Chagas' disease: trends in immunological research and prospects for immunoprophylaxis

ANTONIO R. L. TEIXEIRA

Acute infection with Trypanosoma cruzi usually subsides spontaneously but the mortality rate encountered in individuals with the chronic infection is high. Much evidence has accumulated in the last five years that autoimmunity plays an important role in the pathogenesis of the myocarditis that is common in the chronic phase. A negative relationship has been observed between the demonstrable parasitaemia and the presence of severe cardiac lesions. This myocarditis is characterized by lymphocytic infiltrates and destruction of normal heart cells, in the absence of the parasite in situ. Furthermore, the demonstration in vitro of heart cell lysis by T. cruzi-sensitized T lymphocytes is strong evidence of autoimmunity in Chagas' disease.

Acquired immunity plays a major role in the course that T. cruzi infections may run in the mammalian host. As a result of the immune mechanisms induced by the parasite, the infection is controlled at subpatent levels, and the immune host does not develop acute T. cruzi infection again. At present there are several means of achieving immunoprotection against experimental T. cruzi infections, but it is not known whether vaccinated animals might develop chronic Chagas' disease and die many months or years later. Studies on immunoprotection against Chagas' disease should therefore not be limited only to the acute phase of the infection. Furthermore, the involvement of autoimmunity in the production of the lesions of Chagas' disease indicates that research in this area should be conducted with caution. The definition of an animal model for chronic Chagas' disease is essential to further development of immunological research devoted to immunoprophylaxis.

Trypanosoma cruzi infects more than one hundred species of domestic and wild mammals, including members of several orders. Man is considered to be the definitive host of this protozoan because of all the species of mammal infected by T. cruzi, man has the longest life span. T. cruzi is essentially an intracellular parasite and does not multiply in the blood of vertebrate hosts, but its metacyclic trypomastigote form may be present transiently in the peripheral blood and tissue fluids at the time of transfer from one cell to another.

The acute phase of T. cruzi infection—Chagas' disease or American trypanosomiasis—can be totally asymptomatic and goes unrecognized in approximately two-thirds of infants and children infected. The initial infection usually subsides spontaneously within 2–3 months. Then the infection becomes latent for a long period during which time parasitaemia is subpatent, but antibodies to T. cruzi can be demonstrated by serological tests. In asymptomatic patients diagnosed by serological tests as having Chagas' disease, the onset of recurrent palpitations, electrocardiographic abnormalities, or heart failure are ominous signs. The mortality in the population with chronic T. cruzi infection reaches 12.8/1000, of which 57% die as a direct result of Chagas' disease. Of these, 58% die from cardiac insufficiency, and 4% from a non-cardiac cause; 37.5% are stricken by

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1 Associate Professor, Immunopathology Laboratory, Faculty of Health Sciences of the University of Brasilia, Brazil.
sudden death. Recent estimates indicate that at least 12 million people in Latin America are infected. Thus, Chagas' disease is a major public health problem in endemic areas of Latin America.

Clinical and laboratory evidence has shown that human subjects and experimental animals do not undergo a second acute phase of the disease. This observation has suggested that the eventual development of a practical immunoprophylactic procedure is feasible. However, because of the involvement of autoimmunity in Chagas' disease the development of a vaccine for use in man will require much further research. This article is concerned with the immunological research that may eventually lead to effective immunoprophylaxis.

CLINICAL MANIFESTATIONS OF CHAGAS' DISEASE

Acute phase

Acute Chagas' disease usually occurs in children and is characterized by the microscopic finding of trypomastigotes in the peripheral blood. Until very recently, clinical and epidemiological studies had shown low prevalence of acute Chagas' disease in endemic areas where the prevalence of chronic T. cruzi infection was high. This discrepancy is now explained by the description of an inapparent form of the acute stage of the disease. This subclinical infection is totally asymptomatic, not being perceived by the patient or the physician. Inapparent cases of acute Chagas' disease are diagnosed by seroconversion of previously negative individuals living in an area endemic for Chagas' disease, and studies have shown that the inapparent cases are approximately twice as frequent as the apparent cases. These studies have thus demonstrated that the initial T. cruzi infection is usually benign and asymptomatic. It seems that only a small percentage (not yet determined) of acute cases eventually develop myocarditis or meningoencephalitis.

Latent phase

The individuals who recover from acute Chagas' disease may harbour T. cruzi in their bodies for life, although they do not show any symptoms of Chagas' disease. However, haemodynamic studies and ECG recordings continued for longer than usual have shown early, otherwise unsuspected signs of cardiac involvement. Pathological study of the hearts of patients with positive serological tests but no clinical signs (i.e., with the indeterminate form of the disease) has shown small areas of myocarditis and heart cell destruction with no apparent involvement of the parasite in the lesion.

Chronic phase

Patients diagnosed by serological tests as having Chagas' infection may present, many years or decades later, with electrocardiographic abnormalities and digestive disturb-

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ances. Cardiac insufficiency appears predominantly between 20 and 50 years of age. Once decompensation is established, life expectancy is usually 7 months–2 years. When these patients die of chronic Chagas' disease their hearts are enlarged. Microscopic examination of the myocardium reveals striking myocarditis, but parasites are not seen in the cardiac fibres. Other common manifestations of chronic Chagas' disease are the "mega" conditions. Segments of the oesophagus and colon are dilated and hypertrophied, conditions related to the destruction of ganglion cells in the parasympathetic myenteric plexus.

Influence of the level of parasitaemia on clinical manifestations

Acute Chagas' disease is defined as the initial phase of T. cruzi infection in which the flagellates are found in the peripheral blood by direct examination of wet preparations. The descriptions above of clinically asymptomatic acute Chagas' disease and of the natural history of T. cruzi infections in vertebrate hosts, leave open to question, however, the relationship between the number of parasites in the blood or encysted in tissue cells and the severity of Chagas' disease and raise two major questions:

1. Why are the majority of the initial T. cruzi infections asymptomatic?
2. How can the observation be explained that although the initial infections are frequently benign the infected individuals die many decades later when the infection is subpatent?

Adequate answers to these questions have now been provided by studies on the immunopathology of Chagas' disease and will be discussed later.

In patients with chronic Chagas' disease the parasite cannot be detected in the peripheral blood unless special methods are used to concentrate the parasites after a long period of multiplication in the insect vector (xenodiagnosis) or in a synthetic medium. These studies have shown that the majority of Chagas' patients with negative xenodiagnosis are found between the 3rd and 5th decades of life. This is interesting because clinicians and pathologists know that the highest rate of morbidity and mortality in Chagas' disease also occurs in this same age group. These observations also indicate that in a large number of patients with chronic Chagas' disease even repeated xenodiagnosis does not demonstrate parasites in the blood. Furthermore, a negative relationship has been observed between the level of parasitaemia and the presence of the severe cardiac and digestive disturbances that are frequently responsible for the high morbidity and mortality of Chagas' disease. These results therefore indicate that the clinical manifestations of Chagas' disease are not related to the level of parasitaemia.

MECHANISMS OF RESISTANCE AGAINST TRYPANOSOMA CRUZI

Natural immunity

Cold-blooded animals and birds are generally refractory to Trypanosoma cruzi. The peripheral blood trypomastigote forms derived from infected mammals do not infect

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reptiles and amphibians; within 3 hours all trypanosomes disappear from the site of inoculation and cannot be found alive in the blood. Further, if the animals are killed 7 days later they do not show amastigote forms encysted in the tissues. There is some indication that sera from fowl, frogs, and toads have a lytic effect on metacyclic trypanosomes (i.e., trypomastigotes) but the mechanism of natural resistance has not been fully explained.

*Trypanosoma cruzi* does not enter the blood of avians because the parasites are destroyed in the skin at the site of inoculation. The resistance of chickens to *T. cruzi* appears at hatching; *T. cruzi* infection can be established in the embryo, but no signs of infection are found in chicks hatched from infected embryos. Although it appears that serum factors are involved in the natural resistance of avians to this protozoan, there are indications that the main natural defence mechanism against the infective trypomastigotes is probably phagocytosis by macrophages.

When trypomastigotes are inoculated into fowl, frogs, and toads, some parasites reach the bloodstream but are taken up by macrophages. Also, when *T. cruzi* forms of low virulence are injected into non-immune mice the parasites are largely destroyed by macrophages at the site of inoculation. On the other hand, highly virulent forms multiply in macrophages by binary fission and, although many are destroyed in the phagocytic cell, some become trypomastigotes that escape to infect other "nonphagocytic" cells. Any tissue or cell type can be parasitized, but cysts containing amastigotes are most often found in cardiac muscle, skeletal muscle, and glial cells.

In view of the description given in the previous paragraphs, it becomes evident that further research is required to elucidate the mechanisms of natural resistance of these vertebrates against *T. cruzi*. For instance, although many studies have been carried out on the uptake of *T. cruzi* by macrophages from susceptible mammalian hosts, there is no information on the uptake and intracellular killing of *T. cruzi* by macrophages from animals not susceptible to *T. cruzi* infections.

The genetic control of resistance to several protozoan organisms has been studied in inbred mice, but there are few data on such genetic control of resistance in *T. cruzi* infections. Data obtained by Trischmann et al. do indicate, however, a strong genetic influence, but one that is independent of the major histocompatibility complex, and hence probably unrelated to known Ir genes. Three general categories of genetic resistance are observed. Resistant strains, such as C57BL/10, when challenged with a standard inoculum develop a transitory parasitaemia, but all mice survive. In contrast, susceptible strains, such as C3H and A/J develop a high parasitaemia and die of infection. A number of other strains, including BALB/c, SJL, DBA1, DBA/2, and AKR mice have intermediate susceptibility. On the other hand, congenitally athymic nude mice (nu/nu), despite having a BALB/c background, are highly susceptible and develop an exceptionally high parasitaemia before death, suggesting that the level of parasitaemia attained and possibly, as a consequence, the degree of resistance of the host may be related to the immune response of the host. In all cases, resistance has been found to be associated with the genetic background and to be independent of the H-2 genes. Thus, C3H.SW are as susceptible as C3H, and B10.BR are as resistant as C57BL/10. Trischmann et al. compared peritoneal macrophages from C3H and C57BL/10 mice for susceptibility to infection and clearance of parasites *in vitro* and found them to be

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indistinguishable in their resistance to infection with trypomastigotes, suggesting that resistance is not mediated directly by macrophages.

Circumstantial evidence strongly suggests that individual susceptibility is involved in the prevalence of Chagas' disease. For instance, it is well known that under the same conditions not all family members acquire *T. cruzi* infections with equal readiness. Therefore, attempts should be made to identify the factors responsible for individual susceptibility to *T. cruzi* infections.

**Acquired immunity**

There is abundant evidence indicating that man, as well as animals, develops partially effective acquired immunity to *T. cruzi*. Although some progress has been made, many aspects of the basic mechanisms involved are still poorly understood.

**Humoral immunity**

*T. cruzi* is a strongly immunogenic parasite and high titres of complement-fixing, haemagglutinating, and precipitating antibodies occur in Chagas' disease. The highest antibody titres are detected in the serum during the acute phase, when many parasites are circulating in the blood. These antibodies are mainly of the IgM type. Later, when the parasitaemia becomes subpatent, lower levels of specific antibodies are still present in the serum and during the latent and chronic phases of Chagas' disease these are in the immunoglobulin classes IgG and IgA.

Studies of humoral antibodies in Chagas' disease have shown that immune sera from a wide range of mammals can lyse epimastigote forms of *T. cruzi* in culture. However, trypomastigotes and amastigotes, the parasite forms found in the mammalian host, are not lysed. For example, there is no lysis when trypomastigote forms are incubated with immune sera containing high titres of IgM and IgG antibodies from either patients with Chagas' disease or rabbits infected with *T. cruzi*. By direct microscopic observation of the parasite in the wells of a Terasakky plate it is possible to observe cyclic changes initiated by the rounding up of the trypomastigote forms and multiplication by binary fission. In this way, clumps of amastigote forms are identified, which can change to epimastigotes. All these changes can occur in immune sera, regardless of the presence of complement factors, and the infectivity of these forms of *T. cruzi* to Swiss mice is not lost after incubation with fresh immune sera. Similar results are obtained when trypomastigotes of the Emestina strain of *T. cruzi* are incubated with fresh, normal, or immune chicken sera. It is thus unlikely that the refractoriness of chickens to *T. cruzi* infections is related to serum antibodies or complement factors.¹

The mechanism whereby *T. cruzi* eludes the host's humoral immune response is not clear. The absence of demonstrable peaks of parasitaemia and, also, the absence of IgM antibodies in the chronic phase of *T. cruzi* infections appear to indicate that antigenic variation, the mechanism that enables African trypanosomes to evade the host humoral immune response, is very unlikely to occur in Chagas' disease. Nevertheless, this and

¹ Abelho, J. & Teixeira, A. R. L. The effect of immune sera from mammals and birds on trypomastigote and amastigote forms of *T. cruzi* (abstract). In: XV Congresso de Sociedade Brasileira de Medicina Tropical, Campinas, São Paulo, 1979, p. 46.
other mechanisms that might enable T. cruzi to escape the humoral antibody response should be investigated.

**Cell-mediated immunity**

Immune responses to antigens of T. cruzi of the type usually attributed to cell-mediated hypersensitivity are well documented in man with Chagas' disease as well as in animals infected with T. cruzi. The inflammatory reactions in Chagas' disease, including the reaction at the portal of entry of T. cruzi — Romaña's sign in the eyes, or chagoma in the skin—are characterized by lymphocytic infiltration and granulomatous lesions. Further, the histopathological lesions found in patients with Chagas' disease resemble those found in other delayed hypersensitivity diseases, like tuberculosis, syphilis, and rheumatic fever. A delayed skin response to T. cruzi antigens appears to be correlated with the degree of delayed-type hypersensitivity in patients with Chaga's disease, in the same way as the PPD skin response is correlated with delayed-type hypersensitivity in patients with tuberculosis. *In vitro* correlates of cell-mediated immunity have also been reported in experimental animals and patients with Chagas' disease. Migration of macrophages, migration of leukocytes, blast transformation and migration of sensitized lymphocytes, have all been investigated in the presence of antigens of T. cruzi, but more practical and sensitive tests, both *in vivo* and *in vitro*, are essential for studies on the role of acquired cell-mediated immunity in Chagas' disease.

The importance of the lymphocyte-macrophage system in acquired resistance to T. cruzi infections has been pointed out and the reactions of lymphocytes and macrophages have been compared in immunized and non-immunized mice. Blocking the reticuloendothelial system of mice with thorium dioxide or silica particles before challenge with T. cruzi resulted in higher parasitaemia, a more prolonged patent period, and higher mortality, while injection of mice with agents that stimulate macrophage activity, such as diethylstilbestrol, resulted in enhanced resistance to T. cruzi as indicated by lower parasitaemia and lower mortality. Other studies have shown that mice whose macrophages have been activated by BCG, *Toxoplasma*, or *Besnoitia* are more resistant to T. cruzi than controls. *In vitro* studies have shown that macrophages from mice immunized with BCG were more resistant to T. cruzi than macrophages from non-immunized mice. These studies indicate that survival of T. cruzi in a macrophage is dependent on the escape of the parasite from the phagocytic vacuole.6,7

Evidence of cell-mediated immunity is also drawn from experiments showing that administration of antilymphocyte serum, along with neonatal thymectomy, results in greater parasitaemia and mortality in treated than in control animals. Moreover, the thymectomized mice produced a normal quantity of antibody, suggesting that the lack of cell-mediated immunity was responsible for exacerbated T. cruzi infection in these animals. Cell-mediated immunity can be transferred from animals with Chagas' disease to normal recipients by lymphoid cells. Furthermore, mice that received homologous lymphocytes from chagasic donors were resistant to the virulent Y strain of T. cruzi.

Investigations have also been carried out to determine whether immune effector cells from Chagas' disease donors are directly cytotoxic to parasitized target cells *in vitro*. In

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one of these experiments, significant killing of $^{51}$Cr-labelled, infected, syngeneic fibroblasts by spleen cells obtained from sensitized mice was reported. The in vitro destruction of infected rabbit heart cells by peripheral blood lymphocytes from sensitized rabbits has also been described. This demonstration of cell-mediated cytotoxicity of immune lymphocytes to parasitized host cells has led to suggestions that it is the main mechanism for eliminating intracellular infection by *T. cruzi*. However, the demonstration of significant killing of $^{51}$Cr-labelled infected human heart cells by T lymphocytes of patients with Chagas’ disease indicates that this cell-mediated immune mechanism may be very dangerous during the acute phase of the infection when the heart may be heavily parasitized. The immune destruction of thousands of muscle fibres may jeopardize the functioning of the heart and lead to severe myocarditis and heart failure.

There is, therefore, abundant laboratory evidence that cell-mediated immunity plays an important role in resistance to *T. cruzi*. Unfortunately, the cell-mediated immune mechanisms seem also to have a secondary effect that may harm the host organism. This toxic (or “allergic”) component of the cell-mediated immune system appears to be responsible for the tissue damage in Chagas’ disease.

**IMMUNOPATHOLOGY OF CHAGAS’ DISEASE**

In the acute stage of Chagas’ disease some lesions are probably related to the presence of parasites; however, others are probably not produced directly by the parasites. For example, both quiescent parasitism of tissue cells and inflammatory lesions in which parasites are not seen are known to occur. It seems that the latter type of lesion might be produced by immunological means such as those known to be active in the chronic phase of the disease.

In the chronic stage of Chagas’ disease the lesions seen in the heart and digestive tract are characterized by diffuse lymphocytic infiltrates; there are no parasites in situ and many authors consider these lesions to be the result of an altered allergic state of the host. In the chronic cardiac form of Chagas’ disease the typical microscopic lesion is characterized by myocytolysis by lymphocytes lying on the surface of the cytoplasm of non-parasitized heart cells. This lesion has also been found in the hearts of patients with the indeterminate form of Chagas’ disease who died suddenly or who died in car accidents. In any of these cases, the most striking pathological feature is the destruction of normal, non-parasitized heart cells by lymphocytes. Electron microscopic studies of samples of heart tissue obtained by biopsy from patients with chronic Chagas’ cardiopathy have shown a close relationship between lymphocytes and muscle fibres, with imbrication of plasma membranes and disappearance of basal laminae. In the neighbourhood of the lymphocytes, definite cytology of muscle cells can be found. These observations, plus the existence, *in vitro*, of a cell-mediated response against human heart cell antigen, suggest that a lymphocyte-mediated immune response against heart tissue is involved in the pathogenesis of lesions found in patients with Chagas’ disease.

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Further evidence of the participation of immunological mechanisms in the inflammatory lesions of Chagas’ disease is indicated by observations in mice injected with the virulent Y strain of *T. cruzi*. The large majority of these mice die of infection within ten days of inoculation and show very intensive parasitism of tissue cells but no myocarditis. In those animals that survive twelve days after inoculation the number of parasites in the heart decreases, while the occurrence of inflammatory lesions of the myocardium increases. Further, the mice that survive the acute infection and pass to the chronic phase show severe myocarditis with destruction of normal heart cells, in the absence of parasites *in situ*. These observations suggest that the absence of myocarditis during the first week of infection in mice at the peak of parasitism of heart cells, may be explained by the absence of an immune response so early in the course of the infection. However, the sensitization of the immune system and the development of cell-mediated immune reactions during the second week of infection appear to be associated with decreasing parasitism of the body and the occurrence of inflammatory lesions.

**Autoimmunity in Chagas’ disease**

Reports of the presence of anti-heart antibodies in the sera of patients with Chagas’ disease and experimental animals infected with *T. cruzi* have raised the possibility of autoimmunity, and some investigators have postulated that these antibodies play a role in pathogenesis. Others believe that anti-heart antibodies are the consequence rather than the cause of heart cell destruction. Further, antibodies reacting against endothelial cells, vascular structures, and heart and striated muscle cells (EVI antibodies) have been described in adults and children with acute or chronic infections by *T. cruzi*. These autoantibodies have been reported in 95% of patients with the active chronic myocarditis of Chagas’ disease and in 45% of asymptomatic patients with positive serological tests. Investigations of the anti-heart antibodies in sera from patients with Chagas’ disease have suggested that there are antigenic determinants common to mammalian tissues and *T. cruzi*. However, the role of these antibodies in the pathogenesis of the disease is not clear. Fetal rabbit heart cells can be cultivated in the presence of rabbit anti-*T. cruzi* serum and no toxic effect on the cultured heart cells is observed. In addition, when *T. cruzi*-sensitized lymphocytes from rabbits with chronic *T. cruzi* infection are incubated with homologous fetal heart cells, destruction of the normal heart cells is observed. Further, a heart cell microsomal antigen has been demonstrated, which inhibits the migration of *T. cruzi*-sensitized mononuclear cells. This observation indicates the presence of an antigenic determinant common to both heart cells and *T. cruzi*. Therefore, the recognition of the cross-reactive antigen of heart cell by *T. cruzi*-sensitized lymphocytes might be the pathogenic basis for subsequent host cell injury in Chagas’ disease (see footnote j).

Further studies have shown that release of ⁵¹Cr from human fetal heart cells is mediated by T lymphocytes from patients with the acute, latent, or chronic forms of Chagas’ disease (see footnote k). These observations indicate that T lymphocytes from patients without clinical manifestations of Chagas’ disease, as well as T lymphocytes from patients with clinic evidence of cardiopathy, are cytotoxic to cardiac cells. These

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experimental results thus suggest that heart cell destruction by immune T lymphocytes can occur any time in the course of an infection by *T. cruzi*. The rapid progress of the disease in humans, at the time when the patients pass from the totally asymptomatic latent phase to the chronic phase, in which the cardiac and digestive symptoms may be manifested, can be considered a threshold effect that is apparent only after the capacity of the organ concerned to compensate for the loss of cells reaches its limit.

Other manifestations of Chagas' disease are related to the presence of the "mega" conditions in the chronic phase of the *T. cruzi* infections. The depopulation of parasympathetic ganglion cells of the myenteric plexus that has been observed in this disease has allowed many investigators to establish a direct relationship between this morphological finding and the presence of megalo-oesophagus and/or megacolon. Various degrees of neuronolysis and lymphocytic infiltration of the parasympathetic ganglia have been described in experimental animals and in persons with Chagas' disease. Many hypotheses have been considered to explain the pathogenesis of these "mega" conditions. The possibility of neuronal cell destruction by the parasite or by a toxin released by the parasite can be discarded on the basis of the evidence available in the literature. Furthermore, neuronal cells present in cultures of fetal nervous tissue are not parasitized by *T. cruzi*, whereas glial cells are easily parasitized.

The possible role of autoimmune mechanisms in the destruction of neuronal cells of the digestive tract cannot be discarded in view of the evidence that *T. cruzi*-sensitized rabbit immune lymphocytes have selective affinity to those cells. Yet, further studies are required to clarify many aspects of the pathogenesis. The molecular basis of autoimmune reactions leading to host cell destruction in Chagas' disease is not known. There are indications that very complex molecular structures present in the subcellular microsomal fraction of *T. cruzi* share common antigenic determinants with mammalian host cells. However, the mechanisms whereby the host immune system induces suppression or exacerbation of these autoimmune reactions are not clear. There is some evidence indicating that the capacity of immune T lymphocytes to destroy heart cells in vitro bears a direct relationship to the intensity of a delayed-type skin reaction to *T. cruzi* antigens.

**IMMUNODEPRESSION IN ACUTE CHAGAS' DISEASE**

Two types of patient with acute Chagas' disease have been identified. The patients in whom the acute disease is apparent have a positive delayed-type skin response to *T. cruzi* antigen. In these patients, leukocyte migration is significantly inhibited in the presence of this antigen. In contrast, the patients in whom the acute disease is inapparent do not have positive delayed-type skin response to *T. cruzi* antigen and no significant inhibition is observed when their cells migrate in the presence of this antigen. It is also of interest that none of these patients is capable of developing contact sensibility to 2,4-dinitrochlorobenzene, whereas the normal control subjects show a positive contact reaction after sensitization to this drug. These observations suggest that T lymphocyte function is depressed in patients with the clinically inapparent acute Chagas' disease. This immunodepression seems to be acquired during the course of the *T. cruzi* infection because all patients tested showed positive delayed-type skin response to at least one

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ubiquitous microbial extract, thus indicating previously normal T cell function. The possibility exists that *T. cruzi* antigens may directly stimulate specific suppressor T cells, acting by way of antigen-specific suppressor factors. A soluble *T. cruzi* antigen has been found in fresh serum collected from animals with acute *T. cruzi* infection, but the role that this soluble antigen plays in the modulation of the immune phenomena observed in the acute phase of Chagas' disease has not been determined. However, in view of the observation that the impending severity of the disease is related to the host thymus-dependent immune response to the parasite, it is possible that the factors involved in the regulation of the host cell-mediated immune response to *T. cruzi* are important in determining the fate of patients with Chagas' disease. In favour of this interpretation is the demonstration that the immune destruction of heart cells in Chagas' disease is mediated by *T. cruzi*-sensitized T lymphocytes.

In view of the observations described above, one can say that strong autoimmune mechanisms may be established early in the course of acute Chagas' disease and that these mechanisms may be perpetuated in the course of chronic Chagas' disease by continuous antigenic stimulation. Further, the demonstration that the immune T lymphocyte cytotoxicity to heart cells bears some relationship to the intensity of the delayed-type skin response to *T. cruzi* antigen seems to indicate that the Chagas' patients with a strong skin response to this antigen might carry the potential to develop the cardiac lesions seen in this disease.

Investigations of the role of immune mechanisms in the pathogenesis of Chagas' disease seem to explain, satisfactorily, the variable severity of the acute phase and, also, the latency and the late manifestations of chronic Chagas' disease. In conjunction with natural mechanisms of resistance, acquired immunity is only partially effective in controlling *T. cruzi* infections in mammals and, in spite of a decreasing parasitaemia, the immune host remains infected with *T. cruzi* for life. Unfortunately, acquired immunity in Chagas' disease seems to have a secondary effect, since available evidence indicates that the lesions of Chagas' disease are produced by autoimmune mechanisms. This explains why the highest prevalence of clinical manifestations of the disease is seen in patients with negative parasitaemia.

**VACCINATION AGAINST CHAGAS' DISEASE**

Experimental evidence leaves little doubt that primary infection in animals induces significant immunity to acute Chagas' disease. This observation suggests that it should be feasible to develop practical immunoprophylactic procedures. However, the involvement of autoimmune mechanisms in the production of the lesions seen in Chagas' disease must be taken into account and the development of a vaccine for use in man will require much more work.

The procedures used and the results obtained in vaccination studies in laboratory animals have recently been reviewed.\(^6\)\(^,\)\(^7\) In general, immune protection to homologous *T. cruzi* strains whose virulence has been attenuated by drugs, by serial passage in culture, by treatment of the virulent *T. cruzi* infection with pharmacological agents, or by

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ionizing radiation, results in various degrees of protection. Usually, all the immunized animals survive a challenge infection with the same strain, despite the presence of parasites in the blood. Partial protection to heterologous T. cruzi strains can also be obtained, despite the fact that trypanosomes from a primary infection survive side-by-side in the immune host with the trypanosomes from the challenge infection.

Although the use of live vaccine against acute Chagas’ disease has serious limitations because the so-called avirulent strains could produce chronic Chagas’ disease, the above-mentioned experiments on immune protection, both with homologous and heterologous strains, have shown that attenuated live vaccines can induce acquired resistance to the acute stage of T. cruzi infection. None of these experiments, however, have dealt with the question of the subsequent mortality of the vaccinated animals from chronic Chagas’ disease. The involvement of autoimmunity in Chagas’ disease suggests that the outlook for the development of a vaccine with avirulent or attenuated strains of T. cruzi is not promising.

Attempts have been made to use parasites of the family Trypanosomatidae for immune protection against T. cruzi. Primary infection of mice with some of these trypanosomes does not protect them against a lethal challenge with T. cruzi. However, primary infection of mice with Leptomonas pessoai, a monoxenous trypanosome, induces some degree of protection against challenge with the lethal Y strain of T. cruzi. Further research is needed to investigate cross-protection between T. cruzi and other cruzi-related trypanosomes of the genus Schizotrypanum (Chagas 1909), which are not pathogenic for man or other mammals. In view of the possibility that previous infection with these living trypanosomes can give strong immune protection to superinfection with T. cruzi, they may be safe for prophylactic vaccination. The demonstration that Leptomonas pessoai gives some degree of immune protection to challenge with virulent T. cruzi would indicate that other species of trypanosomes should be tested. Therefore a research line should be initiated to attempt the isolation and identification of new species of monoxenous flagellates of insects and low vertebrates that could eventually lead to more effective immune protection against virulent T. cruzi infections.

Immune protection has also been obtained with killed T. cruzi and its products with different degrees of success. In general, physical methods of obtaining antigens have so far been superior to chemical methods. The survival of mice immunized with these antigens and challenged with a lethal inoculum of T. cruzi has been complete in some experiments. However, some of these immunized mice have shown transient, low parasitaemia after challenge with a virulent T. cruzi strain. Further, the influence of vaccination on the development of the pathological lesions of chronic Chagas’ disease remains unknown.

The possibility of producing a vaccine with purified T. cruzi antigens appears to be worth pursuing. Previous reports have shown that the soluble substances derived from homogenates of T. cruzi induced high titres of humoral antibodies, whereas the cell-mediated immune responses elicited by these substances did not render immune lymphocytes cytotoxic to host cells. Studies on cross-reactivity to target host cells of immune lymphocytes sensitized to other subcellular antigens of T. cruzi should be encouraged. Also, further studies should be undertaken on possible methods of increasing cell-mediated and/or humoral mechanisms for trypanosome inactivation. Clarification of the mechanisms whereby T. cruzi evades the host’s immune responses is clearly essential to further progress in the prophylaxis of Chagas’ disease.

Results obtained during the last five years indicate that vaccination against Chagas’
disease should not be limited to immunoprotection against only the acute phase of *T. cruzi* infections. Attempts should be made to develop a vaccine that would give effective protection against chronic Chagas’ disease, but these studies will require a suitable animal model for chronic Chagas’ disease. The lack of such a model has precluded further progress in studies of the immunology and immunopathology of Chaga’s disease and of vaccination and chemotherapy.

Although the chemotherapeutic agents at present available are effective in eradicating parasites from the blood, the intracellular amastigote forms remain in the host tissues and there is no clinical evidence that the prognosis of Chagas’ disease is favoured by such treatment. However, there is some experimental evidence suggesting that the cell-mediated immune response is decreased in these patients. Therefore, the effects of these chemotherapeutic agents on the immune responses to *T. cruzi* appear to be worthy of further investigation.

**RÉSUMÉ**

MALADIE DE CHAGAS: ORIENTATION DES RECHERCHES IMMUNOLOGIQUES ET PERSPECTIVES EN MATIÈRE D’IMMUNOPROPHYLAXIE

Selon les estimations, 12 millions au moins de personnes vivant en Amérique latine sont infectées par *Trypanosoma cruzi*, agent de la maladie de Chagas. Dans la plupart des cas, l’infection initiale est asymptomatique et apparentement bénigne, mais le parasite peut subsister chez l’individu touché durant toute la vie de celui-ci. Dans l’infection chronique, les manifestations pathologiques sont souvent constatées en l’absence de parasitémie décelable et la mortalité atteint 12,8/1000. Les troubles cardiaques en relation avec l’infection sont le plus souvent à l’origine du décès, ce qui explique que la maladie de Chagas ait pu être qualifiée de forme la plus courante de cardiomyopathie dans le monde.

L’existence d’une immunité naturelle contre l’infection à *T. cruzi* a été mise en évidence chez les animaux à sang froid et les oiseaux et, chez l’homme, la susceptibility individuelle peut varier même au sein d’une famille. Quant à l’immunité acquise, dont l’existence chez l’homme et l’animal est bien établie, elle met en jeu soit la réponse humorale, avec des titres élevés d’anticorps, soit la réponse cellulaire, laquelle constitue la principale forme de résistance acquise à l’égard de *T. cruzi* mais est sans doute à l’origine d’effets allergiques nuisibles à l’organisme de l’hôte. La forte immunogénicité de *T. cruzi* a été démontrée par l’évolution clinique aussi bien que par les épreuves de laboratoire. Des titres élevés d’anticorps de type IgM sont décelés au cours de la phase aigüe de l’infection, et les anticorps IgG et IgA sont présents dans le sérum pendant la phase chronique. La réponse immunitaire à médiation cellulaire se manifeste par des réactions cutanées de type retardé, la transformation blastique de lymphocytes stimulés, l’inhibition de la migration des macrophages et la cytotoxicité des lymphocytes activés à l’égard des cellules hôtes parasitées.

Les immunsérum provenant de divers mammifères peuvent entraîner la lyse de *T. cruzi* cultivé *in vitro*. Mais les formes parasitaires présentes chez l’hôte mammifère — soit les stades amastigote et trypomastigote — résistent à l’action de ces immunsérum; le mécanisme qui permet à *T. cruzi* d’éluder la réponse immunitaire humorale de l’hôte
n'a pas encore été éclairci. Dans la réponse cellulaire, les réactions du système lymphocytes-macrophages capables de juguler l'infection chez les animaux immunisés ont été analysées. Lorsque des parasites de faible virulence sont injectés à des souris, ils sont en grande partie phagocytés par les macrophages. Cependant les souches hautement virulentes se multiplient dans les macrophages et une partie des parasites s'en vont infecter des cellules non phagocytaires. Des études in vitro ont montré que la survie de *T. cruzi* dans un macrophage était due à son évasion de la vacuole phagocytaire.

On a démontré la présence chez des sujets souffrant d'une cardiomyopathie concomittante à la maladie de Chagas d'anticorps réagissant contre l'endocarde ainsi que les membranes vasculaire et interstitielle du cœur. Ces anticorps (EVI) fixent le complément et sont absorbés par les épimastigotes de *T. cruzi*; ceci suggère qu'ils sont induits par les antigènes du parasite. Leur rôle dans la pathogenèse de la cardiopathie décelée n'a pas encore été tout à fait éclairci. On a cependant pu établir que des lymphocytes T stimulés par *T. cruzi* et provenant de patients atteints de la maladie de Chagas étaient capables de détruire in vitro des cellules cardiaques humaines non parasitées. On a aussi pu établir qu'un déterminant antigénique présent dans les cellules cardiaques inhibait la migration des cellules mononucléaires activées par *T. cruzi*. Il s'agit donc d'un déterminant antigénique commun aux cellules cardiaques et à *T. cruzi*, et la reconnaissance par les lymphocytes activés de cet antigène à réaction croisée pourrait être à l'origine des lésions cardiaques de la phase chronique.

D'autres manifestations courantes au cours de la phase chronique de la maladie (mégaoesophage et/ou mégacôlon) sont dues à la raréfaction des cellules glanglionnaires parasymphathiques du plexus myentérique de l'appareil digestif. L'existence d'une affinité sélective des lymphocytes activés par *T. cruzi* pour ces cellules a été démontrée, et ceci indique que l'autoimmunité peut jouer aussi un rôle dans la pathogenèse des lésions de l'appareil digestif au cours de la maladie de Chagas.

L'immunisation contre l'infection aiguë au moyen de souches non virulentes ou atténuées du parasite permet en général d'obtenir une protection de degré variable contre les infections à *T. cruzi*. Tous les animaux ainsi immunisés ont habituellement survécu à l'infection d'épreuve en dépit du fait que des trypanosomes d'épreuve persistent côte à côte avec des trypanosomes de l'infection immunisante. L'emploi de vaccins vivants doit faire l'objet de la plus grande prudence car des souches réputées non virulentes peuvent provoquer la forme chronique de la maladie. En outre, il est encore trop tôt pour qu'on puisse être renseigné sur la mortalité ultérieure chez les animaux vaccinés. Une infection primaire suscitée chez la souris par un trypanosomidé monoxène, *Leptomonas pessoai*, lui confère une certaine protection contre une souche létale de *T. cruzi*. La possibilité qu'une infection antérieure par des trypanosomides non pathogènes pour l'homme suscite un haut degré d'immunité à l'égard d'une superinfection par *T. cruzi* pourrait justifier l'emploi de ceux-ci pour la vaccination prophylactique. Quant au degré de protection obtenu avec *T. cruzi* tué et ses produits, il est variable. Les substances solubles dérivées d'homogénats de *T. cruzi* peuvent susciter des titres élevés d'anticorps huméraux sans induire chez les lymphocytes activés de cytotoxicité à l'égard des cellules de l'hôte. La survie des souris immunisées de cette manière et éprouvées par une inoculation normalement létale de *T. cruzi* a été de 100 % dans certaines expériences.

Les perspectives de mise au point d'un vaccin au moyen d'antigènes purifiés de *T. cruzi* possédant une forte immunogénicité semblent donc prometteuses. Il ne faut toutefois pas perdre de vue que la vaccination peut jouer un rôle dans la pathogenèse des
lésions associées à la maladie chronique, et cette éventualité doit faire l'objet d'études approfondies. D'autre part, l'immunité à médiation cellulaire suscitée par le vaccin peut aussi être dangereuse lorsque de nombreux parasites sont présents dans le cœur au cours de la phase aigüe de l'infection. Quoi qu'il en soit, la protection contre la forme chronique de la maladie demeure le problème essentiel et, si l'on veut produire un vaccin acceptable, il importe en premier lieu de définir un modèle animal pour la maladie de Chagas chronique.