PATHOGENESIS AND IMMUNOPATHOLOGY OF CHRONIC CHAGAS' DISEASE

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Trypanosoma cruzi is transmitted in natural conditions by strictly haematophagous triatomid-bugs. These vectors harbour in the digestive tract metacyclic trypomastigotes, infective stages which are eliminated through the faeces and infect the human host by contamination of skin lesions or intact mucous membranes. However, T. cruzi may by-pass the vectors and be transmitted to man by a number of alternative mechanisms, namely, blood transfusion, congenital transmission, laboratory accidental infection, organ transplantation and oral route. Whatever the route of infection the trypomastigotes penetrate into a variety of cells, mostly muscle cells and macrophages; they change into amastigote stages which readily multiply in the cells and differentiate into trypomastigotes. The flagellates then disrupt the host cells and after circulating in the blood for a variable period of time reach other cells to accomplish their intracellular cycle. The T. cruzi-host cell interaction involves a complex process of mutual recognition mediated by surface membrane receptors, mainly glycoproteins and free carbohydrates.

The exponential parasite intracellular multiplication results in a gradual increase of parasitemia usually detectable by fresh blood examination. Most patients are able to mount an immune response which
controls the parasite proliferation and curbs parasitemia to a subpatent level. In mice the outcome of the early *T. cruzi* infection is under the influence of the parasite strain and the host genetic background but the role played by such factors in the human infection is not yet known. Also in mice the acute phase induces a massive blast transformation of lymphocyte classes indicating the existence of polyclonal activation, a phenomenon characterized by the emergence of B-cells highly reactive to antigens non-related to the parasite followed by the host immunosuppression. Human acute cases display unusual high levels of antibodies against laminin (a basement membrane glycoprotein that participates in the attachment of epithelial and endothelial cells) which contrast with the low levels of specific anti-*T. cruzi* antibodies, a finding which might be an expression of polyclonal activation.

The acute phase in humans roughly corresponds to the period of patent parasitemia, lasts for 1 to 2 months and is clinically characterized by signs of portal of entry of the parasite, fever, oedema, lymph nodes enlargement and a severe myocarditis related, at least at its early phase, to the presence of intracellular parasites. An "innaparent" form of the acute phase has been described in which symptoms were mild and the patients apparently immunoregulated. In most acute cases the clinical symptoms subside spontaneously and, interestingly, are followed by an apparent complete recovery of the heart myocarditis. In untreated cases lethality has been reported to occur in 2% to 8% of the patients, mainly in children.

The acute phase gradually merges into the chronic phase, a life-long disease displaying the following general characteristics: parasitemia is subpatent and bloodstream parasites can only be detected by tedious and time-consuming parasitological methods as xenodiagnosis and hemoculture; tissue parasitism is accordingly very scanty; spontaneous parasitological cures are certainly very rare or do not occur at all; spontaneous reagudization has not been reported either; at least in experimentally infected hosts reinfection does not induce new outbreaks of acute disease and patent parasitemia.

At the chronic phase hosts build-up a steady immune response against *T. cruzi*. Protective (or "lytic") antibodies directed to surface membrane epitopes present only in living trypomastigotes are associated to resistance; in contrast, conventional serology antibodies (CSA) which are involved in the serodiagnosis and recognize *T. cruzi* fixed parasites,
subcellular fractions or purified antigens, do not participate in the host resistance. Only protective antibodies, but not CSA, are able to mediate effector immune mechanisms in Chagas' disease such as complement-mediated lysis, antibody-dependent cell cytotoxicity and phagocytosis. The ineffectivity of vaccines so far tested in Chagas' disease has been suggested to be due to their ability to generate CSA but not protective antibodies.

The fate of chagasic who overcome the acute stage of the disease and enter the chronic phase is usually unpredictable. The majority (50%-60%) remains apparently "asymptomatic" for long periods of time or even for life, and are considered as being in the "indeterminate" form; about 20-30% develop within 10 to 20 years infection a myocardopathy of variable severity; finally, 8% to 10% present a digestive form characterized by pathological dilatations of the esophagus and/or colon (megaesophagus and megacolon). A certain percentage show an association of cardiac and digestive manifestations. Why chronic chagasic patients develop such different clinical forms is the first enigma of this intriguing disease.

In order to be included in the indeterminate form chronic chagasic patients should fit into the following minimum clinical criterion: positivity of serological and/or parasitological tests for Chagas' disease; absence of clinical manifestations of the disease; normal conventional electrocardiogram; normal X-Ray of heart, colon and esophagus. Nevertheless, the definition of this group as whole is not as simple as here described. Ergometric tests performed with these patients often show results similar to those observed in normal controls in relation to functional capacity, maximum load attained, arrhythmias, etc; however, more refined methods (for instance, ECG taken after administration of ajmaline, a potent conduction depressing drug) or more prolonged electrographic monitorization are able to disclose in a percentage of patients heart disturbances otherwise undetected. Interestingly, a number of "indeterminate" patients who die from causes not related to Chagas' disease (accidents, suicide, etc) display heart lesions similar but much less severe than those found in patients with the cardiac form who die suddenly or from cardiac failure. Although longitudinal epidemiological studies performed in endemic areas show that conversion of the indeterminate form to cardiac occurs at a rate of about 3% per year, the prognosis of the former group is in general far better and the survival time longer than the cardiac form. A 10-year follow-up of chronic chagasic patients in the brazilian endemic area of
Bambui demonstrated that the survival time of the group displaying normal ECG at the beginning of the observation was of 97.4%, whereas in the group with abnormal ECG it was 69.8% for females and 54.8% for males.4

The patients from the cardiac form develop a myocardiopathy of variable severity clinically expressed by an extensive range of manifestations from minor electrocardiographic alterations to heart failure or sudden death; practically all sort of electrocardiographic abnormalities related to impulse formation and conduction can be detected in such patients. The chagasic myocardiopathy results from a long process of chronic, progressive and fibrotic myocarditis.5 Parasites at this stage are hardly found in the heart and other organs.

In the digestive form one of the earliest manifestations of the megaesophagus is a slowness of the esophagus normal peristalsis which is later on followed by dilatation, dysphagia and related symptoms. Prolonged obstipation is the main symptom of megacolon which presents different degrees of organ dilatation.

The pathogenesis of the digestive tract lesions in Chagas' disease seems to be related to a destruction of the neuron cells from the intrinsic nervous system which plays a fundamental role in the motor coordination of the digestive tract. Interestingly, a significant destruction of the neuron cells from the heart parasympathetic gangliae are also detected in cardiac chagasic patients. We will discuss later the possible factors involved in the "denervation" in Chagas' disease.

Another puzzle in the disease is the geographical variability in prevalence of the clinical forms. In relation to the digestive form, for instance, there is a marked difference between its occurrence in Brazil where circa 10% of the chagasic patients show some degree of esophagopathy, and in Venezuela or Central America countries where chagasic megaesophagus has an extremely low prevalence or is practically absent.

Finally, the most controversial problem in Chagas' disease is the identification of mechanisms by which late lesions occur in different organs at the chronic phase, after a long latent period and when parasites are very scarce. Although the bulk of evidence indicates that intracellular parasites play a negligible role in the pathogenesis of Chagas' disease, recent papers suggest that intracellular parasitism might be more frequent than previously thought. Thus, systematic and tedious search of parasites carried out by various authors has demonstrated their presence in scanty numbers not only
in the heart but also in other organs. Intriguingly, a higher number of intracellular forms has been detected in the central vein of the suprarenal. An important observation is that only in the myocardium are the parasites associated to chronic inflammatory reactions, an evidence of the participation of other factors than only parasites in the pathogenesis of the chagasic myocarditis. Parasites and hosts factors have been incriminated in the pathogenic of the disease as we will now review.

*T. cruzi* comprises a pool of highly heterogeneous populations which circulate in nature among humans, vectors and reservoirs. Once isolated these different parasite strains display a great variability in their experimental behaviour and can be identified by a number of markers. From the biological point of view they may differ in the course of infection, *in vitro* infectivity to host cells, *in vivo* distribution of intracellular parasites, sensitivity to immune response, etc. Biochemical characterization involves differences in surface membrane carbohydrates detectable by lectins, isoenzyme patterns, kinetoplast-DNA (K-DNA) fragments yielded by restriction endonucleases and sequences of K-DNA detected by molecular probes. The availability of such biological and molecular markers raised expectations that a correlation could be established between morbidity expressed by the clinical forms and the characteristics of *T. cruzi* strains isolated from the different patients. However, because of the lack of well designed clinical-epidemiological studies and, also, the existence of subpopulations in *T. cruzi* strains this correlation could not be achieved. There has been demonstrated that cloned organisms derived from single *T. cruzi* developmental stages present a great variability. Moreover, since selection and deletion of these subpopulations may occur during the process of isolation and maintenance of strains in experimental conditions, and as infections by more than one single strain may also take place in nature, there is now some uncertainty about the characterization and stability of *T. cruzi* strains isolated from patients.

There is, however, some scarce circumstantial evidence relating morbidity to parasite characteristics. For instance, mice that survive a severe acute phase (an expression of parasite virulence) show at the chronic phase a higher rate of myocarditis and fibrosis than mice surviving a mild acute infection. In relation to human disease differences in the prevalence of the digestive forms in Brazil and Venezuela have been related to dissimilar isoenzyme patterns of *T. cruzi* strains in both countries, a
finding which has been considered as more circumstantial than factual evidence.

The doubtful role played by the parasite in the pathogenesis of Chagas' disease has reinforced the alternative possibility that lesions may be induced by auto-immune mechanisms. There has been demonstrated that monolayers of allogeneic rabbit heart cells parasitized by *T. cruzi* but also non-parasitized cells were lysed by lymphocytes obtained from rabbits chronically infected with *T. cruzi* or immunized with its subcellular fractions, an evidence for the existence of cross-reacting antigens between heart cells. These data and the possible role of cell-mediate auto-immune response in Chagas' disease myocardiopathy suggested by the authors have been disputed on grounds that in this kind of experiments cytotoxic T cells would only bind to target cells bearing both specific antigens and MHC determinants, a requirement not present in the allogeneic system described in the paper. There has been then suggested that the described cytotoxicity could be effected by cells which are not MHC restricted (NK, lymphocyte activate killer cells and cytotoxic macrophages). Since then, however, other data point out towards the participation of misdirected cellular immune response in Chagas' disease pathology have been published. Thus, heart lesions have been apparently induced in rabbits injected with *T. cruzi* subcellular fractions; myositis and mild myocarditis were produced by adoptive transfer of lymphocytes from *T. cruzi*-infected to naive mice; muscle cell lines were lysed by T cells from chronic chagasic mice; chronically infected mice developed splenic lymphocyte cytotoxicity directed against normal syngeneic neonatal cardiac myofibers.

In relation to participation of humoral auto-immune response in Chagas' disease an antibody against heart endothelial cells, vascular structures and interstitium (EVI antibody) has been detected in a high percentage of chagasic patients; however, its importance in the pathogenesis of heart lesions still remains controversial. More recently there has been demonstrated that laminin is the only or the major antigen inducing EVI antibodies. Since laminin is an important component of basement membranes to which epithelial and endothelial cells are attached, the presence of antibodies against this glycoprotein might have practical implications. The interpretation of the meaning of these antibodies is complicated by the fact that laminin has been also detected in the surface membrane of *T. cruzi* trypomastigotes. Specific antibodies against neuron cells and
Peripheral nerves have been also detected in most chronic chagasic patients. Anti-neuron cells antibodies would be particularly important to explain denervation occurring in heart gangliae and digestive tract nervous autonomic system. In general, however, correlation between these antibodies against host tissues and morbidity has been considered as circumstantial and no definitive demonstration of this relationship has been provided.

There is also in the literature some scarce direct demonstration of the existence of antigens shared by T. cruzi and host cells. A monoclonal antibody raised against rat root ganglia could recognize epitopes on T. cruzi surface membrane; on the other hand, two monoclonal antibodies against T. cruzi were able to define antigenic determinants from murine brain and spinal cord. Finally, serological tests seemingly demonstrated in T. cruzi an epitope antigenically related to sarcoplasmic reticulum adenosine triphosphatase preparations (SRA), an enzyme from rabbit skeletal muscle that participate in the contraction of striated muscle.

Another auto-immune mechanism suggested by in vitro experiments was the destruction of infected and non-infected stand-by cells which either express or passively adsorb parasite antigens in the surface membrane and therefore turn into targets for the immune response.

Immunoregulation in patients with different clinical forms has been investigated by examining the reactivity of peripheral blood mononuclear cells to T. cruzi antigens. Although the patients have shown differences in their responses (about 30% were considered as "low responders"), no relationship could be established between immune responsiveness to T. cruzi antigens and their clinical forms. On the other hand, an enhancement of the myocarditis was observed in dogs chronically infected with T. cruzi and submitted to treatment with low doses of cyclophosphamide. As this treatment does not induced multiplication of parasites suggestive of T. cruzi-specific immunodepression, the authors propose that the exacerbation of cell-mediated lesions may be caused by selective destruction of T suppressor cells or other components of the host immunological network. Experiments carried out to investigate the role of T8-suppressor cells in the immunoregulation of chronic chagasic patients demonstrated that whereas cells of 91.0% of patients in the indeterminate form presented different levels of suppression in their proliferative response to PHA, only 28.0% of those in the cardiac form had their response suppressed.

There has been also suggested that anti-self clones which emerge during
the polyclonal activation induced by *T. cruzi* acute experimental infections and persist for long periods of time might participate in the auto-immune process at the chronic phase.\(^2\) Finally, recent work has demonstrated in chagasic patients the existence of auto-antidiotype-T cells which proliferate after being stimulated by purified anti-*T. cruzi* epimastigote polyclonal antibodies; moreover, the cells are apparently more efficiently stimulated by pooled anti-epimastigote antibodies from cardiac patients than from the indeterminate form, suggesting that Id/anti-Id network may participate in the pathogenesis of Chagas' disease.

This review indicates that the pathogeny of Chagas' disease is still a controversial issue. The diversity of immune responses to host antigens, the complex antigenic make-up of *T. cruzi* which apparently includes also antigens shared by the host, the early stage of investigations on the peculiarities of the immunological network in Chagas' disease and the lack of experimental models in which active *T. cruzi* infection or immunization with parasite antigens would regularly result in lesions resembling human pathology - all these problems together indicate how far we probably are from answers to the "enigmas" in the pathology of Chagas' disease.

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