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INVITED REVIEW

CHAGAS DISEASE CARDIOMYOPATHY: CURRENT CONCEPTS OF AN OLD DISEASE

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SUMMARY

Chagas disease continues to be a significant public health problem, as ca. 10 million people are still infected with *T. cruzi* in Latin America. Decades after primary infection, 30% of individuals can develop a form of chronic inflammatory cardiomyopathy known as Chagas disease cardiomyopathy (CCC). Data from both murine models and human studies support the view that an autoimmune response as well as a parasite-driven immune response involving inflammatory cytokines and chemokines may both play a role in generating the heart lesions leading to CCC. This review aims to summarize recent advances in the understanding of the immunopathogenesis of Chagas disease cardiomyopathy.

KEYWORDS: Chagas disease; Cardiomyopathy; Autoimmune disease; Pathogenesis.

CHAGAS DISEASE: AN OVERVIEW

In 1909, Brazilian physician Carlos Chagas took a significant step in the advancement of Medicine by discovering Chagas disease and its etiological agent, the protozoan parasite *Trypanosoma cruzi*. However, despite efficient vector control programs to restrict the spread of Chagas disease over the century since its discovery, the World Health Organization estimates that ca. 10 million people are still infected by *T. cruzi* in Latin America (SCHOFIELD *et al*., 2006), which makes Chagas disease an important public health problem. Unfortunately, Chagas disease remains a neglected disease, with no vaccines available so far, and only very few anti-parasitic drugs proven efficient for treating the acute phase of the disease. Moreover, the millions of people that were infected decades ago are still in need of appropriate treatment, and require the attention of the scientific community.

The general consequences of acute infection can range from flu-like symptoms and asymptomatic myocarditis, which affects the majority of infected individuals, to intense myocarditis accompanied by high blood and tissue parasitism in about 10% of those infected (CHAGAS, 1909; DIAS *et al.,* 1956). This subset of individuals may develop fulminant myocarditis, which is often fatal (PARADA *et al.,* 1997; ANDRADE, 1999). On the other hand, the majority of infected individuals remain free of any clinical symptoms (the so-called indeterminate patients, IND), but decades later may develop a form of inflammatory cardiomyopathy known as chronic Chagas disease cardiomyopathy (CCC). CCC affects 30% of infected patients and, in some endemic areas, nearly 10% of all adult deaths are due to CCC. Clinical progression and survival are significantly worse in CCC patients as compared with patients with dilated cardiomyopathy (DCM) of other etiologies (FREITAS *et al.,* 2005) due to the lack of effective therapies. The mechanisms that govern the pathogenesis of CCC remain 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 (TANOWITZ *et al.,* 1992; KIERSZENBAUM, 1996; KALIL & CUNHA-NETO, 1996; KIERSZENBAUM, 1999; ENGMAN & LEON, 2002; LEON & ENGMAN, 2003; CUNHA-NETO *et al.,* 2004). This review aims to summarize recent advances in the understanding of the immunopathogenesis of Chagas disease cardiomyopathy.

IMMUNOPATHOGENESIS OF CHAGAS DISEASE CARDIOMYOPATHY

Autoimmunity X parasite persistence: CCC lesions are consistent with a process of inflammation and myocardial remodeling, which includes T cell/macrophage-rich myocarditis, hypertrophy, and fibrosis with heart fiber damage (reviewed in HIGUCHI *et al.,* 2003). Since inflammation is one of the hallmarks of CCC lesions, one might speculate as to its role in disease outcome. HIGUCHI and co-workers showed that the frequency of myocarditis was higher among patients with severe CCC than in patients with less severe forms of the disease

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(HIGUCHI *et al.,* 1987). Accordingly, our group showed a significant correlation between myocarditis and fibrosis and ventricular dilation in the Syrian hamster model of CCC (BILATE *et al.,* 2003). However, in spite of the substantial evidence supporting a role for immune responses in myocardial damage, the nature of the antigen eliciting the destructive immune response remains elusive. The near absence of parasites from severely inflamed heart tissue initially suggested that lesions may be autoimmune in origin (the "autoimmune hypothesis," reviewed in CUNHA-NETO *et al.,* 2006). The later discovery of substantial autoimmune responses in both humans and experimental animal models of disease reinforced this idea (reviewed in CUNHA-NETO *et al.,* 2006). On the other hand, the presence of parasite antigen and DNA in host tissues during the chronic phase of Chagas disease (reviewed in HIGUCHI *et al.,* 2003), although very scarce, may be relevant for maintaining *T. cruzi*-specific immune responses. Furthermore, *T. cruzi*-specific CD8+ T cells have been isolated from endomyocardial biopsies of a CCC patient (FONSECA *et al.*, 2005), providing evidence for the recruitment and expansion of *T. cruzi*-specific T cells in the myocardium, possibly related to the presence of the parasite antigen. In experimental *T. cruzi* infection, a higher inoculum (BILATE *et al.*, 2003) or higher parasite load in the acute phase (MARINHO *et al.,* 1999) are associated with more aggressive heart disease, suggesting a relationship between parasite burden and severity of disease in animal models. However, *T. cruzi* DNA - but not intact *T. cruzi* - was detected in the hearts of both IND and CCC patients (reviewed in CUNHA-NETO *et al.,* 2006), which does not support an association between parasite presence and CCC in humans. Other factors must therefore be operating along with parasite persistence to lead a subgroup of *T. cruzi*-infected individuals towards heart damage. This led to the hypothesis that the breakdown of immunological tolerance with heart tissue damage could be secondary to an originally protective response directed to a given *T. cruzi* antigen bearing structural similarities to a tissue-specific heart component. Several mechanisms have been suggested to play a role in triggering autoimmunity after *T. cruzi* infection (reviewed in CUNHA-NETO *et al.,* 2006), including (i) antigen exposure secondary to tissue damage, followed by sensitization in an appropriate inflammatory environment (i.e., bystander activation); (ii) molecular mimicry, where T and B cells recognize parasite antigens that share structurally similar epitopes in host antigens, generating crossreactive autoimmune responses (Table 2); and (iii) polyclonal activation leading to autoantibody production. In the following paragraphs, we discuss a number of significant findings supporting some of these mechanisms.

Table 1
Autoreactivity after *T. cruzi* infection

Host component	Host	Molecular definition
Neurons	H	Serum IgG
Sciatic nerve homogenate	H	Serum IgG
Heart homogenate	H	T cells
Cardiomyocytes	H, Rb	T cells
Heart homogenate	M	T cells
Cardiac myosin	M	CD4+ T cells, serum IgG
43 kDa muscle glycoprotein	M	Serum IgG
Nervous tissue, Heart and skeletal muscle	M	Serum IgG
2nd extracellular loop, M2 cholinergic receptor	H, M	Serum IgG
2nd extracellular loop, β1 adrenergic receptor	M	Serum IgG
Small nuclear ribonucleoprotein	H	Serum IgG

M, mouse; H, human; Rb, rabbit. Adapted from CUNHA-NETO *et al.,* 2004.

T. cruzi-infected mice display autoantibodies specific for various autoantigens contained in cardiac, nervous, and other tissues. These include antibodies to structural proteins (e.g. desmin, myosin and actin) and proteins with functional activities, such as β1-adrenergic and M2-muscarinic cholinergic receptors (Table 1) (reviewed in CUNHA-NETO *et al.,* 2004). Immunization of mice with the *T. cruzi* protein cruzipain induced autoantibodies to skeletal and cardiac myosin and to the cardiac muscarinic cholinergic receptor, leading to muscle damage and heart conduction abnormalities (GIORDANENGO *et al.*, 2000). 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 (MOTRAN *et al.,* 2000). See Table 2 for a complete list of all *T. cruzi* cross-reactive antigens described so far.

Cellular autoimmunity directed at heart autoantigens has also been observed in experimental Chagas disease. CD4+ T cells from *T. cruzi*-infected mice recognize cardiac myosin but not actin (RIZZO *et al.,* 1989; LEON *et al.,* 2001). Passive transfer of CD4+ T cells or T cell lines from chronically *T. cruzi*-infected mice to noninfected syngeneic mice cause myocardial tissue inflammation (RIBEIRO-DOS SANTOS *et al.,* 2001). Tolerance induction with a myosin-enriched cardiac homogenate ameliorated chronic myocarditis and fibrosis in a mouse model of *T. cruzi* infection (PONTES DE CARVALHO *et al.,* 2002).

In humans, sera from Chagas disease patients but not from healthy individuals was shown to contain autoantibodies specific for various autoantigens expressed in cardiac, nervous, and other tissues (Table 1). One study reported finding anti-neuron antibodies in serum of over 80% of patients, accompanied by net loss of neurons from the autonomic system. Functional antibodies against adrenergic and muscarinic (M2) cholinergic receptors were found in serum from arrhythmic CCC patients. These antibodies were capable of inducing arrhythmia in explanted rabbit hearts (DE OLIVEIRA *et al.* 1997; Tables 1 and 2). The presence of functionally active anti-receptor antibodies may also correlate with dysfunction of the autonomic nervous system, rather than with heart symptomatology (GOIN *et al.*, 1997).

Sera from chronic Chagas disease patients also contain antibodies crossreactive with human and *T. cruzi* proteins, many of which are evolutionarily conserved (Table 2). In particular, antibodies crossreactive with cardiac myosin heavy chain and the *T. cruzi* protein B13 are more frequent in sera from CCC than in indeterminate Chagas disease patients (CUNHA-NETO *et al.,* 1995). CD4+ T cell clones derived from an endomyocardial biopsy sample taken from a CCC patient crossreactively recognized *T. cruzi* protein B13 and cardiac myosin, but not actin (CUNHA-NETO *et al.,* 1996). *In vitro* sensitization of human peripheral lymphocytes with B13 protein

Table 2
Molecular mimicry after *T. cruzi* infection

Host component	*T. cruzi* antigen	Host	Molecular definition
Neurons, liver, kidney, testis	?	M, R	Mab
Neurons	Sulphated glycolipids	H, R	Mab, sera
47 kDa neuron protein	FL-160	H	rDNA, AS
Heart and skeletal muscle	Microsomal fraction	H, M	Mab, serum IgG
Smooth and striated muscle	150 kDa protein	H, M	Serum IgG
Human cardiac myosin heavy chain	B13 protein	H	rDNA, Ab T cell clones
Human cardiac myosin heavy chain	Cruzipain	M	Ab
95KDa myosin tail	*T. cruzi* cytoskeleton	M	Mab
Skeletal muscle Ca++ dependent SRA	SRA	H, Rb	AS, serum IgG
Glycosphingolipids	Glycosphingolipids	H, M	Serum IgG
MAP (brain)	MAP	H, M	rDNA, AS
Myelin basic protein	*T. cruzi* soluble extract	M	Serum IgG, T cells
28 kDa lymphocyte membrane protein	55 kDa membrane protein	H, M	Mab
23 kDa ribosomal protein	23 kDa ribosomal protein	H	Ab
Ribosomal P protein	Ribosomal P protein	H	rDNA, Ab, SP
38-kDa heart antigen	R13 peptide from ribosomal protein P1, P2	M	IgG1, IgG2
β1-adrenoreceptor M2 muscarinic receptor	Ribosomal P0 and P2β proteins	H	rDNA, Ab, SP
β1-adrenoreceptor M2 cholinergic receptor	150 kDa protein	H, M	Mab
Cardiac muscarinic Acetylcholine receptors(mAChR)	?	H	Ab
Cardiac muscarinic Acetylcholine receptors(mAChR)	Cruzipain	M	Immunization with cruzipain
Cha antigen	SAPA, 36KDa TENU2845	M	Ab, T cells

M, mouse; H, human; Rb, rabbit; R, rat; AS, antiserum; Ab, patient antibody; Mab, monoclonal antibody; rDNA, recombinant DNA; SP, synthetic peptides. Adapted from CUNHA-NETO *et al.*, 2004.

peptides elicits cardiac myosin-crossreactive T cell clones (ABEL *et al.,* 1997; IWAI *et al.* 2005). The presence of molecular mimicry between cardiac myosin and *T. cruzi* protein B13 suggests, at least in part, that a cellular effector response against cardiac myosin triggered by autoimmune recognition might be involved in the development of CCC heart lesions.

Autoimmune and *T. cruzi*-specific responses secondary to parasite persistence are not incompatible or mutually exclusive in Chagas disease, and it is likely that a combination of both should be involved in the establishment of heart tissue lesions. A consensus among the scientific community is that inflammation - triggered either by the parasite or by autoantigens - certainly has a role in CCC pathogenesis. In this scenario, inflammatory cytokines seem to be the key players, as discussed below.

Inflammation and the role of cytokines: Data from murine models show that inflammatory cytokines play a central role in *T. cruzi* infection. The innate and adaptive immune responses triggered by the parasite and its derived surface molecules, such as GPI anchors, during the acute phase lead to exacerbated production of inflammatory cytokines, including IL-12, TNF-α, and IFN-γ, and chemokines such as CCL3 (MIP-1α), CXCL10 (IP-10), and CCL5 (RANTES) (CUNHA-NETO *et al.*, 1998; TEIXEIRA *et al.,* 2002). Macrophages and dendritic cells endocytose parasites and, with the ensuing expression of IL-12 and costimulatory molecules, prime IFN-γ-producing specific T cells that migrate to the target organs in response to chemokines produced in infected tissues (reviewed in TEIXEIRA *et al.,* 2002). These steps, which occur shortly after infection, help to build up cellular and humoral immune responses to *T. cruzi*, leading to control - but not complete elimination - of parasitism.

The initial innate immune response against *T. cruzi* is believed to be mediated in part by Toll like receptors (TLR), pattern recognition receptors that play an essential role in the recognition of microbial components by the innate immune system (CAMPOS & GAZZINELLI, 2004). Infection of mice deficient of TLR2 and its adaptor molecule MyD88 resulted in impaired production of inflammatory cytokines, along with enhanced parasitemia and mortality (CAMPOS *et al.,* 2004). Moreover, OLIVEIRA and co-workers showed that TLR4 is important in the recruitment of neutrophils after injection of *T. cruzi* surface glycoinositolphospholipids, and infection of C3H/HeJ mice, a strain naturally deficient in TLR4, resulted in higher parasitemia and earlier mortality as compared to TLR4 sufficient mice (OLIVEIRA *et al.*, 2004). Recent data show that mice deficient in TLR9 and both TLR9 and TLR2 display decreased *in vivo* IL-12/IFN-γ responses upon *T. cruzi* infection and thus enhanced susceptibility to infection (BAFICA *et al.,* 2006), suggesting that TLR2 and TLR9 cooperate in the control of parasite replication. These results indicate that *T. cruzi* is recognized by TLRs, and that the activation of cytokine production by these innate receptors plays an important role in host defense.

The use of cytokine deficient mouse models has provided important findings that helped understand the mechanisms of immunity to *T.*

cruzi. Production of inflammatory cytokines is associated with protection against *T. cruzi* infection, and TNFR1 or IFNγ deficient mice succumb to infection more rapidly than wild type mice (ALIBERTI *et al.*, 2001; MICHAILOWSKY *et al.,* 2001). On the other hand, complete absence of anti-inflammatory cytokines has severe negative effects on the infected host. IL-10 deficient mice infected with *T. cruzi*, despite efficient parasite control, develop a syndrome similar to endotoxic shock due to the enhanced production of TNF-α and IFN-γ (HOLSCHER *et al.*, 2000). Furthermore, it has been reported that susceptible mouse strains display higher production of TNF-α than resistant strains upon *T. cruzi* infection (RUSSO *et al.*, 1989). Moreover, elevated levels of TNF-α were associated with higher parasitemia, wasting, and mortality during acute *T. cruzi* infection (TRUYENS *et al.,* 1999), suggesting a negative role for elevated TNF-α production during the acute phase. Collectively, these results point to the importance of both inflammatory and anti-inflammatory responses during *T. cruzi* infection.

More recently, using a single combination of parasite strain (Y strain) and a genetically heterogeneous host (Syrian hamster) our group observed two distinct disease profiles upon acute *T. cruzi* infection: (1) absence of systemic acute phase signs such as lethargy and weight loss, low cardiac parasitism, and low mRNA expression of TNF-α, IFN-γ, IL-10 and CCL3 (MIP-1α); and (2) presence of acute phase signs, high cardiac parasitism and markedly increased expression of cytokines (BILATE *et al.*, 2008 in press). The presence of acute phase signs and high cardiac parasitism in a subset of infected hamsters could be the underlying reason for the increased expression of cytokines and chemokines. The dichotomy with respect to clinical signs and intensity of cardiac parasitism in *T. cruzi*-infected hamsters suggests that a subset of animals is able to control parasitism - either systemically or locally - in a more effective manner. Since Syrian hamsters are outbred, it is possible that this difference may be based on a genetic component.

Recent reports have addressed the role of TGF-β, a cytokine produced by several cell types, including T cells and fibroblasts, in *T. cruzi* cell invasion cell cycle. WAGHABI and co-workers showed that SB-431542, an inhibitor of the TGF-β type I receptor, impairs cardiomyocyte invasion by *T. cruzi* and trypomastigote differentiation and release, and reduces the number of parasites per infected cell (WAGHABI *et al.,* 2007). Previous work from the same group suggested that *T. cruzi* uses host-cell TGF-beta to maintain its own intracellular life-cycle (WAGHABI *et al.,* 2005). These findings suggest that TGF-β could be a potential target for the treatment of Chagas disease.

The inflammatory infiltrate found in heart tissue of CCC patients contains macrophages, granzyme-expressing CD8+ T cells, and CD4+ T cells (HIGUCHI *et al*, 1993). Data from CCC patients show that cytokine profile is shifted towards Th1 cytokines such as IFN-γ with suppression of Th2-type cytokines such as IL-4 (RIBEIRÃO *et al.,* 2000; ABEL *et al.,* 2001), and elevated plasma levels of TNF-α (FERREIRA, 2003). Furthermore, peripheral blood mononuclear cells from chronic CCC patients produce more IFN-γ and less IL-10 (ABEL *et al.*, 2001; GOMES *et al.,* 2003) than indeterminate Chagas disease patients, reinforcing the hypothesis that CCC patients develop an exacerbated Th1 immune response. Moreover, increased myocardial expression of adhesion molecules, HLA class I and II, chemokines MCP-1, IP-10 and MIG and their receptors CCR2 and CXCR3, and cytokines IFN-γ, TNF-α, IL-15, IL-6 and IL-4 has also been reported (reviewed in CUNHA-NETO *et al.,* 2006; FONSECA *et al*., 2007). Gene expression profiling of CCC myocardial tissue showed that 15% of genes known to be selectively up-regulated in CCC are IFN-γ-inducible (CUNHA-NETO *et al.,* 2005). Exposure of neonatal murine cardiomyocytes to IFN-γ and MCP-1 upregulates expression of atrial natriuretic factor (CUNHA-NETO *et al*., 2005), a marker of cardiomyocyte hypertrophy and heart failure, suggesting that IFN-γ may directly modulate gene expression in myocardial cells. Together, these observations suggest that IFN-γ-mediated chronic myocardial inflammation could contribute to the pathogenesis of CCC.

Finally, work from our group and others has brought to attention the complex role of inflammatory cytokines in CCC pathogenesis. Given that TNF-α levels are increased among CCC patients as compared to IND individuals (FERREIRA *et al*., 2003; TALVANI *et al.,* 2004), and that T cells infiltrating the heart of CCC patients predominantly produce IFN-γ and TNF-α (ABEL *et al.,* 2001), one may hypothesize that inflammatory cytokine inhibition could ameliorate CCC progression. In order to test this, we used the hamster model for CCC (RAMIREZ *et al.,* 1994; BILATE *et al.*, 2003) to evaluate whether TNF-α blockade could attenuate disease progression. To our surprise, blocking TNF-α with a soluble TNF-α receptor (Etanercept) during the chronic phase of *T. cruzi* infection worsened CCC, as shown by impaired cardiac function, a hallmark of human CCC (BILATE *et al*., 2007). Severity of cardiomyopathy was not due to increased tissue or blood parasitism, direct drug toxicity on intact myocardium, increased myocardial fibrosis, or increased myocarditis and inflammatory cytokine expression in the heart. However, Etanercept treatment induced increased expression of IL-10 and decreased expression of iNOS mRNA in the myocardium, which could be associated with the observed outcome either direct or indirectly. The worsening of ventricular function in Etanercept-treated animals suggests that the absence of TNF activity during the chronic phase of *T. cruzi* infection in hamsters was detrimental to cardiac function.

To summarize, the data discussed above support the notion that inflammatory cytokines and chemokines are essential for immune-mediated control of parasite dissemination. On the other hand, regulatory or anti-inflammatory cytokines are absolutely necessary to counteract the otherwise destructive effect of exacerbated inflammation, which often results in tissue damage and may lead to death of the host. Collectively, the results obtained so far highlight the importance of maintaining the balance between a strong effector immune response that leads to tissue damage and a regulated immune response that will help maintain tissue homeostasis.

Genetic studies: Differential clinical progression of the disease occurs in the presence of persistent, low-grade 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 (ZICKER *et al.,* 1990). It is thus likely that gene polymorphisms affecting the immune response could also influence differential progression towards CCC among *T. cruzi*-infected patients. However, HLA-disease association studies yielded conflicting results (FERNANDEZ-MESTRE *et al.,* 1998; DEGHAIDE *et al.,* 1998; COLORADO *et al.,* 2000; FAÉ *et al.,* 2000), and have failed to disclose relevant genes leading to the clinical

dichotomy observed. This may have been due to the genetic heterogeneity of the populations in the different regions and countries under study. Polymorphisms in immune-response genes have also been assessed for association with CCC. The 59029A allele of chemokine receptor CCR5, reported to induce increased expression of the receptor, was shown to be more frequent among asymptomatic patients in a Peruvian study (CALZADA *et al.,* 2001), suggesting a protective effect. RODRIGUEZ-PEREZ *et al.* have reported an association between tumor necrosis factor-α promoter polymorphisms (TNF -308A promoter allele) and susceptibility to cardiomyopathy in a Mexican population (RODRÍGUEZ-PÉREZ *et al.,* 2005). A longitudinal study conducted by our group showed that Brazilian patients carrying the TNF2 or TNFa2 alleles display a significantly shorter survival compared to those carrying other alleles (DRIGO *et al.,* 2006). However, recent data show no significant differences either between CCC and IND patients, or among CCC patients according to severity of cardiomyopathy with respect to TNFa or -308 TNF promoter polymorphisms (DRIGO *et al.,* 2007). A similar lack of association was observed in a Peruvian study of the -238 TNF promoter polymorphic site (BERAÚN *et al.,* 1998). Together, these results indicate that, at least in South America, TNF polymorphisms are associated neither with CCC development nor with progression to more severe forms of cardiomyopathy in Brazilian Chagas disease patients, although the same polymorphisms may be important for mortality among end-stage CCC patients. More recently, further studies have disclosed an association between CCC and polymorphisms in several immune response genes. Innate immunity genes are attractive candidates for studying differential susceptibility in the development of CCC since parasitism during the acute phase is controlled by the innate immune response. Variants of BAT1, a putative anti-inflammatory gene associated with down-regulation of TNF-α and IL-6, are predictive of the development of CCC (RAMASAWMY *et al.,* 2006). The chemokine MCP-1 (CCL2) variant behaves as a genetic modifier of clinical outcome in *T. cruzi* infection, subjects with the CCL2 -2518AA genotype showing 4-fold greater risk of developing CCC than those without this genotype (RAMASAWMY *et al.,* 2006). Another associated gene is *IKBL*, which is homologous to IκBα, an inhibitor of NFκB that plays a key role in innate immunity. The likelihood of developing CCC is three- and two-fold higher among *T. cruzi*-infected individuals bearing the *IKBL* - 62 genotypes AA and AT, respectively, than among homozygous TT individuals infected with *T. cruzi*. Similarly, patients with genotype - 262AA show three-fold higher risk of developing CCC (RAMASAWMY *et al.,* 2008). The presence of such variants among CCC patients may lead to less efficient downregulation of inflammatory responses, thus contributing to the elevated production of inflammatory cytokines in patients with CCC.

Proteomics: Novel technological approaches, such as high-throughput proteomics, now allow for direct evaluation of large-scale protein profiles and identification of differentially expressed proteins (ARRELL *et al.,* 2001), contributing to our understanding of disease mechanisms and aiding the discovery of potential therapeutic targets.

Our group has recently described an inventory of myocardial proteins from patients with severe CCC. This has allowed us to identify proteins with high and low abundance in the tissue, including proteins with structural, metabolic, stress, apoptosis and immune response functions (TEIXEIRA *et al.,* 2006). It would be interesting to investigate whether the expression of cardiac proteins differs between severe CCC patients and mild CCC or IND subjects. However, since this comparison cannot be performed due the impossibility of obtaining samples from the two latter groups, one must rely on animal models in order to clarify this issue. By combining the outbred hamster model for Chagas disease with proteomic analysis, our group has also shown that susceptibility to acute *T. cruzi* infection is associated not only with high parasite load and cytokine expression in the heart, but also with differential myocardial expression of structural/contractile, stress response, and metabolism proteins (BILATE *et al.*, 2008 in press). These results suggest a link between parasitism, inflammation and cardiomyocyte response. Although the functional data needed to ascertain the role of myocardial protein expression in the pathogenesis of the disease are still lacking, it is very likely that *T. cruzi* infection per se and/or parasite-triggered inflammation can play a role in cardiac damage.

Concluding remarks: Chagas disease cardiomyopathy is an inflammatory disease caused by protozoan *T. cruzi* that affects only 30% of the infected individuals. Data from both murine models and human studies support the view that autoimmune, as well as parasite-driven immune responses may both play a role in generating heart lesions, along with inflammatory cytokines and chemokines. Moreover, the ability of the host to cope with the infection may determine the balance between the inflammatory vs. anti-inflammatory immune responses.

Although much is known about the immunopathogenesis of Chagas disease, certain questions remain open. These include the factors involved in the dichotomy of clinical outcomes among cardiomyopathy patients and indeterminate individuals, and the mechanisms by which parasites manage to survive - essentially unnoticed - for such extended periods within the host. Knowing how the parasite is able to invade cells and circumvent the host's immune response will certainly aid in the development of effective drugs and immunotherapy for the millions of patients still afflicted by this neglected disease.

RESUMO

Cardiomiopatia chagásica: conceitos atuais de doença antiga

A doença de Chagas continua sendo importante problema de saúde pública uma vez que cerca de 10 milhões de indivíduos ainda estão infectados pelo *T. cruzi*. Décadas após a infecção primária, aproximadamente 30% dos indivíduos podem desenvolver uma cardiomiopatia inflamatória crônica, a chamada Cardiomiopatia Chagásica Crônica (CCC). Dados de modelos murinos e de estudos em humanos apóiam a visão de que tanto respostas auto-imunes como as determinadas pelo parasita em conjunto com citocinas e quimiocinas inflamatórias participam da geração das lesões cardíacas típicas da CCC. A presente revisão tem como objetivo sumarizar os recentes avanços no entendimento da imunopatogênese da Cardiomiopatia Chagásica Crônica.

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