The idea that selection during epidemics or longer periods of exposure to infectious diseases may have had significant effects in modifying the constitution of the human genome is not new. In 1931, A.E. Garrod, in his book "The Inborn Factor in Disease", suggested that infectious diseases may have been a major selective force in human evolution and in shaping the biochemical individuality (1).
Various studies have suggested that host genetic factors are major determinants of susceptibility to infectious diseases in humans. For example, HLA gene polymorphism has been associated with susceptibility or protection to malaria, tuberculosis, leprosy, AIDS, hepatitis virus, etc. (Table I). Likewise, the polymorphism in cytokine and cytokine receptor genes have been associated with several infectious diseases (2).

A major factor for establishing and maintaining pathology in some chronic parasitic diseases is the presence of high levels of the causative agent. However, in Chagas’ disease, an infection caused by the protozoan parasite Trypanosoma cruzi, the number of parasites in the host decreases dramatically as the disease progresses from the acute to the chronic phase, either with or without characteristic symptoms and clinical manifestations. Although the presence of the parasite is critical to trigger the series of reactions that lead to chronic pathology, the parasite may not be solely responsible for its maintenance. The presence of autoreactive antibodies and the identification of cross-reactive antigens between host and parasite have lead to the hypothesis on the involvement of autoimmune-type reactions in the generation and maintenance of chagasic morbidity (3). T and B lymphocytes have been shown to play an important role in the development of pathology in human and experimental Chagas’ disease but the exact mechanisms through which these cells mediate pathology are not completely understood (4).

Considering the involvement of an active immune response in the development and establishment of pathology in Chagas’ disease, several studies have been dedicated to determine the possible association between human leukocyte antigens (HLA) and the susceptibility or resistance to T. cruzi chronic infection and to the development of Chagas cardiomyopathy. Recently, other studies have analyzed changes in the T-cell receptor (TCR) repertoire induced by this parasite. Here, we will review the most important immunogenetic studies realized on Chagas disease.

CHAGAS’ DISEASE

Chagas’ disease or American trypanosomiasis is a parasitic disease caused by the protozoan Trypanosoma cruzi. Nearly 90 million individuals are living in endemic areas and 18-20 million are already infected. Actually, it is estimated that there are more than 550,000 new cases and 50,000 deaths associated with this condition every year. This disease leads to an annual loss of 2.7 million disability-adjusted years, being the largest parasitic disease burden in our continent and only third on a global scale after malaria and shistosomiasis (5).

In humans and other susceptible mammals, the disease begins with a short acute phase characterised by elevated parasitemia followed by a life-long chronic phase maintained with scarce parasites. Heart and/or digestive organs (oesophagus and colon) are affected in the chronic phase, usually 20-30 years after infection (6).

Table I

<table>
<thead>
<tr>
<th>Disease</th>
<th>HLA</th>
<th>Population</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>B53↓ DRB1<em>1302-DQB1</em>0501↓</td>
<td>Gambia</td>
<td>35-36</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>DR2↑</td>
<td>India, Russia</td>
<td>37-40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indonesia</td>
<td></td>
</tr>
<tr>
<td>Lepra</td>
<td>DR2↑</td>
<td>India</td>
<td>41-43</td>
</tr>
<tr>
<td>HIV progression</td>
<td>B35↑ A1-B8-DR3↑ B27↓</td>
<td>USA, Europe</td>
<td>44-47</td>
</tr>
<tr>
<td>Hepatitis B persistency</td>
<td>DRB1*1302↓</td>
<td>Gambia, Germany</td>
<td>48,49</td>
</tr>
<tr>
<td>Hepatitis C persistency</td>
<td>DR5↓</td>
<td>Italy, UK</td>
<td>50</td>
</tr>
<tr>
<td>Dengue shock syndrome</td>
<td>A2↑ B blank↑ B13↓</td>
<td>Bangkok</td>
<td>51</td>
</tr>
</tbody>
</table>

↑: Antigen allele/haplotype which confers susceptibility
↓: Antigen allele/haplotype which confers protection

From Hill A.V.S., 1998 (2).
BIOLOGY OF THE PARASITE AND MODES OF TRANSMISSION

The complex life cycle of *T. cruzi* involves mammalian hosts and insect vectors. Transmission to mammals, including humans, occurs when faeces of bloodsucking triatomine insects (reduvid bugs) containing infective organisms contaminate a bite, mucosal surface, or conjunctive. After local intracellular multiplication, organisms are released when the host cells die. Then infectious organisms are spread hematogenously to distant sites where new cells are parasitised. In this way, a cycle is established that alternates asynchronously between intracellular multiplying forms (amastigotes) and non-dividing but infective forms (tryomastigotes) that circulate in the bloodstream (7). The tryomastigotes enter in macrophages by phagocytosis. In the phagolysosomes, tryomastigotes, by a mechanism that is unclear, enter the cytoplasm and convert to replicating amastigotes which eventually produce new tryomastigotes. In the phagolysosome system there is opportunity for trypanosome antigens to be captured by class II MHC molecules, transported to the cell surface and presented to CD4+ T cells. During replication in the cytoplasm there is the possibility for trypanosome antigens to be captured by class I MHC molecules, transported to the cell surface and presented to CD8+ T cells. Such presentation of *T. cruzi* antigens could stimulate CD8+ cytotoxic T cells, leading to destruction of infected cells and interference with parasite replication (8). The cycle is completed when reduvid bugs ingest blood that contains infective forms (tryomastigotes). Not surprisingly, the transfusion of blood donated by persons with chronic *T. cruzi* infection often results in transmission of the parasite (7) and constitute a serious public health problem in endemic and non-endemic areas of America (9). Moreover, parasites occasionally pass from mother to fetus, resulting in spontaneous abortion or congenital Chagas’ disease, which in some areas may occur in 2 to 5% of infants born of infected women. Finally, laboratory workers can be infected accidentally and such infection occurs with alarming frequency (7).

IMMUNOPATHOLOGY OF CHAGAS’ DISEASE

The acute infection of Chagas’ disease last for 30-90 days and gradually merges into a chronic phase in which the parasite proliferation is under control of a continuous immune response. The destiny of the chronic patients that overcome the first years of the disease is uncertain. However, about 20-30% of chronically infected patients develop a cardiomyopathy of variable severity 10-20 years after infection and in 8-10%, a digestive form characterised by pathologic dilatations of the oesophagus and/or colon (megaoesophagus and megacolon). Most patients (50-60%) are apparently asymptomatic for long periods of time or for life, and are considered as indeterminate; these patients display positive serology and/or parasitology tests but negative clinical manifestations. The ratio of conversion from patients of indeterminate form to cardiac disease is approximately 2-3% per year. The asymptomatic patients have a better prognosis and their survival time is longer than that of cardiac chagasic individuals (6).

A large number of studies have been dedicated to the understanding of the immunopathology of Chagas’ disease. Together with the specific immunological response, the acute phase of infection by *T. cruzi* is associated with mechanisms of evasion of host immunity, as well as with polyclonal B and T cell activation, which could lead to immunosuppression due to clonal depletion. The chronic phase of the disease is also associated with autoimmune responses. This is a point of special relevance, since it has been suggested that after its development, probably as a consequence of molecular mimicry between parasites’ antigens and host cellular components, it continues including in absence of the parasite (5). In fact, the human chronic Chagas’ disease cardiomyopathy could be considered as an organ-specific autoimmune disease according to the revised criteria for autoimmune disease by Rose and Bona (Table II) (10).

Recent data using advanced methods have challenged the role of autoimmunity in the pathogenesis and progression of the cardiac lesions in the chronic phase of Chagas’ disease. First, using PCR-based method, specific parasite DNA sequences have been detected in characteristic inflamma-

<table>
<thead>
<tr>
<th>Table II</th>
<th>Rose and Bona criteria of classification for autoimmune</th>
</tr>
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<tbody>
<tr>
<td>Circumstantial evidence:</td>
<td></td>
</tr>
<tr>
<td>Scarcity of parasites in heart lesions (52,53)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytic infiltration of target organ (heart) (54)</td>
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<tr>
<td>Restricted T-cell receptor variable gene usage in situ (30)</td>
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<tr>
<td>Statistic association with a particular MHC haplotype (23-26)</td>
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<tr>
<td>Indirect evidence:</td>
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<tr>
<td>Passive transfer of disease in murine models by CD4+ T cells (55)</td>
<td></td>
</tr>
<tr>
<td>Molecular mimicry between <em>T. cruzi</em> and target organs (56)</td>
<td></td>
</tr>
<tr>
<td>Heart disease-associated molecular mimicry between heart specific epitope and <em>T. cruzi</em> antigen (57)</td>
<td></td>
</tr>
<tr>
<td>Isolation of self reactive T cells infiltrating the lesions in target organ (56)</td>
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</table>

From Kalil J. and Cunha-Neto E. 1996 (56).
tory lesions observed in chronic Chagas heart disease. No parasite DNA has been detected in heart tissue of chagasic patients without cardiac inflammation or in patients with megacolon but without myocarditis (11). Neonatal hearts transplanted into mice chronically infected with T. cruzi do not display signs of autoimmune-type rejection or any inflammatory response. These transplanted hearts survived for more than 1 year and were free of parasites as determined by in situ PCR analysis. However, they were rejected only when directly injected with live parasites, leading to a rapid and dramatic inflammatory response with cessation of heart function. These results suggest that parasitism of heart tissue is both necessary and sufficient for the induction of heart tissue damage in Chagas disease (12). Second, in many cases there are autoreactive antibodies of heterophilic nature and largely irrelevant to the pathogenesis of Chagas' disease, in spite of the fact of the reported similarity (either in amino acid sequence or structural conformation) between molecules or segments of molecules of T. cruzi and those of its host (13). Third, sera from patients with overt Chagas heart disease recognised the C-terminal regions of the T. cruzi ribosomal P proteins, the human ribosomal P proteins and the acidic epitope of the second extracellular loop of the β1-adrenergic receptor. The authors observed that this anti-P-antibodies showed a marked preference for the parasite proteins and had low affinity for the human P proteins (14). Moreover, it has been shown that these antibodies can produce stimulatory or inhibitory effects on heart activity (15) and may play a role in the induction of functional myocardial impairments observed in chronic Chagas heart disease (14). Finally, it has been demonstrated that, in experimental infections, the elimination of T. cruzi with specific chemotherapy causes reversion of cardiac fibrosis (16) and that the treatment of chronic chagasic patients with benznidazole induces a marked reduction in the occurrence of electrocardiographic changes and lower frequency of deterioration in their clinical condition (17). These results provide evidence on the need of the parasite's presence for the induction of the pathogenic response characteristic of chronic Chagas disease (12), as well as on the fact that the elimination of the parasite is a prerequisite to arrest the evolution of the disease (5).

HLA AND CHAGAS' DISEASE

An association between HLA molecules and susceptibility to the development of chagasic cardiomyopathy has been previously reported. In 1983 our laboratory studied the distribution of HLA antigens in Venezuelan individuals serologically positive for T. cruzi using classical serology and mixed lymphocyte culture methods. We reported a statistically significant increase of the HLA-Dw22 antigen (pc<0.001) (DRB1*1602 DQA1*0501 DQB1*0301) in the patients compared to healthy subjects. However, neither the patients with arrhythmia nor those with congestive heart failure showed HLA class II or I antigens frequency differences compared to the asymptomatic patients (18). Furthermore, the distribution of HLA class I antigens in serologically positive Chilean individuals showed a statistically significant difference in the HLA-B40 Cw3 haplotype frequency present in cardiomyopathies compared to non-cardiomyopathies (19). Data obtained during the V Latin American Histocompatibility Workshop did not confirm the significant decrease of the HLA-B40 Cw3 combination observed among Chilean cardiopatients. However, by testing the subtypes of B40 (B60 and B61), the Workshop data demonstrated a diminution of B60 (0.043 vs 0.086), an increment of B61 (0.067 vs 0.017), and a slight increase of Cw3 (0.14 vs 0.10) among cardiopatients as compared with asymptomatic chagasic patients (20). Nevertheless, a study recently done in our laboratory demonstrated that HLA-Cw*03 allele is significantly increased among cardiopathic patients. In addition, HLA-Cw*03 showed linkage disequilibrium with HLA-B*40 or B*15 in patients with cardiac damage and asymptomatic. Contrary to what has been observed among Chilean chagasic patients, both the B*40 Cw*03 and the B*15 Cw*03 haplotype were more frequent among Venezuelan chagasic with damaged heart than among asymptomatic patients (OR = 4.8 and 3.2, respectively). These results suggest that the HLA-Cw*03 allele could confer susceptibility to the development of cardiomyopathy among Venezuelan T. cruzi seropositive individuals and contrast with the protective effect conferred by the HLA-B40Cw3 haplotype among Chilian chagasic patients. The differences between both studies could be due to differences in population ethnicity, environmental factors or prevalence of distinct parasite strains (21). During the V Latin American Histocompatibility Workshop, the serological distributions of HLA class I and II antigens was evaluated among Caucasian and Mestizo chagasic patients living in several countries. The study reported an increase of HLA-A31, B39, DR8 and a decrease of HLA-DR4, DR5, DQ1, DQ3 antigens in chagasic mestizos with cardiomyopathy as compared to mestizo controls (20). Recently, Deghaide et al. evaluated the HLA class I and II profiles of a large group of patients with Chagas' disease using serology and oligotyping analysis. These patients were classified into subgroups: patients with cardiomyopathy and heart failure, patients with cardiomyopathy and without heart failure, patients with digestive tract manifestations, patients with positive serology for chronic T. cruzi infection. The HLA-A30 antigen was increased in the total group...
of patients (pc<0.001) and in the subgroups compared with healthy individuals. However, the comparison between the subgroups of patients and control subjects lost significance after correction of the p values. The relative risk (RR), indicates how often the disease occurs in individuals with the antigen/allele compared to those without it) and the etiologic fraction (EF; estimates how much the antigen/allele contributes to the susceptibility to disease) conferred by the group of patients and for the subgroups with solely cardiac or digestive manifestations were similar. This suggests that HLA-A30 may be a shared susceptibility marker for both forms of disease. The frequency of HLA-DQ1 antigen was increased and the frequency of HLA-DQ7 was decreased in the total group of patients with respect to healthy individuals. The comparison of HLA-DQ1 frequency among the subgroups of patients showed variable values of RR and EF, suggesting distinct patterns of association according to the form of disease presentation. The protection conferred by the HLA-DQ7 antigen was similar for the total group of patients and for the subgroups with cardiac and/or digestive tract manifestations. Molecular analysis of HLA-DQ specificities showed that HLA-DQB1*06 allele was significantly decreased in the total group of patients and the subgroup with cardiomypathy and heart failure. Although, patients with pure digestive tract disease showed a nonsignificant decrease of HLA-DQB1*06 allele, the RR and preventive fraction (PF, estimates the protection against development of the disease) conferred by this allele were similar for the total group and for the subgroup with cardiomypathy. This suggests that this allele could be a marker of protection against the development of the disease, independently of the form of presentation (either cardiac or digestive disease) (22).

In 1998, our laboratory reported that HLA class II genes might be associated with the development of a chronic infection and with heart damage in Chagas' disease. In this study a decreased frequency of DRBI*14 and DQB1*0303 allele in the patients compared with healthy individuals was found. The analysis of HLA alleles revealed the increased frequency of DRBI*08 among seropositive subjects with arrhythmia and congestive heart failure compared with asymptomatic individuals. Increased frequencies of DRBI*0101, DQB1*0501 and of the haplotype formed by them were found in the patients with cardiac tissue damage compared with asymptomatic patients (23). In 2000, Nieto et al. reported a highly significant increased frequency of the DRBI*14 allele in healthy individuals compared with chagasic patients of the district of Arequipa (Perú) (24). This suggests that this allele, in haplotypic combination with DQB1*0301 or another factor linked to them, are involved in resistance to chronic infection by T. cruzi (24), thus confirming the association that our laboratory had previously reported (23).

The finding of an increased frequency of DRBI*08 among seropositive subjects with arrhythmia and congestive heart failure (16.7% vs 8.1% in asymptomatic patients) in our study (23), confirmed the serological data reported during the V Latin American Histocompatibility Workshop (20) and the study carried out in Brazil (25). The three studies showed positive association of HLA-DR8 with cardiomypathy among chagasic individuals, suggesting that the HLA-DR8 could confer susceptibility to the development of cardiomypathy among T. cruzi seropositive individuals (Table III).

More recently, our laboratory confirmed the association of the DRBI*0101 DQB1*0501 haplotype with susceptibility to the cardiac disease. Molecular analysis of the HLA class II DP gene polymorphism showed an increased frequency of DPBI*0401 and DPBI*2301, and of the combination of DPBI*0401 with *0401, *2301 or *3901 alleles in patients with cardiomypathy as compared with asymptomatic patients. The increased frequency of these combinations of alleles may imply susceptibility to the development of Chagas' cardiomypathy (26). However, the influence of the HLA background and the identification of mechanisms to explain it are obstructed by the difficulty to identify individuals that develop an effectively protective immune response against the parasite in endemic areas and the possibility that autoimmune cardiomypathy in Chagas' disease may be present in only a fraction of these patients (23).

The results suggest that several HLA class I and II genes may be associated with development of cardiac pathology in Chagas' disease. However, these studies can not be considered as conclusive due to the fact that the associations between infectious diseases and MHC polymorphism are difficult to show because the low coefficient of selection of MHC alleles requires the study of very large population samples (27).

CHAGAS' DISEASE AND TCR REPERTOIRE

T lymphocytes play a central role in the ability to generate humoral and cell mediated immune responses. Although their effector functions are diverse, T-cells recognise antigenic peptides presented by molecules encoded within the major histocompatibility complex (MHC). The fine specificity of a T-cell is determined by the receptor for antigen (TCR), which is displayed on the cell surface as a heterodimer composed of an α and β chain or a γ and δ chain. Thus, the TCR plays a potentially crucial role in mediating the human
immune response (28). Recently, the identification of T-cells involved in a variety of autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and type I diabetes mellitus has been the focus of immunological research. These studies have been inspired by the observation that in several experimental models of autoimmunity, the responses to the antigens inducing disease show a prominently high degree of restricted heterogeneity with respect to the T-cell receptor (29). Many years after the primary infection in Chagas' disease, a group of infected individuals develop severe heart disorders including electrical conduction abnormalities and dilated cardiomyopathy, associated with a lymphocytic inflammatory infiltrate in the affected heart (30).

The involvement of cell populations related to autoimmune processes in the development of chagasic pathology has been studied. Dutra et al. showed that elevated proportions of activated CD4+ and CD8+ T cells as well as CD5+B cells are present in high levels in the peripheral blood of chagasic patients (31). Furthermore, activated CD8+ T cells are the major component of the inflammatory infiltrate in chagasic cardiac lesions. The presence of these cells, which are capable to exert cytotoxic activity and IFN-γ production in the site of the lesions, suggests that they may play a critical role in the damaging processes leading to severe inflammation and cardiac pathology. Other studies have correlated high levels of T cells expressing HLA-DR with autoimmune pathology (3).

In Chagas' disease, the importance of T cells for protective immunity has been demonstrated (32,33). However, T cells can also contribute to the pathogenesis of the chronic inflammation. For example, CD4+ autoirreactive T cell clones have been isolated from the heart of chronic chagasic patients. A restricted TCR V gene usage in human hearts and CD4 T cells reactivity to alpha cardiac myosin also favour the hypothesis that chronic inflammation caused by T. cruzi is T-cell mediated (34).

Cunha-Neto et al. studied the participation of T cell population in the pathogenesis of Chagas cardiomyopathy, looking at the variable region family usage from both the α and β chains of the TCR expressed in the heart tissue of chagasic patients and healthy individuals. The results demonstrated that heart-infiltrating T cells used a limited number of VαTCR, but not Vβ families. On the other hand, in normal heart tissue, diversity of Vα and Vβ TCR was similar among the scarce circulating T cell population (29).

Recently, Costa et al. analysed the expression of VβTCR repertoire in CD4+ and CD8+ T cells from acute and chronic chagasic patients using flow cytometry. Vβ expression was determined in peripheral blood mononuclear cells isolated from patients, as well as after in vitro stimulation with antigens derived from epimastigote (EPI) or trypomastigote (TRP). The analysis showed an increase in Vβ5 expression in the CD4+ T cell population from chronically infected patients with cardiac disease, but not in patients with the indeterminate clinical form. On the other hand, the frequency of Vβ5 CD4+ T cells from acutely infected individuals was decreased with respect to healthy individuals. The results demonstrate that the frequency of Vβ5 expressing T cells is variable in different phases and clinical forms of the disease. Similarly, the analysis of the VβTCR repertoire after in vitro stimulation with antigens derived from EPI or TRP showed that both antigenic preparations led to preferential expansion of CD4+Vβ5+ T cells suggesting a possible involvement of these cells in the pathology of Chagas' disease (3).

**CONCLUSIONS**

The infection with the protozoan T. cruzi is apparently associated with genetic components (HLA genes and non-HLA genes, such as TCR genes) which need to be studied to understand the immunological mechanisms which control the replication of T. cruzi during chronic infection, the immunopathogenesis and the immunogenetics of the chronic disease. Such knowledge...
may contribute new approaches to treat and/or avoid the development of symptomatic forms of Chagas' disease (6). Furthermore, the study of genetic factors in the infection by T. cruzi in humans offers the possibility to answer two important questions: why some of the individuals living in endemic areas are resistant to become infected, and why only a fraction of the chronically infected develop cardiac disease (21). It is necessary, however, to emphasise the necessity of housing and nutritional improvement in endemic countries, community education and use of insecticides against triatomine vectors to prevent human infection and to control disease transmission.

References