Review

THE FUNDAMENTAL NATURE OF CHAGAS' DISEASE: A VIEW PROVIDED BY IMMUNE RESPONSES AND IDIOTYPES

DANIEL G. COLLEY AND MICHAEL J. HOWARD

Received November 1 1990/Accepted December 2 1990

Abstract: Chagas' disease is caused by the protozoan Trypanosoma cruzi which infects 10-15 million people in endemic areas throughout Latin America and is naturally transmitted by insect vectors of the family Reduvidae. Infection can also occur by congenital passage, oral ingestion, laboratory accident, and in organ transplants and blood transfusions. There are 3 life-cycle forms of T. cruzi. Epimastigotes multiply in the midgut of the insect vector, differentiate in the hind-gut into infectious trypomastigotes, and are excreted with the feces or urine after a blood meal. They enter the body by mucous membranes or abraded skin, enter mammalian host cells, escape the phagolysosomal vacuole, and differentiate into amastigotes. Intracellular amastigotes multiply in host cells, redifferentiate to trypomastigotes, and are released upon cell rupture.

Acute Chagas' disease can be asymptomatic, or a mild to severe illness. Morbidity can be localized and/or systemic and is usually accompanied by general immunosuppression with blood and tissue parasitemia. It can be fatal. Usually, symptoms and parasitemia decrease within months, followed by a life-long, chronic infection with little morbidity and few apparent parasites. Serologically-positive, chronic asymptomatic patients are termed indeterminate (I). Morbidity develops in 20-30% of chronic patients after 10-30 years and often involves myocardopathy (Cardiac disease; C), ranging from minor electrocardiographic changes to sudden death by heart failure. Severe "digestive" megasyndromes can also develop. Chagasic myocardial inflammatory infiltrates are associated with cardiac muscle and neuron destruction, followed by progressive fibrotic replacement. Asymptomatic I-patients who die of unrelated causes have identical (but less intense) lesions.

Responses against T. cruzi antigens, their immunoregulation, and responses against related idiotypes (Ids) correlate with the presence of the different clinical forms of the infection. Although there are numerous findings of autoimmune lymphocyte and antibody reactivities in chagasic patients, a causal relationship is always difficult to prove. Chagasic patients' peripheral blood mononuclear cells (PBMC) respond to T. cruzi epimastigote antigens (EPI) with varying vigor. Almost all low responders are C-patients, and their responses are usually augmented by removal of adherent macrophage suppressor cells or the addition of indomethacin. Chronic I-patients are medium or high responders, and exhibit little adherent cell-mediated immunoregulation. Western blotting

Veterans Affairs Medical Center, and the Departments of Microbiology and Immunology, and Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, U.S.A.
studies indicate patients' Ab and cell-mediated responses to EPI show differences between C- and I-patients. PBMC from I-patients responded more often to high molecular weight components (100-150 kD), while both I- and C-patients responded well to moieties between 28-32 and 48-57 kD. All chagasic patients' sera had Abs and PBMC responses to T. cruzi GP57/51 antigen.

Chronic Chagas' patients have peripheral blood anti-Id T cells that respond to anti-EPI Abs purified from patients' sera. Some patients' PBMC anti-Id responses to anti-EPI Ids from C-patients (Id-C) are inhibited by chloroquine (Group 1), but some are not inhibited by chloroquine, anti-HLA Class II antigens, or sodium azide (Group 2). Most patients in Group 1 are asymptomatic, but all Group 2 patients have severe disease. Direct (non-processed; non-MHC-presented) stimulation of anti-Id T cells from C-patients by Ids expressed on anti-EPI Abs from C-patients could be immunopathogenic. Anti-Id specific rabbit sera detect Id differences in the anti-EPI Abs from pooled or individual C vs. I-cases. Competitive ELISA assays and Western blot analyses of Abs show that Ids on I-patients' anti-EPI Abs are primary structure expressions, while Ids on C-patients' anti-EPI Abs are defined by intact Ab molecules.

The chronic, endemic nature of Chagas' disease allows maternal/neonatal Id interactions that might influence later immune response and immunoregulatory abilities of children born of infected mothers. Such interactions occur because cord blood mononuclear cells from chagasic mothers' children respond to Ids on anti-EPI Abs. A hypothesis based on Id-induced pathology and immunoregulation will be described that could account for certain aspects of the immunology and pathology of Chagas' disease.

History and prevalence

Chagas' disease (South American Trypanosomiasis) is an endemic zoonosis produced by Trypanosoma cruzi which is found presently only in the Americas. The disease is named for the 29 year old Brazilian physician, Carlos Chagas, who in 1909 identified and described the parasite in the hind-gut of insects from the family Reduviidae. Dr. Chagas later observed its pathogenicity in mammals and located and described many of the domestic (dogs, cats, goats, etc.) and wild (armadillo, opossum, etc.) reservoirs. He went on to demonstrate this infection in humans and described its acute and chronic stages. Recent epidemiologic studies in South America suggest there may be 10 to 15 million people serologically positive for T. cruzi (Brener, 1982; Chagas, 1988). Reports on the incidence of infection crudely estimate it to be over 800,000 new cases each year with an annual mortality of around 60,000 (Dias, 1987).

Like many parasitic infections, Chagas' disease is most common in, but not confined to, people occupying the lower socioeconomic classes. Contact with the vector and transmission have been correlated most strongly to poor housing construction and inadequate vector control (Marsden, 1984). These factors play a major role in transmission of Chagas' disease as the vector and trypanosome are much more widely distributed than is human infection (Grogl et al., 1984; Beard et al., 1988; Yaeger, 1988).

Transmission in endemic areas is largely vector-related and thus limited to the Americas, but recent reports have shown that congenital passage (Azogue et al., 1985), laboratory accidents (Brener, 1987), organ transplants and blood transfusions (Kirchhoff, 1989) play a considerable role in transmission in endemic countries and may become a serious problem for non-endemic countries as well. Two recent reports of transfusion related T. cruzi infection in North American hospitals have stressed the danger which T. cruzi
contaminated blood could pose for non-endemic countries (Grant et al., 1989; Nickerson et al., 1989). These cases involved two patients undergoing chemotherapeutic treatments for leukemias. Each of these patients had received platelets from Latin American immigrants. These blood products were screened for conventional infectious contaminants and deemed fit for use, but there are no protocols in the United States of America or Canada which consider T. cruzi contamination. Screening of donors within endemic areas of Latin America is becoming more common and there are highly sensitive and specific serological assays available (complement fixation, indirect immunofluorescence and ELISA) which should help decrease the risk of transfusion or transplant related infection. Presently, Brazil reports 10,000 to 20,000 transfusion related T. cruzi infections a year (Brener and Camargo, 1982), and 5-51% of the blood units recently tested in Bolivia were serologically positive (Carrasco et al., 1990). With the recent increase in immigration of people of Japanese heritage from Brazil to Japan, transfusion related Chagas’ disease may become something that the Japanese medical community will need to consider in the future.

**Biology of Trypanosoma cruzi**

*Trypanosoma cruzi* has a complex life cycle involving both vertebrate and invertebrate hosts (Pereira, 1990). Under natural conditions, the trypanosome is transmitted to its definitive mammalian host via the feces or urine of an infected reduviid bug of the genera Triatominae, Rhodnius, or Panstrongylus (Ghauri, 1973). Those strictly hematophagous reduviids which are the most efficient vectors of human infections defecate shortly after taking a blood meal. The feces and urine contain the infectious trypomastigote stage which develops from the non-infectious, asexually dividing epimastigote form found in the hind-gut of the vector. Trypomastigotes cannot penetrate intact epidermis but enter through breaks in the skin or through intact mucous membranes. Therefore, infection is not due to a direct inoculation by the insect vector, but rather indirectly through the victim wiping and scratching at the bite and subsequently contaminating the wound, their eyes or mouth. Evidence of primary infection is sometimes seen at the site of entry as localized inflammatory reaction resulting in an indurated nodule at the bite (chagoma) or by unilateral periorbital swelling and conjunctivitis (Romana’s sign).

The non-dividing blood stage trypomastigote form has the well described ability to avoid complement lysis (Joiner et al., 1988; Rimoldi et al., 1988) and phagocyte killing (Osuna et al., 1986; Reed, 1988; Reed et al., 1989) and is capable of receptor mediated entry of a wide variety of host cell types (Velge et al., 1988; Davis and Kuhn, 1990). Once inside the cell the trypomastigote produces a recently described perforin-like molecule, analogous to C9 of the complement cascade, and passes from the parasitoferous vacuole into the cytoplasm of the cell (Andrews et al., 1990). The trypomastigote differentiates into the amastigote form in the cytoplasm. Amastigotes multiply asexually and develop into trypomastigotes while liberating antigens into the host cell cytoplasm (Andrews et al., 1988). These antigens theoretically would be expressed in a Class I restricted manner on the surface of infected cells. Whether acute and chronic lesions are related to host cell lysis by either immune cytotoxicity or passive rupture due to parasite load is not clear. The release of parasites results in trypomastigotes and amastigotes in the circulation. These organisms are capable of disseminating and parasitizing diverse tissues throughout the host or can be taken up by a feeding reduviid to complete the cycle (Andrews et al., 1987).
The intracellular, dividing amastigote stage is readily identified in histologic sections of cardiac, intestinal and cerebral tissues from infected individuals early in the course of infection and is localized predominantly to neuronal or conductive fibers within the parenchyma of these organs (Andrade et al., 1978, 1984; Said et al., 1985). Sequestration of the parasite in these intracellular sites confers a significant survival advantage to T. cruzi. The intracytoplasmic residence of these organisms means that effective sterilizing chemotherapy regimens would need to achieve trypanocidal concentrations of drugs inside host cells. This is not thought to occur with the current drugs of choice (nifurtimox and benznidazole) and poses a major challenge in the area of drug development and chemotherapy (Filardi and Brener, 1987; McCabe, 1988). The intracellular location of the parasite also allows it to largely avoid the immune response. The host's early immune responses induce a resolution of blood parasitemia but they are unable to clear the persistent, intracellular infection. It is commonly assumed that intact host cells with resident intracellular amastigotes do not elicit inflammatory responses early in infection. The intense focal inflammation typical of acute infection is believed to occur only after the rupture of parasitized cells which exposes the host to concentrations of parasite antigen. However, during chronic infection parasites are scarce in blood and are usually not found within the observed diffuse inflammatory responses in host tissue. This has lead to the widely proposed, but still controversial, theory of an autoimmune basis for Chagas' disease (Takle and Hudson, 1989; Hudson and Hindmarsh, 1985).

Clinical aspects of infection

Chagas' disease, like many chronic endemic parasitic infections, demonstrates a spectrum of clinical presentations. The initial infection can go completely unnoticed or result in lethal cardiac infarctions. Chronic patients range in presentation from asymptomatic but serologically-positive to severe debilitation and death. This heterogeneity is no doubt due to a multitude of parasite and host factors but recent reports have begun to link specific host immune responses during infection to the various clinical stages (Gazzinelli et al., 1988a, b; Reis et al., 1989; Gazzinelli et al., 1990; Colley et al., 1990). Interestingly, although the acute phase of primary infection does occur in endemic human populations it is rarely seen by health care workers. Oftentimes, acute cases of parasitic diseases in non-endemic people present as severe infections in contrast to those of endemic peoples (von Lichtenberg, 1987; Nash et al., 1982; Ottesen, 1984). These findings suggest that there are many individuals within endemic populations who are somehow able to better modulate the severity of disease in the acute, and possibly chronic, phases of infection with parasites.

When acute chagasic infection is clinically apparent it is usually defined by a marked parasitemia with symptoms ranging from mild fatigue and fever to severe myocarditis and death (5-10% of acute patients) (Nogueira, 1986). Diagnosis is made by demonstrating parasites in stained blood smears or live parasite isolation (xenodiagnosis or hemoculture) and is occasionally aided by description of a chagoma or Romana's sign. Diagnosis of acute Chagas' disease by serological testing is generally of little use possibly due to the rapid and severe immunosuppression seen in these patients (Brener, 1980; Kuhn, 1981; Beltz et al., 1989).

Because the acute phase is not often recognized, data on cellular and humoral events during acute Chagas' disease are generally not available. A few reports discuss the humoral response progression during acute accidental infection of laboratory workers from sero-
negativity through to seroconversion and frank parasitemia (Brener, 1984, 1987; Hofflin et al., 1987). But little is known about cellular reactivities during this phase of human infection. The acute phase of experimental Chagas’ disease has been widely studied (Teixeira et al., 1975; Tarleton and Scott, 1987). Acute murine infection with T. cruzi demonstrates a tremendous polyclonal stimulation involving both B and T cells. Nearly half of all spleen cells appear to be blast cells undergoing mitosis. This response is truly polyclonal: all Vh-gene families studied (covering more than 95% of the repertoire of the entire locus) are stimulated (Minoprio et al., 1989). The majority of these activated splenic and lymph node plaque forming cells are not specific for the parasite (Minoprio et al., 1988). There are no reports in the literature concerning the existence or the extent of this polyclonal activation in humans but its presence has been proposed to play a role in the marked immunosuppression and possible autoimmunity seen in human trypanosomiasis (Petry and Eisen, 1989). Similar panlymphocytic, polyclonal proliferation has been reported in viral infections, bacterial (leprosy) infections, and Leishmania infections (Petry and Eisen, 1989). All of these organisms develop in macrophages. During the acute phase of infection T. cruzi also largely infects macrophages (McCabe et al., 1984; Villalta and Kierszenbaum, 1984). Because of this unifying observation, several researchers have proposed roles for cytokines produced by the infected macrophages as being responsible for the immunological disturbances seen in T. cruzi infections (Kierszenbaum and Wirth, 1987; Reed, 1988; Wirth and Kierszenbaum, 1988; Tarleton 1988; Reed et al., 1989).

The chronic phase of the disease usually presents 20–30 years after initial infection and often without history of acute disease (Nogueira, 1986). Intracellular parasites are believed to maintain a lifelong tissue infection but trypomastigotes are typically scarce or absent in the blood as parasitemia is tightly controlled by the host’s immune response. This becomes very apparent in those chronic patients who, for reasons unrelated to their Chagas’ disease, receive immunosuppressive therapy and subsequently develop a new “acute” infection complete with patent parasitemia (Brener, 1980; Kierszenbaum et al., 1983; Hudson and Britten, 1985). In the chronic phase, demonstration of parasitemia is generally very difficult because of the low numbers seen in the peripheral circulation. Simple blood smears are completely useless for diagnostic purposes. This requires very sensitive techniques which rely on a long multiplicative phase either in uninfected reduvid bugs allowed to feed on patients’ blood (xenodiagnosis) or in vitro culture (hemoculture) of parasites from patient blood directly. For this reason serology is usually the method of choice for positive diagnosis of chronic Chagas’ disease (Brener, 1982). New approaches using molecular biological techniques such as the polymerase chain reaction (PCR) for highly sensitive and specific detection of organisms are now being explored (Moser et al., 1989; Sturm et al., 1989).

Most chronic patients are asymptomatic and typically present with positive serology and sub-patent parasitemia. Between 60% and 80% of people infected with T. cruzi fall into this category and are termed “indeterminate” (Brener and Camargo, 1982). By definition, these people do not suffer from disease related morbidity and mortality, but this does not mean that they are free from pathology caused by their infections. A series of autopsies performed on “sudden death” cases in an endemic area indicate varying degrees of sub-patent chagasic cardiac pathology are present in indeterminate individuals (Brener and Camargo, 1982; Pereira-Barretto et al., 1986). This has lead to the hypothesis that the clinical forms of Chagas’ disease represent a progression rather than distinct and unrelated disease states.
Why certain infected individuals progress more rapidly to morbidity while others regulate the infection their entire lives without clinically apparent disease is not known.

The minority of chronic patients (20-30%) demonstrate a more severe chronic course (Brener and Camargo, 1982). Patients with the "cardiac" clinical form demonstrate severe lesions to the conducting and muscular structures of the heart which commonly lead to a dilative cardiomyopathy and congestive heart failure. Lesions of the conducting system of the heart may lead to right bundle branch block and life threatening arrhythmias. This is evident in electrocardiographic changes, and rich inflammatory infiltrates can be demonstrated in post-mortem sections of the heart—often in the absence of demonstrable parasites or parasite antigens (Andrade et al., 1978). The clinical finding of chagasic cardiopathy include recurrent palpitations and ECG changes combined with cardiac insufficiency and heart enlargement. The electrocardiographic changes commonly involve right bundle branch block with occasional complete atrio-ventricular block. Fibrosis and chronic inflammation are prominent features of the histology of chronic chagasic cardiac pathology (Ribeiro dos Santos and Rossi, 1985; Carrasco-Guerra et al., 1987; Brener and Krettli, 1990). It is this cardiopathy which contributes to Chagas' disease being among the leading cause of death in adults in some endemic areas.

A further subgroup of chronic patients with severe Chagas' disease present with aperi-stalsis and dilation of the esophagus, large bowel or both. These patients have "megadisease" or the "digestive" clinical form. Histology of lesions typically shows mononuclear infiltrates in the myenteric plexus of the gut wall with demyelination and sclerosis of surrounding conductive fibers. The loss of autonomic control and subsequent dilatation are believed due to the inflammatory destruction of the ganglionic plexus and nerve fibers. However, direct destruction by intracellular parasitism no longer observed at the site cannot be ruled out (Hudson and Hindmarsh, 1985; Hudson and Britten, 1985). A small minority of patients demonstrate both cardiac and digestive involvement.

Lesions and their possible immune etiology

The etiology of chronic chagasic pathology has been examined in a number of experimental animal models and in human infection. In 1974, Cossio and co-workers (Cossio et al., 1974) reported a series of experiments concerning antibodies reacting with endocardium, vascular structures, and interstitium of striated muscle (EVI antibodies) present in the sera of patients with chronic chagasic cardiopathy. This was one of the first findings which suggested a mechanism for the lesions to heart and digestive tract tissues and implicated an autoimmune etiology. These EVI antibodies reacted with human, mouse, bovine and guinea pig heart tissue from non-infected subjects. In these studies, similar cross-reactive antibodies were found in over 90% of patients with chronic chagasic cardiopathology. Later findings that these antibodies could be absorbed by epimastigotes and by laminin lent further support to this theory (Szarfman et al., 1982; Brener et al., 1983; Gazzinelli et al., 1988d). However, these results have been difficult to reproduce, and it is now known that EVI-autoantibodies are also found in the sera of over 40% of indeterminate patients and in patients with other parasitic infections (malaria, leishmaniasis, African trypanosomiasis and Trypanosoma rangeli) (Khoury et al., 1983; Avila et al., 1984; Kierszenbaum, 1986; Avila et al., 1987; Towbin et al., 1987). These findings have fueled debate over the significance of these antibodies in the pathogenesis of T. cruzi (Kierszenbaum and Hudson, 1985).
Many mouse and rat monoclonal antibodies exist which are reported to cross-react with *T. cruzi* and host tissue. Some of these antibodies and the parasite and host epitopes recognized have been defined in detail. For example, Eisen and colleagues have described two anti-*T. cruzi* monoclonals, VESP 6.2 and VESP 8.2, which were shown by indirect immunofluorescence to recognize different glycolipids on the surface of fixed, CL-strain trypomastigotes and also epitopes on cerebellar neurons in culture and frozen sections of heart, digestive tract and peripheral nervous tissues (Petry and Eisen, 1989). Another mouse monoclonal antibody (CE5) raised by Wood and colleagues against membranes of rat dorsal root ganglia also recognizes epitopes on the surface of fixed Y-strain epimastigotes (Wood et al., 1982). Western blots using CE5 demonstrate a complex banding pattern of proteins (50–100 kD) in both epimastigote and amastigote antigen preparations which may reflect a common amino acid sequence in the distinct proteins of these two life cycle stages. CE5 also recognizes several bands on Western blots of preparations from rat heart, intestine and brain but not from tissues known not to be affected in Chagas' disease. Snary and his co-workers have used monoclonal antibodies derived from immunized and infected mice to demonstrate cross-reactivity between epitopes on the parasite surface and those found on mouse central and peripheral neurons and glia (Snary et al., 1983). All of the monoclonal antibodies discussed were shown to have no activity against the culture medium or fetal bovine serum used to raise the parasites, making it unlikely that these cross-reactivities are due to antigen scavenging by *T. cruzi*.

The existence of cross-reactive clones such as those described above is not unexpected in situations of intense lymphocyte polyclonal activation. This has been shown in, and is correlated with, pathogenesis in a number of autoimmune diseases (Klinman and Steinberg, 1987). If Chagas' disease does have an autoimmune component, its etiology may be related to polyclonal stimulation of lymphocytes such as that observed in experimental *T. cruzi* infection of mice. It is interesting to note that some systemic autoimmune disease in both humans and mice are characterized by hypergammaglobulinemia and abnormally high levels of serum autoantibodies, two things known to occur in acute human and experimental Chagas' disease (Minoprio et al., 1989). How these observations correlate with pathology and what is responsible for the breakdown of normal control of humoral autoreactivity in these diseases are not clear at present.

Cell transfer studies by Laguens and colleagues gave the first evidence suggesting a T cell–mediated autoimmune etiology in experimental Chagas' disease (Laguens et al., 1981). These studies used "parasite-free" non-adherent spleen cells isolated from infected mice to create lesions in non-infected syngeneic recipient mice only 4 days after cell transfer. In similar studies, some recipient animals later died of *T. cruzi* infections so that parasite generated pathology could not be ruled out. Recently, Hontebeyrie-Joskowicz and co-workers have used CD4+ T cell clones to create truly parasite free pathology in an MHC-restricted manner in healthy mice. These CD4+ clones were shown to have specificity for both epimastigote antigen and syngeneic murine sciatic nerve antigen giving strong support for a cell-mediated autoimmune mechanism in chagasic cardiopathy (Hontebeyrie-Joskowicz et al., 1987; Ben Younes-Chennoufi et al., 1988).

The above observations have lead to several theories on the development of the pathogenesis associated with *T. cruzi* infection. The most widely tested hypothesis involves generation of autoreactivity due to infection. The mechanisms involved in the development
of this auto-reactivity are unknown but several models are commonly addressed in the literature. A few of these include: 1. Antigen mimicry; 2. Polyclonal blastogenesis of both B and T cells, stimulating a B cell repertoire already naturally expressing a large number of anti-self reactivities; 3. Development of autologous responses to the idiotypes or the isotypes of the original antigen specific clones. How these possibilities might occur and how they would relate to specific lesion development in chronic infection with T. cruzi is only beginning to be examined.

The spectrum of immunologic responses

The clinical spectrum observed in patients infected with T. cruzi allows speculation regarding differential host immunoregulatory mechanisms that may contribute to these differences in morbidity. The remainder of this article will summarize findings concerning responses against T. cruzi antigens, and their immunoregulation, and responses against idiotypes (Ids) associated with these anti-T. cruzi responses. It will present, analyze and hypothesize about immunologic profiles which correlate with the presentation of different clinical forms of the infection.

In Chagas' disease, peripheral blood mononuclear cells (PBMC) proliferate to soluble (Morato et al., 1986) or nitrocellulose-fixed (Gazzinelli et al., 1990) parasite antigen. If the in vitro responses to soluble epimastigote antigens (EPI) are subdivided into levels of responsiveness and analyzed, almost all of the low responders are clinically classified as "cardiac" (C) patients (Gazzinelli et al., 1988a; Colley et al., 1990; Morato, M.J.F. et al., submitted for publication). The responses of these patients are also augmented more, relative to those of "indeterminate" (I) patients, by the removal of adherent cells (Morato, M.J.F. et al., submitted for publication). PBMC from chronic I patients usually respond well to EPI and these responses and are not apparently regulated by adherent cells. These data, coupled with the observations that C patient PBMC responses are often partly augmented by the addition of indomethacin (Morato, M.J.F. et al., submitted for publication), suggests a role for prostaglandin-mediated adherent macrophage suppression in the development or expression of clinical disease. It is possible that strong cellular responses against some of the parasite components in EPI are essential to hold the parasite in check, thus decreasing the chance of parasite-induced related morbidity.

There have been a number of studies examining humoral responses of patients infected with T. cruzi (Kuhn, 1981; Snary, 1985; Nogueira, 1986). Recent research has compared patients' antibody responses by Western immunoblotting against separated EPI in parallel with their cell-mediated responses by T cell-Western blotting (Gazzinelli et al., 1988a, 1990). These experiments have shown some differences between C and I patients. PBMC from I patients responded more often to high molecular weight EPI components (100–150 kD) while both C and I patients cells responded to molecules in the 28–32 kD and 48–57 kD ranges. But while there was an apparent difference in the recognition by T cells of separated antigens, none of the specific antigen bands were recognized preferentially by sera of patients with one clinical form vs. another. These studies further describe a glycoprotein component of EPI which migrates diffusely between 57 and 51 kD (GP57/51) (Scharfstein et al., 1986; Murta et al., 1988). GP57/51 antigen is recognized by all patients' sera and cells, and a given patient's PBMC responses to this purified antigen correlate with their responsiveness to crude EPI. It appears that a major portion of the cellular reactivity against crude EPI is due to responses
specific for GP57/51. The biological significance of GP57/51 and its potential use as a diagnostic antigen are the focus of ongoing studies.

The role of Id/anti-Id interactions in the regulation and maintenance of the immune response was first proposed by Jerne in 1974 (Jerne, 1974). It has been demonstrated since that time that repeated, long-term exposure to antigens can lead to the development of dominant cross-reactive Ids (CRI), and that these CRI and anti-Ids against them may play multiple roles in the responses to immunizations or during infections (Bona, 1987; Kearney, 1989; Cerny and Hiernaux, 1990). Id/anti-Id interactions have been demonstrated to be integral to the ability of the host to regulate some responses to immunologic challenges and have also been implicated in the pathogenesis of certain autoimmune diseases. We have begun to explore the possibility that Id/anti-Id systems, which occur in Chagas' disease, are related to the clinical differences that occur in this infection.

In studies which parallel findings in human schistosomiasis mansoni (Colley et al., 1989), chagasic patients' PBMCs proliferate upon exposure to anti-EPI immunoaffinity-purified antibodies (anti-EPI) from patients' serum (Gazzinelli et al., 1988b, c; Colley et al., 1990). As in schistosomiasis, PBMC from patients who have never been exposed to T. cruzi are not stimulated by these antigen specific antibodies. Controls for these experiments demonstrate that neither normal Ig or immunoaffinity-purified antibodies to antigens of other non-related parasites stimulate PBMC of chagasic patients to proliferate.

Gazzinelli and co-workers (Gazzinelli et al., 1988b) observed that anti-EPI purified from sera of chronic chagasic patients with cardiac disease (Id-C) were generally more stimulatory for PBMC (or T lymphocytes) than anti-EPI Id isolated from sera of indeterminate patients (Id-I). This was seen regardless of the clinical form of the donor of the responding cells. This was the first demonstration of anti-Id T lymphocytes in Chagas' disease, and the correlation with clinical disease suggested a possible role for Id/anti-Id responses in the generation of chronic chagasic cardiopathy.

This Id stimulation of auto-anti-Id T cells from Chagas' patients was observed to require the presence of an adherent cell population. Recently, a difference in the requirements for chronic chagasic T cell-recognition and response to Id-C and Id-I became evident while examining this role of adherent cells in antigen processing (Colley et al., 1990; Gazzinelli, R. T. et al., submitted for publication). As expected, antigen (EPI) stimulated responses of all patients are completely ablated in the presence of 25 µM chloroquine. This level of chloroquine is known to block lysosomal enzymatic degradation by raising the pH of lysosomes and thus blocking processing and presentation of antigen to T cells (Unanue, 1984). Chloroquine also inhibited Id-I stimulation of PBMC from either C or I patients, but strikingly, when Id-C preparations were used as the stimulus, two different response patterns were observed in the presence of chloroquine (Colley et al., 1990; Gazzinelli, R.T. et al., submitted for publication). The PBMC responses of one group of patients (Group 1) resembled the responses to either Id-I or EPI. Their anti-Id-C responses were markedly inhibited by chloroquine (responses were decreased by greater than 40%). In contrast, a second group of patients (Group 2) had anti-Id-C responses which were not significantly inhibited or were augmented in the presence of chloroquine. Most Group 1 patients were I, and surprisingly, all 14 Group 2 patients had severe Chagas' disease-related morbidity. This is a striking clinical correlation. It is possible that the direct (non-processed; non-MHC-presented) stimulation of anti-Id T cells seen with cells and Ids from C-patients could be related to the immunopatho-
genesis of the disease.

Recent experiments in progress (Reis et al., 1989) have shown that anti-Id specific rabbit sera can detect Id differences in the anti-EPI Abs from pooled or individual sera from C vs. I patients (i.e. Id-C vs. Id-I). Preliminary competitive ELISA assays and Western immunoblot analyses of these Ids confirm that the Ids on Id-I are associated with the primary structure of Ig heavy and light chains, while the Ids on Id-C are defined by conformation, requiring the presence of intact, non-denatured immunoglobulin molecules. These data are completely compatible with those results from the in vitro anti-Id T cell proliferation studies where processing and presentation was required for Id-I stimulation, but not Id-C stimulation, and delineated the different clinical forms.

Presently, it is conjectural how differential processing requirements for anti-Id T cell responses among patients with different clinical forms of Chagas’ disease is related to pathogenesis. It is at least possible that the lesions could result from repeated, unregulated direct stimulation of anti-Id T cells, and thus be a unique form of autoimmunity based on an Id etiology. Further dissection of the response at both the cellular and Id levels may clarify why T. cruzi infection in one patient stabilizes and remains asymptomatic, while in another it leads to severe morbidity.

Maternal fetal effects of idiotypes in Chagas’ disease

In areas endemic for Chagas’ disease individuals become infected early in life and maintain life-long, chronic infections. In areas of high prevalence, this pattern predicts that many women of child bearing age are actively infected at the time of their pregnancy. This situation could create an opportunity for the mothers’ infection, or their immunological responses to that infection, to greatly influence the developing repertoire and future reactivity of her child. An active infection at the time of pregnancy would lead to placental or milk transfer of the mothers’ high titer IgG antibodies (bearing their Ids) and circulating T. cruzi antigens to the developing child. The impact of these Ids or antigens on the future development of pathology when that child becomes infected with T. cruzi is currently only speculative. This may, however, explain the observation in many parasitic infections that people from non-endemic areas (i.e., who would be unlikely to be born of infected mothers) are much more likely to suffer severe disease than are residents of the endemic area. People born of infected mothers may be primed in utero to elicit regulatory or non-pathogenic Id/anti-Id responses by specific maternal Ids or anti-Ids on antibodies developed during their chronic infections (Bona, 1987; Kearney, 1989; Cerny and Hiernaux, 1990).

Specific anti-Id priming in utero (possibly caused by antigen, Id or anti-Id) has been demonstrated in humans by Eloi-Santos and her colleagues in studies of the responsiveness of cord blood mononuclear cells (CBMC) from neonates born of mothers with Chagas’ disease (Eloi-Santos et al., 1989). CBMC from neonates born of mothers infected with T. cruzi responded to soluble antigen from epimastigotes but not from unrelated antigens (Eloi-Santos et al., 1988). Their studies also showed that anti-EPI antibodies, but not anti-schistosome egg antigen antibodies (anti-SEA) or normal human Ig, stimulated these CBMC. Furthermore, anti-EPI Ids did not stimulate CBMC from neonates of uninfected or Schistosoma mansoni-infected mothers. These data strongly support the hypothesis that in utero sensitization of anti-Id T-cells occurs. We further hypothesize that such powerful influences on the developing repertoire of the neonate play an important role in determining the
responses and regulatory mechanisms expressed by most children in endemic areas when they are subsequently exposed to the same chronic infection.

This work was supported by grants from the Financiadora de Estudos e Projetos (FINEP), the Conselho Nacional de Pesquisas (CNPq), the Department of Veteran Affairs and the NIH (AI 26505 and T32-GM-07347: Medical Scientist Training Program Grant).

REFERENCES

17) Brener, Z. (1984): Laboratory acquired Chagas' disease: an endemic disease among par-
186


35) Gazzinelli, R.T., Galvao, L.M., Cardoso, J.E., Cancado, J.R., Krettli, A.U., Brener, Z. and


189


defining antigenic determinants on subpopulations of mammalian neurons and *Trypanosoma cruzi* parasites, Nature, 296, 34-38