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Induction of cardiac autoimmunity in Chagas heart disease: A case for molecular mimicry

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Abstract
Up to 18 million of individuals are infected by the protozoan parasite *Trypanosoma cruzi* in Latin America, one third of whom will develop chronic Chagas disease cardiomyopathy (CCC) up to 30 years after infection. Cardiomyocyte destruction is associated with a T cell-rich inflammatory infiltrate and fibrosis. The presence of such lesions in the relative scarcity of parasites in the heart, suggested that CCC might be due, in part, to a postinfectious autoimmune process. Over the last two decades, a significant amount of reports of autoimmune and molecular mimicry phenomena have been described in CCC. The authors will review the evidence in support of an autoimmune basis for CCC pathogenesis in humans and experimental animals, with a special emphasis on molecular mimicry as a fundamental mechanism of autoimmunity.

Keywords: *Chagas disease cardiomyopathy, autoimmunity, cardiomyocyte,* Trypanosoma cruzi*, molecular mimicry*

Introduction: Chagas disease

Chagas disease, named after the Brazilian physician Carlos Chagas who first described the disease in 1909, is endemic to Central and South America. The World Health Organization estimates that 16–18 million people are infected with *Trypanosoma cruzi*, with about 100 million people at risk in 21 countries [1,2]. *T. cruzi* infection is a major cause of heart disease and cardiovascular-related deaths in endemic areas, with approximately 50,000 fatalities per year [3]. In certain endemic areas, nearly 10% of all adult deaths are due to chronic Chagas disease cardiomyopathy (CCC). Clinical progression and survival are significantly worse in CCC patients as compared with patients with dilated cardiomyopathy (DCM) of other etiologies. In spite of recent advances in the control of the vectorial and transfusional *T. cruzi* transmission [4], Chagas is still a serious public health problem in Latin America [5].

Despite the obvious clinical importance of CCC and the efforts of many investigators during the past century, the pathogenic mechanisms of CCC are still poorly understood. There are at least six proposed mechanisms for CCC pathogenesis including: (i) microvascular spasm, (ii) ischemia, (iii) chronic eosinophilia or neutrophilia, (iv) parasite-mediated toxicity, (v) anti-*T. cruzi* immune responses to parasites or parasite antigen that persist in the heart and (vi) *T. cruzi*-induced autoimmunity (reviewed in references [6–12]). Finally, the finding of kDNA minicircles integrated into the nuclear genome of some individuals with CCC suggests that alteration of host cell gene expression might contribute to pathogenesis [13]. The absence or near absence of parasites from severely inflamed heart tissue initially

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suggested the autoimmunity hypothesis. The later discovery of substantial autoimmune responses in both humans and experimental animal models of disease reinforced this idea. This review aims to summarize the evidence for participation of autoimmunity in CCC pathogenesis with particular emphasis on molecular mimicry as a valid mechanism by which autoimmunity is induced during *T. cruzi* infection.

T. cruzi life cycle and clinical infection

The life cycle of *T. cruzi* involves two intermediate hosts (triatomine insects and mammals) and three developmental stages: epimastigotes, trypomastigotes and amastigotes [14]. The epimastigote forms replicate in the midgut of the reduviid bug insect vector and develop into infective, non-replicative metacyclic trypomastigote forms. When the insects feed on blood, they release their excreta containing metacyclic trypomastigotes that subsequently penetrate the mammalian host through the bite wound or a mucosal surface and initiate cellular invasion. Within the host cells, the parasite differentiates into the replicative amastigote form followed by further differentiation to the bloodform trypomastigotes. A fully parasitized cell will then rupture, releasing bloodform trypomastigotes to the blood stream, infecting adjacent cells, and disseminating infection through the blood, where they can be taken up by a new reduviid bug, thus completing the cycle. Reduviid vector-associated transmission occurs in poor houses in rural areas since the reduviid bug primarily resides in crevices of walls and roofs of such makeshift domiciles. Congenital transmission [15] as well as limited cases of transmission by oral ingestion [16,17] have been reported. Less common routes of parasite transmission including blood transfusion [18–20], organ transplantation [21] and laboratory accidents can occur in non-endemic areas such as the United States and Europe.

There are two stages of human Chagas disease: the acute stage which occurs shortly after the infection and the chronic stage which appears after a silent period that may last many years. The acute stage of the disease, generally seen in children, is characterized by fever, lymphadenopathy and hepatosplenomegaly and local inflammation at the site of infection. In most cases, however, clinical symptoms are either absent or mild and non-specific [5], making it difficult to diagnose disease in the acute stage of infection. Though death occurs in a small number of individuals during the acute phase, *T. cruzi* establishes a lifelong, low-grade infection and approximately 25% of infected individuals [5] progress to a symptomatic chronic phase some 10–30 years later, with irreversible damage to the heart, while ca. 5% develop denervation of the smooth muscle layers of the esophagus and colon, leading to severe dilatation and dysfunction of these organs, while the other 70% remain asymptomatic for life (ASY patients). Through a number of possible mechanisms, all leading to chronic cardiac inflammation, myocyte destruction and fibrosis continue throughout the course of disease, causing a gradual and irreversible decrease in cardiac contractility. Approximately, 10% of all *T. cruzi* infected patients will die from refractory, end-stage heart failure or severe arrythmia [22,23]; CCC has a shorter survival and worse prognosis than cardiomyopahties of non-inflammatory etiology [24]. At present, there is no effective treatment for CCC, other than heart transplantation [25].

Differential clinical progression of the disease occurs in the presence of this persistent infection, and the development of CCC in only one third of infected individuals points to an element of genetic susceptibility. This is reinforced by the finding of familial aggregation of cases of CCC [26]. It is thus likely that gene polymorphisms affecting the immune response could also influence the differential progression towards CCC among *T. cruzi*-infected patients. However, genetic studies in humans have been conflictive over the MHC region [27–31], and have failed to disclose relevant genes leading to the clinical dichotomy observed, which may have been due to the genetic heterogeneity among the populations in different regions and countries under study. Polymorphisms in genes encoding proteins associated to the immune response have also been assessed for association with CCC. The 59029A allele in chemokine receptor CCR5, reported to induce increased expression of the receptor, is associated to asymptomatic patients in a Peruvian study [32], suggesting a protective effect. Rodriguéz-Pérez et al. have reported an association of the Tumor necrosis factor-α promoter polymorphisms (TNF −308A promoter allele) with susceptibility to cardiomyopathy in a Mexican population [33]. However, an analysis of 252 Brazilian CCC and ASY patients failed to confirm such findings (ECN and JK, unpublished results); the same lack of association was observed in a Peruvian study on the −238 TNF promoter polymorphic site [34]. A Brazilian longitudinal study showed that patients positive for the TNF −308A or TNFa2 microsatellite allele 2 display a significantly shorter survival time compared to those carrying other alleles [35]. Association of CCC with polymorphisms in other immune response genes is currently under investigation.

Pathogenesis of heart-specific inflammatory lesions in CCC: Role of local parasitism and autoimmunity

During the course of Chagas disease, $CD4^+$ and $CD8^+$T cells are primed and expanded with *T. cruzi* antigens, and differentiate into memory-activated

effector T cells. Chronic infection with *T. cruzi* induces a systemic shift in the peripheral blood mononuclear cell (PBMC) cytokine profile towards Th1 cytokines with suppression of Th2 cytokines [36–38]. The increased production of IFN-γ in CCC patients [38,39] as compared to ASY patients has been linked to decreased production of IL-10 [38]. All chronically *T. cruzi*-infected patients, even ASY individuals, display increased plasma levels of TNF-α. Furthermore, patients displaying severe CCC present significantly higher plasma levels of TNF-alpha [40]. The proinflammatory and Th1 cytokine profile described above among chronically *T. cruzi* infected patients may be related to the ability of mucin-like glycoconjugates from persisting *T. cruzi* infection to induce the production of IL-12 [41].

After differentiation, effector T cells recirculate and enter the heart, where they mediate inflammation and tissue injury [42]. Histopathological findings in CCC heart lesions are consistent with inflammation and a myocardial remodeling process: T cell and macrophage-rich myocarditis, hypertrophy and fibrosis with heart fiber damage [43]. The T-cell rich inflammatory infiltrate shows a 2:1 predominance of the $CD8^+$ over the $CD4^+$T cell subset [44,45]. This may be due to signaling though survival cytokine pathways (IL-7, IL-15) [SGF, ECN, JK, unpublished data]. Increased local expression of the cytokines IFN-γ and TNF-α [39,46], IL-6 and IL-4, [46] as well as HLA class I and II molecules, and adhesion molecules were reported [47]. Up-regulated production of IFN-γ in *T. cruzi*-infected IL-4 −/− mice enhanced late-phase myocarditis [48]. Real-time PCR analysis showed that the gene expression levels of IFN-γ -inducible chemokines MCP-1, IP-10 and MIG, as well as chemokine receptors CCR2, CXCR3, were selectively up-regulated in CCC heart tissue; moreover, IFN-γ and MCP-1 were found to significantly increase the expression of atrial natriuretic factor, a marker of cardiomyocyte hypertrophy and heart failure, in neonatal cardiomyocytes [49]. Together, these observations suggest that IFN-γ -mediated chronic myocardial inflammation could contribute to CCC pathogenesis. Furthermore, cDNA microarray analysis of CCC and idiopathic DCM myocardium showed significant changes in expression of genes related to energy metabolism [49], which was also observed in hearts of mice infected with *T. cruzi* [50–52]. Proteomic analysis of CCC heart tissue has confirmed some of these energy metabolism changes (ECN, JK, et al. unpublished observations).

A direct role for cardiac parasitism in pathogenesis was proposed after the identification of *T. cruzi* antigen and DNA in CCC hearts by immunohistochemical and PCR techniques [53–55]. However, low-grade parasite persistence is universal in CCC and ASY patients [56,57] and has been found not to be linked to the development of CCC in clinical follow-up studies of patient series [58]. Recent studies, using either immunohistochemistry or *in situ* hybridization to detect *T. cruzi* in cardiac tissue from CCC patients, failed to disclose an association between parasite presence and inflammatory lesions, and *T. cruzi* DNA has been detected in hearts of both CCC and ASY individuals [59–62]. This suggests that *T. cruzi* parasitosis by itself is apparently unable to evoke sufficient heart damage to cause DCM. Thus, some other factor must be operating along with parasite persistence, to lead a subgroup of *T. cruzi*-infected individuals towards heart damage.

The lack of association between high parasitemia and tissue pathology, considered together with the scarcity of *T. cruzi* in CCC heart lesions [59,60,63,64] prompted early investigators [65] to suggest that the lymphomononuclear cell infiltrate in the heart participates in delayed-type hypersensitivity (DTH) responses towards a tissue-specific heart component as a result of chronic *T. cruzi* infection, the so-called autoimmune hypothesis of pathogenesis. It should be emphasized that the notion that autoimmunity seems to play a pivotal role for myocardial damage is not incompatible with a role for parasite persistence, as shown by the identification of autoimmune and *T. cruzi*-specific T cell responses in CCC heart tissue [66,67] (Table I).

The three mechanisms described below have been demonstrated in Chagas disease patients or experimental animals and could generate experienced, effector autoreactive T or B cells capable of inducing tissue damage. Antigen exposure secondary to tissue damage, followed by sensitization in an appropriate inflammatory environment (i.e. bystander activation); molecular mimicry between parasite and host antigens (Table II) and polyclonal activation leading to autoantibody production [68,69].

Animal models of CCC

A variety of animal models of Chagas disease have been employed in order to address a number of issues including mortality, immune function, cardiac pathology, chemotherapeutic agents and autoimmunity. Among the animals analyzed have been dogs [70,71], monkeys, rabbits [72], hamsters [73–75] and more commonly rats [76–81] and mice [82–91]. A number of parasite strains and clones (e.g. Silvio, Brazil, Tulahuen, Y, Colombian, Corpus Christ, etc.) have been used to infect a variety of strains of mice (e.g. BALB/c, C3H, A/J, DBA/2, etc.). While no single parasite-mouse combination recapitulates the entire spectrum of human infection—for instance, experimentally infected mice seldom develop end-stage heart failure during chronic infection—each combination does seem to reflect some particular aspect of the disease, including acute or chronic myocarditis and the ASY infection. One of the critical points in

Table I. Host proteins to which autoimmunity develops during *T. cruzi* infection.

Cell, molecule or substance	Host*	Immune mediator	Reference(s)
Cardiac myosin	M	CD4 + T cells	[100]
Cardiac myosin, p150	M	Serum IgG	[94]
Heart homogenate	M	T cells	[101,182,183]
43 kDa muscle glycoprotein	M	Serum IgG	[146]
Nervous Tissue, heart and skeletal muscle	M	Serum IgG	[147]
2nd extracellular loop, M2 cholinergic receptor	M	Serum IgG	[184]
2nd extracellular loop, β1 adrenergic receptor	M	Serum IgG	[185]
M2 cholinergic receptor	H	Serum IgG	[140]
M2 cholinergic receptor	H	Serum IgG	[135,136,186]
M2 muscarinic acethylcoline receptor	H	Serum IgG	[187,188]
2nd extracellular loop, M2 cholinergic receptor	H	Serum IgG	[137]
Neurons	H	Serum IgG	[127]
Sciatic nerve homogenate	H	Serum IgG	[148]
Small nuclear ribonucleoprotein	H	Serum IgG	[149]
Cardiac myocytes	H	Complement (C5–C9 complex)	[152]
Heart homogenate	H	T cells	[153,154]
Cardiac myocytes	H	T cells	[157]
Cardiac myocytes	Rb	T cells	[189]

* M, mouse; H, human; Rb, rabbit.

validating autoimmunity and molecular mimicry in Chagas disease is the use of animal models that reproduce most components of human CCC. The most common models of *T. cruzi* infection-induced autoimmunity today are: (i) BALB/c mice infected with the Colombian strain for a 150–240 days [92] for the chronic phase of infection, (ii) C3H mice infected with the Silvio X-10/4 clone and (ii) A/J mice infected with the Brazil strain for 7–30 days [93] for the acute phase, though *T. cruzi* related autoreactivity and mimicry have been investigated in other systems as well.

Experimental Chagas disease: Autoimmunity

During *T. cruzi* infection, mice can display antibodies specific for various autoantigens contained in cardiac, nervous and other tissues (Table I). Anti-sera from infected mice has also been found to react with heart homogenate [93] including cardiac myosin, cardiac C protein (unconfirmed) and the intermediate filament protein desmin [94]. Sera from *T. cruzi* infected animals were reactive against nervous system structures (sciatic nerve, spinal cord, brain) [95] and have also caused alterations in sciatic nerve action potentials when injected into naïve animals [96]. While autoantibodies specific for tubulin, actin and myosin are produced during acute murine infection [93,97], lytic autoantibodies are apparently produced only during chronic infection [98,99].

Several lines of evidence support a role for cellular autoimmunity directed at heart-specific autoantigens in experimental chronic Chagas disease as well. $CD4^+$T cells from chronically *T. cruzi*-infected mice from BALB/c or CBA mice proliferate in response to cardiac myosin, but not cardiac actin [100]. In line with these findings, T cells from Brazil strain infected 129Sv mice also displayed *in vitro* proliferation upon stimulation with cardiac myosin (DE, unpublished data). Splenocytes harvested from chronically infected mice elicit lysis of syngeneic myoblasts *in vitro* and induce electrocardiographic abnormalities when transferred to a naïve recipient [84]. Perhaps, the most compelling finding was that $CD4^+$T cells from chronically infected mice mediated the rejection of normal syngeneic newborn hearts transplanted into the ear of recipients [101]. On the other hand, an analogous model system but using a different parasite-mouse strain combination showed that parasites were systematically present in the heart grafts undergoing rejection [102], raising questions of whether the rejection was strictly independent of the parasite. Pontes de Carvalho et al. observed less intense inflammation in heart tissue from *T. cruzi*-infected mice that were tolerized to cardiac myosin-rich antigen by simultaneous administration of anti-CD4 antibody prior to infection with *T. cruzi* as compared to control mice receiving only anti-CD4 treatment [92].

Experimental Chagas disease: Molecular mimicry

The discovery of mimicry between antigens of *T. cruzi* and host in both human CCC and experimental models of disease provides arguable evidence that autoimmunity evolves as a result of parasite-specific immune responses rather than general tissue damage. Benoist and Mathis have proposed stringent criteria to distinguish molecular mimicry from bystander activation [103], which can be summarized as: (i) relationship between a specific microbial infection and a specific inflammatory state, (ii) identification of responsible microbial and self-epitope capable of

Table II. Molecular mimicry during *T. cruzi* infection or after immunization.

Cell, molecule or substance	*T. cruzi* antigen	Host*	Immune mediator	Reference(s)
Neurons, liver, kidney, testis	?	M, R	mAb	[190]
Neurons	?	R	mAb	[191]
Neurons	Sulphated glycolipids	H	mAb	[192–195]
Heart tissue	?	M	Serum IgG	[196]
Heart and skeletal muscle	Microsomal fraction	H	mAb	[197; 198]
Human cardiac myosin heavy chain	B13 Protein	H	rDNA, Ab, T cell clones	[66,168,170,172]
Human cardiac myosin heavy chain	Cruzipain	M	Ab	[199]
Cardiac myosin	*T. cruzi* lysate	M	Ab, DTH	[93,125]
95 kDa myosin tail	*T. cruzi* cytoskeleton	M	mAb	[200]
Skeletal muscle Ca++ dependent SRA	SRA	Rb, H	AS	[158,159]
Smooth and striated muscle	150 kDa protein	H, M	Serum IgG	[201]
Glycosphingolipids	Glycosphingolipids	H, M	Serum IgG	[202]
MAP (Brain)	MAP	H, M	rDNA, AS	[203]
Myelin basic protein	*T. cruzi* soluble extract	M	Serum IgG, T cells	[204]
28 kDa lymphocyte membrane protein	55 kDa membrane protein	H, M	Mab	[205]
47 kDa neuron protein	FL-160	H	rDNA, AS	[206–208]
23 kDa ribosomal protein	23 kDa ribosomal protein	H	Ab	[162,163]
Ribosomal P protein	Ribosomal P protein	H	rDNA, Ab, SP	[164,165]
β1-Adrenoreceptor, M2 muscarinic receptor	Ribosomal P0 and P2β Proteins	H	rDNA, Ab, SP	[139,166,167,209–212]
β1-Adrenoreceptor, M2 cholinergic Receptor	150 kDa protein	H, M	mAb	[213]
38 kDa heart antigen	R13 peptide from ribosomal protein P1, P2	M	IgG1, IgG2	[214]
Cardiac muscarinic acetylcholine receptors (mAChR)	?	H	Ab	[188]
Cha antigen	SAPA, 36 kDa TENU2845	M	Ab, T cells	[143]

*M, mouse; H, human; Rb, rabbit.

eliciting crossreactive T cell responses, (iii) causal relationship between the existence of T cells elicited by the microbe and responsive to both microbe and self-epitopes and the particular autoimmune disease.

A number of crossreactive antigens with both cardiac and non-cardiac specificity have been identified, primarily by serologic approaches, in mouse models of chronic Chagas disease (Table II). Lymphocytes from *T. cruzi* protein extract-immunized animals proliferated in response to MBP stimulation, and vice versa [104], and two regions of the MBP molecule were identified as crossreactive with parasite antigen. Passive transfer of a $CD4^{+}$T cell line recognizing heart and *T. cruzi* antigens derived from a chronically *T. cruzi* infected mouse to BALB/c nude mice immunized with cardiac antigen caused intense heart inflammation [105]. T and B cells from *T. cruzi*-infected mice recognize peptides from the novel autoantigen Cha, which contains regions of homology with different *T. cruzi* proteins, SAPA and a 36 kDa putative gene product. Splenocytes taken from infected animals proliferated to Cha peptide and those taken from animals immunized with recombinant Cha protein recognized *T. cruzi* SAPA peptide, suggesting the existence of T and B cell crossreactivity between *T. cruzi* and host antigens. Passive transfer of T cells appeared to induce heart lesions similar to those resulting from *T. cruzi* infection, but no control was performed for contamination by *T. cruzi*. However, definite proof of T cell crossreactivity can only be demonstrated with T cell clones. Another notable finding was the development of autoreactive anti-heart antibodies and heart functional alterations following the immunization of BALB/c mice with *T. cruzi* ribosomal P1 and P2 protein synthetic peptide. In this case of mimicry, the peptide sequence that corresponds to the C-terminal region of *T. cruzi* ribosomal proteins differs from the eukaryotic ribosomal P consensus sequence only in a non-conservative amino acid substitution [106].

Several investigators have described cardiac myosin as a major antigen of heart-specific autoimmunity in infection-induced disease models [107–111], including murine and human Chagas disease [94,100,112–115], as well as autoimmune myocarditis induced with self-protein immunization [116–122]. The ability of this antigen to induce myocarditis upon immunization, coupled with its repeated appearance as an autoantigen in infectious disease models has made it a target of investigation for molecular mimicry in CCC. Immunization of BALB/c mice with *T. cruzi* antigen, cruzipain, induced autoantibodies to both skeletal and cardiac myosin, leading to muscle damage and heart conduction abnormalities [114,123]. Cruzipain immunization also induced antibodies reactive to the cardiac muscarinic acetylcholine receptor (mAChR), causing a decrease in myocardial contractility characteristic of CCC [124]. In addition, a monoclonal antibody raised against solubilized *T. cruzi* cytoskeletons crossreactively recognized a cardiac myosin tail epitope (95 kDa) [115].

The A/J mice infected with Brazil *T. cruzi* strain has consistently provided evidence of cardiac myosin-specific autoimmunity in forms of both autoantibody production as well as cellular immunity [93]. Infection or immunization of mice with *T. cruzi* lysate induced myosin-specific autoantibody production and DTH even though immunization with *T. cruzi* lysate failed to induce heart inflammation. Interestingly, mice immunized with cardiac myosin developed *T. cruzi*-specific DTH and antibodies [93,125]. Furthermore, myosin tolerization did suppress *T. cruzi* DTH and conversely, *T. cruzi* tolerization (using parasite lysate) suppressed myosin DTH [125]. The induction of bidirectional, crossreactive immunity between *T. cruzi* and cardiac myosin was shown to be specific since such crossreactivity did not occur in Leishmania protein extract or skeletal myosin immunizations. Moreover, the fact that C57BL/6 mice failed to develop cardiac myosin DTH upon immunization with *T. cruzi* extract indicated that the ability to make myosin autoimmunity was immunogenetically restricted. However, *T. cruzi*-induced myocarditis was not affected by myosin tolerization by the antigen-coupled splenocyte method [125], suggesting that other mechanisms contribute to tissue inflammation, perhaps including autoimmunity to other cardiac antigens. Since the peripheral immune tolerization method was effective in preventing autoimmune myocarditis, it will be interesting to see whether tolerization to *T. cruzi* lysate has the ability to inhibit inflammation after myosin immunization due to their crossreactive nature Equally interesting will be to test the efficacy of tolerization using infected heart homogenate, which contains both parasite and heart antigens.

Human Chagas disease: Autoimmunity

In human Chagas disease, sera from over 80% of patients contained anti-neuron autoantibodies, and there is a net loss of neurons from the autonomic system [126,127] (Table I), which may be linked to the autonomic nervous system dysfunction observed in symptomatic and asymptomatic patients [128,129]. Functional antibodies against adrenergic G-protein-coupled and muscarinic (M2) cholinergic receptors were found in serum from Chagas' disease patients [130–140] (Tables I and II), as described in idiopathic DCM [141,142]. However, it has been shown that the presence of such functionally active anti-receptor antibodies does not correlate with heart symptomatology but rather with dysfunction of the autonomic nervous system [137].

Sera from Chagas disease patients display autoantibodies against small ribonucleoproteins and the Cha human autoantigen, as well as its major B cell epitope

Cha (peptide R3) [143,144]. Taken together, these results suggest that functional autoantibodies play a role in the pathogenesis of Chagas disease. On the other hand, the pathogenic role of autoantibodies that do not have functional activity or fail to be associated to disease remains to be elucidated [127,145–150]. Autoantibodies against galectin-1 (Gal-1), a human cardiac protein, are correlated with the severity of cardiac damage in CCC [151]. Complement membrane attack complexes have been identified in the membranes of cardiomyocytes from Chagas disease cardiomyopathy patients [152].

Regarding cell-mediated autoimmunity, early studies have shown that cardiac tissue homogenate induced lymphokine production [153,154] but not proliferative responses among CCC peripheral blood T cells [155,156]. Non-infected cardiomyocytes were targets of cytotoxicity by CCC PBMC [157]. More recent studies have identified crossreactive responses to antigens in molecular mimicry, which will be discussed in the next section.

Human Chagas disease: Molecular mimicry

Several reports of immunological crossreactivity/antigenic mimicry between defined *T. cruzi* and host self-antigens have been described (Table II). Given the evolutionary conservation of primary sequences of many key structural proteins or enzymes from protists to humans, it is not surprising that this kind of crossreactive antigens can be detected [158–165]. In some cases, evidence for a pathogenic role of such crossreactive immune responses comes from functional activity or their association with CCC.

Evidence of crossreactivity between *T. cruzi* ribosomal P0, and the P1 and P2 proteins and human ribosomal P protein, or the β 1-adrenergic receptor, has also been shown [139,165,166]. A recent study demonstrated that peptides from ribosomal proteins P0 and P2b bearing acidic epitopes or the second extracellular portion of the muscarinic acetylcholine receptor could block antibodies from CCC sera with muscarinic activity [167]. It has been shown that affinity-selected anti-human ventricular cardiac myosin heavy chain antibodies from Chagas disease patients' sera specifically recognized a defined *T. cruzi* antigen [168], the recombinant tandemly repetitive protein B13 [169]. Cardiac myosin-B13 crossreactive antibodies (116/140 kDa) were present in sera from 100% of CCC patients but only 14% of ASY patients [168]; sera from 100% of both CCC and ASY patients recognized cardiac myosin.

$CD4^+$T cell clones derived from a biopsy from CCC patient and expanded in the absence of exogenous antigen, crossreactively recognized cardiac (but not skeletal) myosin heavy chain and *T. cruzi* protein B13 [66]. However, none of the 17 tested clones responded to the immunodominant recombinant *T. cruzi* antigens CRA, FRA, JL5 or B12 or to *T. cruzi* trypomastigote lysate [66]. *In vitro* sensitization of peripheral lymphocytes from a *T. cruzi* seronegative individual with B13 protein elicits cardiac myosin-crossreactive T cell clones [170]. Full characterization of B13 T cell recognition was performed and it was found that T cell recognition of B13 protein is restricted by HLA-DQ7, -DR1 and -DR2. One of the variant B13 peptides, S15.4 (KPPPFGQAAAG-DKPP) was preferentially recognized by CCC as compared to ASY HLA-$DQ7^+$ patients [171]. Molecular modelling indicated that variant positions in B13 peptides complexed to HLA-DQ7 were exposed to contact with TCR [171]. In order to identify B13-crossreactive epitopes in cardiac myosin heavy chain, we obtained a T cell clone sensitized against B13 peptide S15.4 from a seronegative individual. This T cell clone crossreactively recognized one partially homologous peptide (EMAVFGAAA-PYLRKS) along with 12 other peptides with low homology with the B13 peptide S15.4 [172]. Taken together, the identification of T cell crossreactive epitopes in B13 and cardiac myosin fulfills one of Benoist's criteria for molecular mimicry. Furthermore, the intramolecular degenerate T cell molecular mimicry involving 12 low-homology myosin epitopes may be an additional mechanism for amplification of anti-cardiac myosin immunity at the T cell clonal level, which may play a role in the myocarditis of CCC.

Although, several reports have cited myosin-specific autoimmunity in the context of CCC [66,168,170–172], it is important to note that levels of anti-myosin antibodies are also significantly increased in patients with heart damage from infectious and non-infectious causes [173,174] suggesting that the antigen exposure by itself could cause the level of anti-myosin immunity to rise in CCC. However, the finding of molecular mimicry indicates that it is antigen receptor cross-reactivity, rather than bystander activation, that causes of anti-myosin autoimmunity in human CCC.

Concluding remarks

Chagas disease is a low-grade, systemic chronic infection with documented autoimmune phenomena. Establishing whether autoimmunity and molecular mimicry are causes or consequences of heart tissue damage in human and experimental models of CCC is key to understanding their roles in CCC pathogenesis. Inasmuch as some authors refuse to classify diseases associated to a known infectious agent as auto-immune, [175,176], the recent identification of persistent virus infection in patients with *bona fide* human autoimmune diseases like multiple sclerosis [177] and insulin-dependent diabetes mellitus [178] may indicate that this might be a common theme among such diseases. When confronting the available data with the criteria of autoimmune disease described

by different authors [103,179], it can be seen that CCC fulfills several of them. The identification of T cell crossreactive antigens (Table II), with reproduction of pathobiological changes by passive transfer in murine models in the absence of *T. cruzi* parasites [101,105], and the amelioration of inflammation as a consequence of tolerance induction to myocardial antigens [92] together with the induction of autoimmune disease after immunization with cardiac myosin [117], the major candidate self antigen in Chagas disease cardiomyopathy, have all been shown. The isolation of cardiac myosin-autoreactive T cells in molecular mimicry with *T. cruzi* B13 protein from affected tissue [66] is considered important indirect evidence. TCR Vα region usage restriction [180] is considered circumstantial supporting evidence according to Rose and Bona's criteria [181]. Together with the demonstration that *in vitro* immunization with B13 protein or B13 epitopes elicits T cell clones crossreactive with cardiac myosin [170] or its epitopes [172], these results suggest a major role for autoimmunity in CCC pathogenesis. However, criteria were individually established with distinct experimental approaches in murine models and patients. What is still missing for the fulfilment of all criteria in a single model is the transfer of tissue lesions by T cell clones crossreactive to known host and parasite epitopes, or a successful trial of tolerance to cardiac antigens among patients. Finally, a direct test of the relevance of B13—or any other parasite antigen—molecular mimicry might be achieved though the use of B13-null parasites for experimental infection. Such a parasite line could permit the direct testing of this molecule in the generation of myosin autoimmunity. The failure of this parasite line to induce myosin autoimmunity and/or myocarditis unless completed with B13 in *trans* would directly imply molecular mimicry in myosin autoimmunity and CCC pathogenesis.

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