with *Trypanosoma cruzi* induces parasite antigen-specific cytotoxic T lymphocyte responses. J Clin Invest 1998; 102:1062-71; PMID:9727076; <http://dx.doi.org/10.1172/JCI3835>
 35. 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; PMID:16879036; <http://dx.doi.org/10.1371/journal.ppat.0020077>
 36. Rosenberg CS, Martin DL, Tarleton RL. CD8⁺ T cells specific for immunodominant trans-sialidase epitopes contribute to control of *Trypanosoma cruzi* infection but are not required for resistance. J Immunol 2010; 185:560-8; PMID:20530265; <http://dx.doi.org/10.4049/jimmunol.1000432>
 37. Plebanski M, Lee EA, Hannan CM, Flanagan KL, Gilbert SC, Gravenor MB, Hill AV. Altered peptide ligands narrow the repertoire of cellular immune responses by interfering with T-cell priming. Nat Med 1999; 5:565-71; PMID:10229235; <http://dx.doi.org/10.1038/8444>
 38. Sidney J, Peters B, Frahm N, Brander C, Sette A. HLA class I supertypes: a revised and updated classification. BMC Immunol 2008; 9:1; PMID:18211710; <http://dx.doi.org/10.1186/1471-2172-9-1>
 39. Moise L, De Groot AS. Putting immunoinformatics to the test. Nat Biotechnol 2006; 24:791-2; PMID:16841062; <http://dx.doi.org/10.1038/nbt0706-791>
 40. Moutafsi M, Peters B, Pasquetto V, Tschärke DC, Sidney J, Bui HH, Grey H, Sette A. A consensus epitope prediction approach identifies the breadth of murine T(CD8+)-cell responses to vaccinia virus. Nat Biotechnol 2006; 24:817-9; PMID:16767078; <http://dx.doi.org/10.1038/nbt1215>
 41. Moise L, Buller RM, Schriewer J, Lee J, Frey SE, Weiner DB, Martin W, De Groot AS. VennVax, a DNA-prime, peptide-boost multi-T-cell epitope poxvirus vaccine, induces protective immunity against vaccinia infection by T cell response alone. Vaccine 2011; 29:501-11; PMID:21055490; <http://dx.doi.org/10.1016/j.vaccine.2010.10.064>
 42. Moise L, Gutierrez AH, Bailey-Kellogg C, Terry F, Leng Q, Abdel Hady KM, VerBerkmoes NC, Szein MB, Losikoff PT, Martin WD, et al. The two-faced T cell epitope: examining the host-microbe interface with JanusMatrix. Hum Vaccines Immunother 2013; 9:1577-86; PMID:23584251; <http://dx.doi.org/10.4161/hv.24615>
 43. Bottino CG, Gomes LP, Pereira JB, Coura JR, Provanance DW, Jr., De-Simone SG. Chagas disease-specific antigens: characterization of epitopes in CRA/FRA by synthetic peptide mapping and evaluation by ELISA-peptide assay. BMC Infect Dis 2013; 13:568; PMID:24299278; <http://dx.doi.org/10.1186/1471-2334-13-568>
 44. Mendes TA, Reis Cunha JL, de Almeida Lourdes R, Rodrigues Luiz GF, Lemos LD, dos Santos AR, da Câmara AC, Galvão LM, Bern C, Gilman RH, et al. Identification of strain-specific B-cell epitopes in *Trypanosoma cruzi* using genome-scale epitope prediction and high-throughput immunoscreening with peptide arrays. PLoS Negl Trop Dis 2013; 7:e2524; PMID:24205430; <http://dx.doi.org/10.1371/journal.pntd.0002524>
 45. Balouz V, de Los Milagros Camara M, Canepa GE, Carmona SJ, Volcovich R, Gonzalez N, Altcheh J, Agüero F, Buscaglia CA. Mapping antigenic motifs in the Trypomastigote Small Surface Antigen (TSSA) from *Trypanosoma cruzi*. Clin Vaccine Immunol 2015; 22(3):304-12; PMID:25589551
 46. Teh-Poot C, Tzee-Arjona E, Martinez-Vega P, Ramirez-Sierra MJ, Rosado-Vallado M, Dumonteil E. From genome screening to creation of vaccine against *Trypanosoma cruzi* by use of immunoinformatics. J Infect Dis 2015; 211:258-66; PMID:25070943; <http://dx.doi.org/10.1093/infdis/jiu418>