Chagas’ Disease and the Autoimmunity Hypothesis

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INTRODUCTION

The last time the clinical, pathological, epidemiological, immunological, biological cycle, vector biology, and other aspects of Trypanosoma cruzi infection (Chagas’ disease [CD] or American trypanosomiasis) were all condensed and compiled in comprehensive book form was in 1979 (14). Since then, reviews and books have sporadically updated the available information on the clinical (116), pathological (116), and immunological (13, 17, 68, 72) aspects of T. cruzi infection, as well as progress in chemotherapeutic approaches (61), experimental vaccination (64), and our knowledge of parasite invasion requirements (19).

The mechanisms underlying the pathology associated with the various forms of CD have been subjects of active research and sometimes polemic discussions. The latter is not unexpected for a disease that presents itself in cardiac, digestive, nervous, and even asymptomatic forms and is caused by a multifaceted parasite displaying variable tissue tropisms and degrees of virulence. Over the years, several hypotheses have been advanced to explain the development of pathologic changes in CD. Of these, the autoimmunity hypothesis is covered in detail in this review while the others are succinctly described in “Non-autoimmune hypotheses for the production of chronic Chagasic tissue lesions” below.

The evidence suggesting a role for autoimmune events in the pathogenesis of CD is presented, interjecting where applicable the pitfalls noted and criticisms raised by skeptics. In addition, and whenever possible, approaches that may help clarify uncertainties or resolve disagreements are suggested.

Over nearly a quarter century, numerous reviews and articles have recorded the evolution of the discussion and controversy surrounding the notion of autoimmunity as a determining factor in the pathologic findings of CD (2, 16, 27, 34, 37, 51, 54, 62, 63, 89, 106, 109, 119). Being the sequel of a review on autoimmunity in CD published by the author in 1986 (63), the present assessment focuses on the more recent literature. Far from engaging in an ineffectual attempt to try to cover exhaustively the entire pertinent literature, this review addresses only the main current trends of thought.

Before tackling the core topics, and for the benefit of readers for whom this may be a new field, it would be appropriate to summarize the implications of the autoimmunity theory for the millions of individuals who suffer from some form of CD or may acquire it. If autoimmunity elicited by cross-reactive T. cruzi antigens were responsible for CD, efforts to develop effective chemotherapy would be worthless and meaningless, since drugs that kill T. cruzi would not arrest an immune response maintained through continuous stimulation by host tissue antigens. Immunosuppressants used to control autoimmunity have been shown consistently to exacerbate human and experimental T. cruzi infections (15, 21, 49, 75, 90, 112) and therefore could not be counted on to control autoimmunity in this case. In addition, and if autoimmunity were a true reflection of immunological cross-reactivity between T. cruzi and host tissue antigens, attempts to develop an effective anti-T. cruzi vaccine would be severely hampered by the need to demonstrate that the selected antigens do not elicit anti-host tissue immune responses. Reliance on negative results is generally poor, and health authorities would be reluctant to use a lack of demonstrable cross-reactivity as a basis for the critical decision about putting millions of people at risk of acquiring the very disease that a vaccine would attempt to prevent. Because CD is a disease whose pathologic findings generally take decades to develop, even the use of human volunteers in vaccine testing would pose major difficulties. On the other hand, if autoimmunity and the above-mentioned immunological cross-reactivity were inconsequential in terms of pathogenesis, the development of effective chemotherapy and vaccines would be goals to be encouraged and strongly supported.

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TRYPANOSOMA CRUZI AND MOLECULAR MIMICRY

The term “molecular mimicry” has been used frequently in the literature on autoimmunity and CD to refer to the similarity in either amino acid sequence or structural conformation...
between molecules or segments of molecules of *T. cruzi* and those of its hosts. Some authors have held the view that molecular mimicry is responsible for misdirecting immune responses originally elicited by parasite antigens toward cross-reactive host tissue antigens, in this way playing an accidental role in the pathogenesis of CD (reviewed in reference 34). The case for molecular mimicry has been strong and, in some cases, well documented. However, as the evidence discussed below indicates, there is no consensus on whether the molecular similarities confirmed to date underlie the development of pathologic findings. Readers are referred to the recent review by Davies (26) for a deeper insight into the general considerations, constraints, and cautions concerning the possible role of epitope mimicsries in autoimmune diseases in general.

**ANTIBODIES TO HOST TISSUE ANTIGENS**

It is a well-established fact that a number of molecules have remained unchanged or minimally modified through evolution on the biological scale. Therefore, it should not surprise anybody that a parasite like *T. cruzi* shares some molecules, stretches of molecules, or some epitopes with its mammalian hosts. Whether this sharing can turn into a disadvantage for the host when *T. cruzi* pays him (her or it) an unwelcome visit remains a debatable subject.

Although the notion that immunological cross-reactivity could play a role in the pathogenesis of CD has been around for over four decades (96, 123), the possibility that autoreactive antibodies could occur in body fluids and be involved in causing tissue damage did not receive much attention until 1974, when a paper (23) that was to be recanted years later (58) was entered into the literature. Although the present review intends to examine mainly the more recent evidence, it is important to discuss this particular paper because, despite its retraction, the reported results have continued to be quoted and cited as if they had remained enduring facts. The initial paper described the presence of serum antibodies reactive with endocardial, vascular, and interstitial (EVI) antigens in a large proportion of chagasic patients. These antibodies were readily removed by absorption with *T. cruzi* epimastigotes (suggesting cross-reactivity of some *T. cruzi* antigens with EVI antigens) and were claimed to be absent in sera from normal individuals or patients with nonchagasic cardiovascular diseases. A subsequent report from the same group described the presence of antibodies to Schwann sheaths of myelinated somatic and unmyelinated autonomic peripheral nerve in the sera of patients acute with CD (aCD) as well as chronic Chagas’ heart disease (cCHD) (59). The retraction of both of these communications came when the investigators realized that the reactivity of the human anti-EVI and anti-nerve tissue immunoglobulins (Igs) was confined to nonhuman tissues (which they had used in their previous immunofluorescence studies) (58). The new observations landed the anti-EVI antibodies in the categories of heterophile and largely irrelevant to the pathogenesis of CD. It was noted later that anti-EVI antibodies were also present in sera from patients with malaria and visceral leishmaniasis (118), cross-reacted with *Trypanosoma rhodesiense* antigens (117), and could bind a carbohydrate epitope expressed by cells from various species (9, 124) as well as several other heterologous antigens (35). However, the auto-reactivity notion did not disappear after the recantation because, by then, other authors had found different cross-reactivities between *T. cruzi* and mammalian hosts antigens (reviewed in reference 63). The more recent findings are presented below.

Van Voorhis and Eisen (128) identified a 160-kDa *T. cruzi* surface protein overlying the flagellum, which they termed FI-160, by screening a parasite DNA expression library with sera from mice with chronic CD; normal mouse serum did not recognize the FI-160 fusion protein. Mouse anti-FI-160 antibodies were shown to cross-react with a 48-kDa protein present in axonal and myenteric plexus cells. Immunofluorescence studies with these antibodies revealed that FI-160 was localized on the flagellum of *T. cruzi* trypomastigotes but was not detectable on either epimastigote or amastigote forms. The antibodies cross-reacted with lysates of nerve and brain tissue but not with lysates prepared with cardiac, skeletal muscle, liver, or kidney tissue. These observations, together with the finding that 44% of the tested chagasic sera displayed reactivity with FI-160 (129), raised the possibility that the anti-FI-160 antibodies were involved in the production of nerve tissue damage, which is seen occasionally in patients with CD. The cognate epitope was mapped to the 12-amino-acid peptide TPQRKTTEDRPO, which was able to block binding of polyclonal anti-FI-160 antibodies to nerve antigens. The sequence of a *T. cruzi* genomic clone including 1,104 bp at the 3’ end of the FI-160 DNA open reading frame encoded regions suggesting that the protein could be linked to the parasite surface via glycosylphosphatidylinositol (129). If this inference were borne out and considering that this type of linkage is often susceptible to cleavage by *T. cruzi* phospholipase C, it might explain why FI-160 has been detected by immunofluorescence on the surface of nonparasitized as well as parasitized cells in *T. cruzi*-infected cell cultures (129). If a similar phenomenon occurred in vivo, it would raise the possibility of a role for anti-FI-160 antibodies in damaging noninfected cells.

Later, Van Voorhis et al. (127) showed that FI-160 was, in fact, a member of a family of closely related *T. cruzi* proteins with similar molecular sizes and characteristics, localized on the surface of the flagellar pocket. The FI-160 protein described above was renamed FI-160-1, and three more molecules were termed FI-160-2, FI-160-3, and FI-160-4. There is approximately 80% homology among these molecules, all of which have the sequence TPQRKTTEDRPO in their C-terminal portions, which mimics a sequence found in mammalian nervous tissue. In addition, the N-proximal segment was found to include a sequence with homology to the fibronectin type III domain. Antibody binding to this epitope could be blocked by recombinant FI-160 bearing the N terminus but not by a variant bearing the C terminus, implying the existence of at least two cross-reactive epitopes in the FI-160 molecule. It is known now that the ability of chagasic sera to react with FI-160 does not correlate with clinical disease (129). This finding seems to have dampened efforts to look for deposition of anti-FI-160 antibody on nerve tissue from chagasic mice and establish if the antibody would mediate lytic or inflammatory effects on nerve cells.

A cross-reactivity between serum antibodies from chagasic patients and sciatic nerve was also described by Gea et al. (44). The sera used in this work were obtained from three groups of patients. Group I consisted of patients with positive anti-*T. cruzi* serologic findings, normal electrocardiograms and no detectable signs of disease; group II included patients presenting abnormalities in their electrocardiograms but no cardiomegaly; and group III included patients with cardiomegaly and congestive heart failure. The antigen used in this study was an acidic *T. cruzi* cytosolic fraction separated by isoelectric focusing. Antibodies to this parasite antigen were present in 50, 92, and 50% of the patients in groups I, II, and III, respectively, whereas reactivity to a soluble extract of human sciatic nerve was demonstrable in 58, 66, and 75% of the patients, respectively. Aliquots of these sera with high reactivity for both sciatic
nerve and *T. cruzi* were separately absorbed with each of the antigens. Absorption with sciatic nerve antigen reduced reactivity with the nerve extract by 48 to 69%, whereas absorption with the *T. cruzi* cytosolic antigen decreased the anti-nerve reactivity by 56 to 75%. Reactivity with the *T. cruzi* antigen preparation was reduced by 12 to 23% after absorption with the nerve antigen and by 53 to 70% after absorption with the parasite material. The cross-reacting nerve epitopes appeared to have a carbohydrate component since the IgG reactivity decreased following treatment of the sciatic nerve antigen with periodate. Because the *T. cruzi* antigen used in this study consisted of cytosolic molecules, it might be assumed that Fl-160 molecules were not present. However, separation of the cytosolic fraction was done after the whole-parasite homogenization, and Fl-160 solubilization during this step cannot be ruled out. It is not known whether native Fl-160 would have been present in the acidic *T. cruzi* fraction under these conditions.

While screening a *T. cruzi* lambda gt11 cDNA library with serum from a chagasic patient with severe heart disease, Levin et al. (77) identified several clones. Of these, the JL5 close was used to map a specific epitope to the C-terminal portion of a *T. cruzi* P ribosomal protein. The EDDDMGFLGLFD peptide in this region is partially homologous to the SD(D/E)DMGFGLFD peptide in the equivalent region of a human P ribosomal protein. Interestingly, the latter endecapeptide is recognized by 15% of sera from patients with systemic lupus erythematosus (SLE). Sera from such patients reacted with dot blots of the JL5 clone (77). It should be pointed out, however, that chagasic and SLE antibodies differ not only in the epitopes and proteins they specifically recognize but also in their mean binding affinity constant. Thus, the constant for chagasic IgG binding the relevant JL5 peptide is five times greater than the constant for H13 binding (H13 is the nearly homologous peptide specifically recognized by antibodies from SLE patients) (55). Skeiky et al. (113) have observed that the JL5 P ribosomal protein and its C-terminal R13 peptide (see below) are distinct from TcP0 (a 38-kDa *T. cruzi* P ribosomal protein) since their sequences are different and a JL5 DNA probe detects a 0.7-kb mRNA species, which is too short to encode a 38-kDa protein. However, anti-JL5 antibodies recognize the 38-kDa antigen on Western blots, indicating the existence of cross-reactivity between JL5 (TcP2β) and TcP0 (77, 113). It is noteworthy that sera from SLE patients do not recognize the 38-kDa TcP0 protein (8, 110).

Reactivity with JL5 was detected in the sera of all 19 patients with overt CD tested by Levin et al. (77). Only weak or non-detectable reactivity was recorded in sera from chagasic patients without clinical evidence of heart tissue damage. On Western blots of *T. cruzi* trypomastigote, epimastigote, or amastigote extracts, the JL5-reactive sera identified a 38-kDa protein. The synthetic R13 peptide (EEEDDMGFLGLFD), which comprises the C-terminal segment of the JL5 protein, defined a linear antigenic determinant reacting with the P-reactive sera from chagasic and SLE patients (85). R13, but not two shorter peptides representing the 7 and 10 C-terminal amino acid residues, was able to partially block serum reactivity with the JL5 recombinant protein, providing a hint about the approximate size of the specific epitope recognized by B lymphocytes. However, R13 blocked no more than 60% of the anti-JL5 reactivity in enzyme-linked immunosorbent assays (ELISA), suggesting that the R13 sequence may not be the only epitope in the *T. cruzi* P ribosomal protein eliciting a humoral immune response in patients with cardiac manifestations of CD. Levels of anti-R13 antibodies (largely IgG1) in the positive chagasic sera correlated with the anti-*T. cruzi* antibody titer (78). Anti-JL5 antibodies purified from chagasic serum but not the fractionated serum, recognized the P ribosomal protein on Western blots (78). A similar observation was made by Bonfa et al. (8) while looking for anti-P0, anti-P1 and anti-P2 reactivities in chagasic sera. This curiosity was tentatively attributed to low concentrations of anti-P protein antibody in chagasic sera or to possible low-affinity binding (8, 78). However, Skeiky et al. (113) did not appear to have encountered difficulties detecting anti-TcP0 activity in whole serum, suggesting that concentrations may vary from patient to patient.

Sera from patients with cCHD mount a humoral anti-P ribosomal protein response, with high levels of antibody against R13 measurable by ELISA and low antibody levels against the highly homologous H13 peptide EESDDMGFLGLFD of the mammalian P protein (79). Moreover, the proportion of chagasic sera testing positive against R13 that were also positive for H13 never exceeded 40%, and the dual-positive sera were the ones that recognized the human P ribosomal protein (85). This is in contrast with the P ribosomal protein reactivity of sera from SLE patients since, in this case, there is no appreciable distinction between antibody levels to R13 and H13 (56). The difference in the anti-R13 and anti-H13 antibody content of chagasic sera (most probably due to the presence of a glutamic acid residue in the *T. cruzi* protein instead of the serine residue found in the C-terminal consensus sequence) would seem to indicate that the humoral response is directed primarily against the parasite epitope. This is not a trivial observation because it goes to the issue of whether autoreactive antibodies originate in responses elicited by parasite molecules or result from stimulation by modified or previously sheltered host antigens released as a consequence of parasite-inflicted tissue damage.

To consider any hypothesis based on the involvement of cross-reactive anti-P ribosomal protein antibodies in the development of chagasic heart tissue lesions, a satisfactory explanation must be provided for how such antibodies could engage an intracellular epitope, normally not accessible to lymphocytes. It is not difficult to envisage that immunological destruction of *T. cruzi* can expose its intracellular molecules, including P ribosomal proteins, to lymphocytes. It is also plausible to consider the release of host P proteins when *T. cruzi*-infected cells burst, although this possibility seems unlikely since serum antibodies from patients with nonchagasic cardiomyopathy fail to detect R13 (85). It is more difficult to visualize how R13-specific antibodies could interact with a cell-sheltered epitope of intact cells, unless the R13 epitope occurred on cardiac cell membranes as well, for which there is no evidence as yet. There is, however, a report showing cross-reactivity of anti-ribosomal P antibody with a membrane-related target of some human cells (70).

It is also noteworthy that mice hyperimmunized with JL5 recombinant protein (currently classified as TcP2β) present an altered electrocardiogram with a net increase in the duration of the QRS complex (79). However, these animals show no histological evidence of cardiac inflammatory lesions. This observation is in keeping with earlier data showing that perfusion of isolated rabbit heart with IgG from chagasic patients by the Langendorff procedure causes an altered electrocardiogram recording, preventable by prior incubation of the IgG with R13 peptide (22).

Aznar et al. (3) used an ELISA to survey R13 reactivity in sera from a group of 161 chagasic patients (including 7 with aCD and 7 with congenital CD), a group of 285 blood bank donors from areas with endemic CD (found to be serologically positive for *T. cruzi*), and a group of 405 European individuals (obviously distant from areas of endemic infection). All of the patients with aCD were found to be unreactive, whereas six of
the seven sera from patients with congenital CD were positive. Among the chronically ill patients and the serologically positive (for *T. cruzi*) blood donors, 60 and 49%, respectively, displayed R13 reactivity, although the percentage of anti-R13-positive sera varied significantly among groups of patients from different countries. Except for the exclusion of patients with digestive or asymptomatic forms of CD (because of their negative R13 reactivity), this study could not establish a clear and precise link between R13 reactivity and other forms of CD because of the lack of definition of the clinical status of each member of the studied population. However, this was feasible for a group of 14 patients with chagasic cardiomyopathy from whom endomyocardial biopsy specimens and blood samples had been taken at the same time. Although close (*P* < 0.06), a statistically significant correlation between high anti-R13 levels and cardiac tissue inflammation could not be established for this group. This type of study is important and deserves renewed attention and repetition with a larger number of patients, preferably from different geographic regions.

Using the ELISA technique, McCormick and Rowland (82) examined sera from mice surviving *T. cruzi* infection (i.e., chronically infected mice) and detected antibodies reactive with a nondenatured mouse heart tissue extract. These antibodies had negligible and less pronounced reactivity with similarly prepared extracts of smooth and striated muscle, respectively, indicating specificity for heart antigens. After immunofinity purification, these antibodies were found to react with an antigen(s) present in the supernatant of epimastigotes broken up by sonication. Although epimastigotes are not found in mammalian hosts, there is sufficient evidence for the sharing of many antigens between these and mammalian forms of the parasite to consider it possible that the antibodies were produced in response to *T. cruzi*, but the original stimulus for the formation of the antibodies was not identified.

In a study of serum samples from 102 patients with cCHD, Bonfà et al. (8) could find only 4 that would stain HEP cells by indirect immunofluorescence. None of these sera had detectable levels of anti-double-stranded DNA, anti-Ro/SSA, anti-La/SSB, anti-Sm, or anti-ribonucleoprotein antibodies, and only 12% of them had somewhat low levels of anti-histone antibodies. On Western blots of a HeLa cell extract, 31 sera displayed weak reactivity against a variety of bands but antibodies from 7 sera coincided in binding weakly to a 23-kDa band. Stronger binding was seen when isolated ribosomes were used instead of the HeLa cell extract. Affinity-purified antibodies specific for the 23-kDa protein cross-reacted with a *T. cruzi* protein with a similar molecular size. The presence of serum antibodies to the 23-kDa protein did not correlate with overt cardiomyopathy, since they were found in chagasic patients with or without this condition. It is noteworthy that these investigators did look for but could not detect reactivity to P0, P1, and P2 in any of the sera they tested.

Kerner et al. (57) screened a lambda gt11 epimastigote cDNA library with sera from mice chronically infected with *T. cruzi* and identified two clones containing a 114-bp sequence coding for a 38-amino-acid repeat with over 60% homology to the *Trypanosoma brucei* microtubule-associated protein (MAP) p320. Expression of one of the inserts produced a β-galactosidase–MAP-like fusion protein (termed K1-7) which was recognized by human chagasic sera. Anti-K1-7 antibodies helped establish the presence of the parasite polypeptide in a 110-kDa band from the *T. cruzi* cytoskeleton and cross-reacted with cytoskeleton bands from NIH 3T3 fibroblasts and bovine brain microtubule preparations. Conversely, monoclonal antibodies specific for bovine brain microtubule reacted with the parasite fusion protein on Western blots. These results suggest immunological cross-reactivity between the *T. cruzi* MAP-like molecules and mammalian cell molecules. Interestingly, immunofluorescence studies showed that the K1-7 *T. cruzi* polypeptide was not expressed on the surface of air-dried trypomastigotes but was, instead, detectable after parasite treatment with acetone-methanol, reaffirming a cytoplasmic localization (57), which implies that KI-7 interaction with host lymphocytes would require parasite destruction.

Curiously, a similar reactivity was present in the normal control sera. The level of anti-CMHc, determined by ELISA, was comparable in cCHD, aCD, and control groups. All of the sera recognized a 210-kDa band comigrating with CMHC on Western blots of a human heart tissue lysate. Affinity-purified anti-CMHc polypeptide from 23 cCHD and 14 aCD patients were tested for reactivity with blots of a *T. cruzi* trypomastigote extract. Of these sera, 14 (61%) and 1 (9%), respectively, detected a doublet of bands of 140 and 116 kDa which was not recognized by sera from normal individuals. Anti-CMHc serum antibodies isolated from all of the 28 patients with cCHD and 14% of the patients with aCD recognized B13, a recombinant *T. cruzi* polypeptide mapped to a 140- and 116-kDa doublet (48). In ELISA, B13 reactivity was blocked in a dose-dependent manner by preincubation of serum from a single cCHD patient with the synthetic CMHC peptide p1439–1453, which includes the sequence AAAALDK, partially homologous to the B13 internal sequence AAAGDK. There was no such competition when serum from an aCD patient was similarly tested. Instead, preincubation of antibody with the S4 (FGQAAAGDK) peptide of B13 blocked the reactivity of sera from cCHD and aCD patients with B13. Cunha-Neto et al. (25) proposed that the reactivity for CMHC displayed by both chagasic and normal sera was attributable to “natural” antibodies and was different from the specific reactivity of the cCHD sera, thus linking “specificity” with chagasic myocarditis. There was, however, an apparent mismatch between the observation that 100% of the sera from cCHD patients displayed CMHC reactivity in the ELISA but only 61% of the antibodies purified from the sera displayed anti-*T. cruzi* reactivity on Western blots. The authors proposed that “natural” antibodies present in the negative sera (39%) might account for the noted difference, but this possibility was not pursued further. “Natural” antibodies are IgM, whereas specific antibodies in chronic patients are largely IgG. Therefore, the explanation could be tested after separation of these two Ig isotypes.

More troublesome, and in conflict with the results summarized above, are the recent observations that the B13 reactivities of sera from cCHD or aCD patients are comparable, very weak, and not too different from the reactivity displayed by sera from patients with idiopathic dilated cardiomyopathy or other cardiopathies (76a). Additional confusion arises from Levin’s inability to independently confirm the finding of Cunha-Neto et al. (25) that B13 binding by antibodies from sera from cCHD or aCD patients in the ELISA is inhibited by the AAAALDK peptide. Such inhibition was absent whether the peptide had been used in free form or coupled to bovine serum albumin (76a).

Also related to the work of Cunha-Neto et al. (25) is an earlier study by Tibbetts et al. (122) with mice, which was designed to establish whether heart tissue-specific antibodies are produced during *T. cruzi* infection. These investigators infected C57BL/6 mice with either the Brazil or Guayas isolate of *T. cruzi*, claimed to cause severe or mild cardiomyopathy, respectively, in mice. C57BL/6 mice infected with the Brazil
isolate produced antibodies reactive with a C57BL/6 mouse heart extract. These antibodies were detected as early as 5 days postinfection (p.i.), i.e., a few days before the appearance of anti-\(T. cruzi\) antibodies (which became detectable on day 10 p.i.). The titers for both antiparasite and anti-heart tissue antibodies increased over time. The autoreactive antibodies present in sera collected over a 150-day period p.i. detected heart protein bands of 200, 150, and 53 kDa on Western blots of a \(T. cruzi\) epimastigote lysate. Heart tissue-reactive antibodies were also present in the sera of mice infected with Guayas flagellates. However, the sera from Brazil-infected mice reacted strongly with the p200 and p150 heart protein bands and a cardiac/skeletal protein band of 53 kDa whereas sera from Guayas-infected animals reacted weakly with bands in the 30- to 40-kDa and 50- to 60-kDa ranges plus an 80-kDa band. These results suggest that some of the antigens exposed to the immune system vary according to the severity of the cardiomyopathy that ensues, the \(T. cruzi\) strain used for infection, or both. However, there was no difference in the ability of sera from Brazil- and Guayas-infected mice to react with an 80-kDa cardiac-skeletal protein antigen. Further analysis revealed that the p200 band was the cardiac isoform of myosin H and the p150 band was desmin, which has no organ-specific isoforms; the remaining bands were not identified. Overall, these results indicate that in \(T. cruzi\)-infected C57BL/6 mice, there is a certain degree of correlation between the appearance of anti-p200 and anti-150 antibodies and the severity of their cardiomyopathy. However, the possibility that the noted variations merely reflected parasite strain differences cannot be ruled out. A major difference from the study of Cunha-Neto et al. (25) was the total lack of cross-reactivity of the mouse anti-heart antibodies with \(T. cruzi\) antigens. Moreover, sera from mice hyperimmunized with a heart tissue homogenate, or even with pure myosin, failed to react with any band in blots of a \(T. cruzi\) epimastigote lysate (122). Conversely, sera from mice hyperimmunized with \(T. cruzi\) failed to detect any muscle tissue antigen. This lack of cross-reactivity suggests that the anti-heart antibodies studied by Tibbetts et al. (122) were produced in response to host antigens, presumably exposed or altered as a consequence of parasite-caused damage. It should be emphasized again that epimastigotes are not found in mammalian hosts and that despite the high degree of cross-reactivity of epimastigotes with mammalian forms of \(T. cruzi\), several stage-specific antigens have been described. Therefore, and however unlikely it may appear to be, the possibility that the antigen(s) responsible for a putative cross-reactivity was absent in the epimastigote lysate used in the study by Tibbetts et al. cannot be ruled out, as the authors themselves recognized. Such a possibility could be easily explored by restesting the materials on lysates of trypomastigotes and amastigotes.

There is an obvious inconsistency between the lack of cross-reactivity of the mouse anti-heart antibodies with \(T. cruzi\) antigens and the cross-reactivity displayed by sera from patients. The disparity might be explained simply on the basis of host species differences or exposure to \(T. cruzi\) or different strains, but it will not be satisfactorily resolved until side-by-side tests of anti-myosin or anti-B13 reactivity in the ELISA are carried out with mouse and human chagasic sera. However, it is doubtful that the results of such a study will receive serious attention before the conflict between the results of Cunha-Neto et al. (25) and Levin (76a) are reconciled.

Some authors have attributed the presence of the (for now putative) anti-CMHC antibodies in sera from cCHD patients to a response to antigens released from damaged heart cells (76). Examples abound for the presence of this type of antibody in cases of nonchagasic myocarditis, myocardial infarction, coronary artery bypass, and heart valve surgery (31). In these instances, it has been assumed that tissue damage precedes rather than follows the onset of the anti-myosin immune response because it stands to reason. In the context of a possible role for anti-CMHC in the pathogenesis of cCHD, several critical questions await answers: (i) whether parasite-mediated heart tissue damage is any different from nonchagasic heart tissue injury in eliciting anti-CMHC antibody production; (ii) whether a \(T. cruzi\) antigen cross-reactive with CMHC really exists and, if so, whether it would trigger an immune response capable of contributing to the development of heart tissue lesions; (iii) in the event that anti-CMHC antibodies were elicited by destroyed host cell antigen, whether they could participate in the aggravation of lesions initiated by parasite infection; and (iv) how anti-CMHC antibodies can mediate the type of preferentially mononuclear cell infiltrate characteristic of chagasic heart lesions rather than the typical granulocytic inflammatory response initiated by immune complexes in conjunction with complement. Relevant to the first of these questions is whether the parasite is really absent from chronic tissue lesions, as negative histological examinations have often revealed (14). This topic is addressed in “Nonautoimmune hypotheses for the production of chronic chagasic tissue lesions” below. While waiting for answers to these questions, readers may be interested in examining the results of Neu et al. (97), published in 1990, indicating that anti-myosin antibodies cannot be involved in initiating heart tissue injury in mice and postulating that such antibodies are secondary rather than primary to heart disease.

Baig et al. (4) designed a study to establish the prevalence and titer of anti-heart antibodies in serum (detected by immunofluorescence) and of specific anti-\(\alpha\)-myosin antibodies (measured by ELISA) in 15 clinically well-characterized cCHD and aCD Argentinean patients with positive anti-\(T. cruzi\) serologic results. None of the sera contained detectable levels of antibodies capable of binding to cardiac tissue or reacting with myosin. A group of Argentinean controls was also found to be negative for either antibody. In contrast, 3.5% of normal European control subjects and fewer than 1% of patients with coronary artery disease had anti-heart antibodies, and 2% of the European controls had serum anti-myosin antibodies. The authors speculated that the higher incidence of positive sera among the European controls and patients with coronary artery disease may have reflected the use of larger numbers of samples. In any case, the total lack of reactivity of the chagasic sera is at odds with the relatively high statistics reported by Cunha-Neto et al. (25) and underscores the need for additional studies with larger and more geographically diverse patient populations. It has become apparent that groups of investigators recording conflicting results and holding dissenting views should exchange samples and conduct double-blind studies to resolve their disagreements. It should be noted that the work of Baig et al. is also at odds with that of Tibbetts et al. (122) (described above), although the use of different host species in the two studies makes a strict comparison more difficult, especially given that different strains of mice can present dramatically disparate courses and outcomes of \(T. cruzi\) infection.

Anti-laminin antibodies, which recognize the galactosyl \(\alpha(1-3)\) galactose epitope occurring ubiquitously on different types of cells from various species, have been found in sera from patients with CD, patients with cutaneous leishmaniasis, and even normal individuals (1, 87, 108, 124). These “natural” antibodies have been postulated to be linked to the thickening of the endothelial basement membrane and myocyte alterations often seen in patients with chagasic cardiomyopathy (108), but they have also been reported to prevent rather than
induce autoimmunity in CD (43). Attempts to correlate anti-laminin reactivity with clinical evidence of CD have yielded negative results (87, 108).

Grauert et al. (47) tested the reactivity of serum samples from an individual who had been accidentally infected with *T. cruzi* with trypomastigote antigens, murine laminin, bovine muscle actin, rabbit muscle myosin, bovine serum albumin, pig brain tubulin and mouse myelin. Although the ELISA titers varied, antibodies against all of these antigens were detected around day 17 p.i. and generally started to decrease after day 30 p.i. Affinity-purified anti-*T. cruzi* IgG from the patient’s samples displayed cross-reactivity with murine laminin. The IgG preparation showed only a slight reduction in anti-laminin reactivity with clinical evidence of CD have yielded an increase in contraction frequency (114). This effect could be inhibited by a specific β-adrenergic antagonist. Because this activity was removed by absorption with turkey erythrocytes (which express β1-adrenergic receptors) but not with guinea pig erythrocytes (which lack these receptors), the authors inferred that the antibody responsible for the noted effect was specific. Later, the same group showed that chagasic IgG inhibited the binding of ([3H]dihydralprenolol to a preparation of rat myocardial cell membranes (9), behaving as noncompetitive inhibitors. This effect was absent in both normal sera and samples from patients with ischemic and rheumatic heart disease. Again in this case, absorption of the chagasic IgG with turkey erythrocytes but not with guinea pig erythrocytes abrogated the inhibitory effect. Because the latter cells have “a high concentration of EVI antigen,” the authors inferred that the activities of the chagasic IgG could not have been due to heterophile antibodies. However, the list of antigens reactive with the “natural” anti-EVI antibodies includes molecules that are not components of guinea pig erythrocytes (23, 47, 58). The uncertainty created by the possible presence of natural antibodies in sera tested in a heterologous system can be avoided by switching to a homologous system. Chronic *T. cruzi* infections could be readily established in rats, from which the atria used in these studies were derived.

Mijares et al. (86) used IgGs from mice with aCD and cCHD to detect activity on L-type Ca2+ channels of isolated guinea pig cardiomyocytes. The antibodies obtained during the acute phase activated Ca2+ channels by stimulation of β-adrenergic receptors, since the effect was inhibited by propranolol. Antibodies obtained during the chronic phase reduced the Ca2+ current by stimulation of the muscarinic receptors, since the effect was inhibited by atropine. Using an ELISA and surface plasmon resonance, Elies et al. (36) screened chagasic sera for antibodies that could bind peptides corresponding to the second extracellular loops of the β1- and β2-adrenoreceptors and the M2 acetylcholine receptor. The prevalence of these antibodies in chronic chagasic sera was anti-M2 acetylcholine receptor > anti-β1-adrenoreceptor > anti-β2-adrenoreceptor. Purified IgG from the identified sera showed in vitro chronotropic effects on rat cardiomyocytes, whose extent increased in the presence of atropine. The β2-selective antagonist ICI 118,551 partially inhibited the IgG effect, suggesting that antibodies against the β2-adrenoreceptor were less functionally important. The specificity of the anti-β1-adrenoreceptor antibody appeared to be directed against the amino acid sequence AESDE, closely homologous to the epitope AESEE of the *T. cruzi* P0 ribosomal protein identified by Ferrari et al. (38). The AESEE peptide exerted a blocking effect on chagasic IgG but not on IgG from patients with idiopathic dilated cardiomyopathy (36), which have also been shown to produce antibodies against the second extracellular loop of the human M2 muscarinic receptor (40). Conversely, a peptide that blocks the activity of IgG from patients with idiopathic dilated cardiomyopathy on the muscarinic M2 receptor did not modify
the effect of the chagasic IgG. These results indicate that antibodies of different specificities recognizing distinct epitopes of the second extracellular loop of the target receptors can produce identical functional effects and cause electrophysiologic disturbances in vitro resembling those in patients with either disease. However, the details and characteristics of the pathologic changes of cCHD are markedly and sufficiently different from those of idiopathic dilated cardiomyopathy to make it difficult to accept that mere stimulation of the receptors by functionally similar autoantibodies could be solely responsible for all of the heart abnormalities associated with cCHD, including the predominantly mononuclear-cell infiltrate typical of chagasic myocarditis. On the other hand, because chagasic IgG preparations contain antibodies against both β-adrenergic and muscarinic cholinergic receptors, which trigger opposite effects on contractility (46), one must wonder what the ultimate balance would be in an in vivo situation if, even in vitro, one effect on rat atria has to be inhibited in order to record the other (46). It should also be considered that agonist-like effects on the receptors recognized by the chagasic IgGs may not contribute significantly to the causation of chagasic abnormalities.

Goin et al. (46) obtained affinity-purified IgG from human and murine chagasic sera specific for the peptide VRTVEDG ECYIQFFSNAAVTFGTA, corresponding to an internal sequence of the second extracellular loop of the human muscarinic acetylcholine receptor. The IgG preparation recognized bands on a Western blot of rat atrial membranes with molecular sizes corresponding to those of the cardiac muscarinic acetylcholine receptor and, not unlike unfractionated chagasic IgG, displayed an agonist-like activity on isolated rat atria, causing decreased contractility, an increase in cyclic GMP levels, and decreased cyclic AMP production (46). These effects were inhibited in the presence of atropine. Of 36 asymptomatic chagasic patients with autonomic nervous system dysfunction, 88.9% had anti-muscarinic cholinergic receptor antibodies in their sera, compared to 26.5% of 49 asymptomatic patients with normal cardiovascular response in tests of autonomic nervous system function. These proportions suggest a link between the presence of these antibodies and dysautonomic syndrome in CD.

Chagasic IgGs also inhibit the action of pilocarpine, in line with their agonist muscarinic cholinergic effect in the heterologous human IgG-rat atrial system (45). Recently, Perez-Leiros et al. (99) showed that chagasic IgG immunoprecipitated purified and reconstituted human M2 muscarinic cholinergic receptor molecules but did not block the binding of radiolabeled specific ligand and therefore did not target or hinder the ligand-binding site of the receptor. The chagasic IgG had an agonist-like effect on CHO cells stably expressing M2 muscarinic acetylcholine receptors, inducing a decrease in binding affinity for agonist and partial desensitization of these receptors. While these results indicate that chagasic IgG include IgGs specific for the human M2 muscarinic cholinergic receptor and can desensitize isolated cells in a controlled in vitro system, it remains to be ascertained whether this would also be the case in the naturally balanced environment of mammalian hosts of T. cruzi and, if so, what the consequences would be in terms of heart damage.

In vitro evidence for the existence of functional antireceptor antibodies in chagasic sera has accumulated for over two decades, and an association between the presence of these antibodies and disease state has been proposed (45). The next logical step would seem to be to find whether passive antibody transfers can cause in normal recipients persistent malfunction or tissue lesions of the type seen in chronic infections. Devices exist to continuously deliver antibodies to normal, freely moving animals for extended periods, simulating the type of continuous production that would be expected to occur in a T. cruzi-infected host. Mice would provide a suitable model system since they produce antireceptor antibodies during T. cruzi infection (46, 86).

**POLYCLONAL ACTIVATION AND AUTOANTIBODY FORMATION**

Studies with T. cruzi-infected mice have shown a substantial, apparently indiscriminate lymphocyte activation with onset during the acute phase and high levels of activation lingering for months (32, 89). Both B and T (CD4+ as well as CD8+) lymphocytes appear to be involved (89). The humoral immune response is polyclonal, since the vast majority of the activated B lymphocytes do not recognize T. cruzi antigens (32). The activated B and T cells eventually acquire effector status, with B cells producing IgGs (frequently IgG2) in relatively large amounts and T cells displaying a cytotoxicity or helper function (89). The administration of anti-CD4 antibodies suppresses polyclonal antibody production (88). Hypothetical explanations have been offered for polyclonal activation in T. cruzi infection, including production of nonspecific mitogens by the parasite and possible depletion or modified function of immunoregulatory cells (89). However, the precise mechanism remains to be elucidated. The immunologic disarray that might be expected from so many lymphocytes being triggered simultaneously has been linked, at least conceptually, to many of the immunologic mishaps seen during T. cruzi infection, from the production of the immunosuppression that develops during the acute phase to the stimulation of autoreactive lymphocyte clones to levels apparently beyond normal homeostatic controls during acute and chronic infection. There is some evidence for the latter, since a number of B cells from T. cruzi-infected mice made into hybridomas produce antibodies that recognize host antigens (32). Whether such B cells would be involved in the production of the tissue injuries characteristic of CD in mice is not known.

In the accidental case of T. cruzi infection described by Grauert et al. (47), there was a marked increase in Ig levels in serum, initially observed on day 17 p.i. Besides T. cruzi antigens, these antibodies recognized laminin, tubulin, bovine serum albumin, and other antigens. The levels of IgGs of the three major isotypes (IgA, IgM, and IgG) were elevated, and various nonspecific reactivities were detected, prompting the authors to suggest that polyclonal B-cell activation may have occurred in this human case.

Freire de Lima et al. (39) described in chronically infected mice the presence of CD4+ spleen cells capable of inducing syngeneic B lymphocytes to produce higher levels of IgM, IgG1, IgG2a, and IgG2b than CD4+ cells from noninfected controls would. This finding is consistent with the notion of polyclonal B-cell activation and suggests CD4+ -cell control of the event. It should be noted, however, that persuasive evidence for massive and persistent polyclonal activation extending to the chronic stage, such as was observed in the mouse model system (33), is not available for human cases of CD.

**CELLULAR ASPECTS OF AUTOIMMUNITY**

The early reports (i.e., those appearing between 1974 and the mid-1980s) suggesting that cCHD may have an autoimmune cellular component mediated by autoreactive T lymphocytes have been the subject of much discussion and analysis condensed in past reviews (51, 63, 84, 89, 106, 109, 119).
recently, and not long after describing the presence of anti-
CMHC antibodies in chagasic sera (25), Cunha-Neto et al. reported the isolation of a collection of T-cell clones (all of which turned out to be CD4\(^+\)) from cells infiltrating the heart lesions of a patient with cCHD (24). Of the 18 clones tested, 2 mounted an in vitro proliferative response upon stimulation with either CMHC or the recombinant \(T. cruzi\) protein B13. These two clones were unable to respond to either skeletal muscle myosin or a \(T. cruzi\) trypomastigote lysate. One major drawback of this study was the use of a beta counter that used a gaseous mixture instead of scintillation fluid, thus considerably reducing the counting efficiency. In this manner, the values that were determined to be either statistically different or not statistically different from the control value were fairly close to each other, weakening the conclusions. Repetition of the above study with larger numbers of T-cell clones, preferably from various patients, and more reliable quantitation would help establish if clonal cross-reactivity indeed occurs. It is also important to resolve the conflict between the results of Cunha-Neto et al. (25) and Levin (76a) regarding the significance and meaning of anti-B13 antibodies in patients with cCHD.

Hontebeyrie-Joskowicz et al. (50) found, the blood of chronically infected C3H/HeJ mice, CD4\(^+\) lymphocytes capable of transferring \(T. cruzi\)-specific delayed-type hypersensitivity (DTH) reactivity to naive recipients. Thus, mice receiving an intravenous injection of a CD4\(^+\)-cell preparation, which contained living \(T. cruzi\), displayed granulomatous lesions in their livers 2 weeks later and one of the mice presented an inflammatory infiltrate in a sciatic nerve. In addition, CD4\(^-\) T-cell lines initiated with blood or lymph node cells from chronically infected mice, cultured with a \(T. cruzi\) or mouse peripheral nerve extract in the presence of interleukin-2, transferred the ability to mount a localized footpad DTH reaction when injected together with \(T. cruzi\) antigens or peripheral nerve antigens. These T-cell lines were also injected into the sciatic nerves of normal mice. Within a week, large granulomas associated with demyelination of neighboring fibers and occasional axon degeneration were observed. Concanavalin A-induced lymphoblasts used as controls did not cause inflammation. As expected, each T-cell line was able to transfer DTH when injected together with the specific antigen used for their propagation, but some T-cell lines propagated with nerve antigen were able to transfer DTH reactivity to half of the recipients when injected together with either nerve or \(T. cruzi\) antigens (50). However, T cells propagated with \(T. cruzi\) antigen were also found to mount a significant DTH response against sheep erythrocytes. The passive transfer of nerve damage capability seen after intraneural injection of sensitized cells was major histocompatibility complex restricted, being demonstrable in syngeneic C3H/HeJ but not in allogeneic C57BL/6 mice.

The transfer of DTH and other T-cell functions to syngeneic recipients by injecting T-cell populations or T-cell clones is a well-established procedure. Therefore, the transfer of DTH reactivity by sensitized cells from chagasic mice, particularly when injected together with their specific antigens, is only to be expected, as exemplified in the work summarized above. It is also known that certain subpopulations of suitably stimulated T cells will produce, among other things, the cytokines and factors required for inflammatory-cell recruitment at their localization sites. While the careful work of Hontebeyrie-Joskowicz et al. (50) demonstrated the presence of such cells in mice with cCHD, it did not answer the critical question whether the T cells from which the cell lines were derived had been sensitized first (in vivo) by damaged nerve tissue or parasitic antigens. Some of the T-cell clones that had been propagated with nerve antigens and were stimulated prior to injection with either nerve tissue or \(T. cruzi\) antigen caused a DTH response in some recipients, denoting cross-reactivity. However, the fact that sheep erythrocytes also triggered a reaction when injected with a \(T. cruzi\)-sensitized clone raised questions about specificity. With respect to this last point, the authors proposed that the particular T-cell clone might have had multiple reactivities since it was also able to provide helper function to syngeneic B cells of various specificities and to proliferate in the presence of syngeneic antigen-presenting cells even without addition of an antigen.

In 1989, Rizzo et al. (103) reported that lymph node cells from BALB/c mice chronically infected with \(T. cruzi\) proliferated in vitro when incubated with rabbit skeletal myosin or a soluble epimastigote antigen preparation but not with rabbit skeletal myosin. The response to myosin was abrogated by pretreatment of the cells with monoclonal anti-Thy-1.2 or anti-L3T4 antibodies plus complement, suggesting that the assay was measuring either CD4\(^+\)-cell proliferation or CD4\(^+\)-cell-dependent proliferation by B lymphocytes. An involvement of B lymphocytes in the antimyosin response of the BALB/c was not ruled out, because the chronic mice had received several inocula of epimastigotes grown in a medium rich in mammalian tissue extracts before being infected with trypomastigotes. However, cells from CBA/J mice infected with trypomastigotes without prior administration of epimastigotes also proliferated upon stimulation with rabbit myosin. Mouse antisera raised against lymphocytes stimulated with rabbit myosin or the epimastigote antigens inhibited the cellular responses to myosin and epimastigotes, respectively. The authors inferred that the cells proliferating in response to each antigen belonged in different compartments of the immune cell repertoire. However, since the presence of myosin and epimastigote antigens in the stimulated cells was likely and had not been ruled out, the noted inhibitory effects could have been mediated by antimyosin and antiepipastigote antibodies, respectively. One puzzling observation made in this study was that pooled sera from the chronically infected mice failed to inhibit myosin- or \(T. cruzi\)-induced proliferation by chronic mouse lymphocytes. While the authors concluded that this pointed to the absence of immunosuppressive factors in serum from mice with cCHD, the same observation would have to be interpreted also as indicative of the absence of both antimyosin antibodies, which is possible, and anti-\(T. cruzi\) antibodies, which is highly unlikely.

A rather interesting and useful model system to study immunological reactions affecting heart and other tissues during the course of cCHD was developed by Ribeiro dos Santos et al. in 1992 (102). The system consisted of an adaptation of the heart transplantation method of Fulmer et al. (41) for the study of heart tissue destruction. The method involves excision of the heart from a newborn mouse and its implantation into the dorsal base of the pinna of one ear of a syngeneic recipient. The implant is subcutaneous and, under normal conditions, starts contractile activity 7 to 10 days after transplantation. Heart rejection is evaluated in terms of the disintegration of the implant, which is accompanied by perivascular, interstitial, and perimyocytic mixed inflammatory cell infiltrates (mononuclear cells and neutrophils) and myocyte necrosis. BALB/c mice chronically infected with either the Y or Colombian \(T. cruzi\) isolates (both virulent for mice) were able to reject their grafted hearts within 20 days posttransplantation (102). The syngeneic grafts were not rejected by normal mice or by animals hyperimmunized with \(T. cruzi\). Passive transfer of anti-C4D but not anti-CD8 antibodies 2 weeks before heart implantation prevented transplant rejection, indicating that graft tissue destruction was dependent upon CD4\(^+\)-lympho-
cyte function. Rejection capacity was not reestablished after interruption of the anti-CD4 antibody treatment and CD4+ cell reconstitution. This was evidenced by the finding that hearts grafted into the ears of mice that had been given passive anti-CD4 antibody 2 months earlier remained intact and free of inflammatory infiltrate for the 100-day observation period. Using an alternative approach, Ribeiro dos Santos et al. (102) injected unfractionated T cells, CD4+ cells, or CD8+ cells from chronically infected mice adjacent to newborn hearts that had been grafted into normal syngeneic mice. Complete graft destruction was seen within 4 days postinjection in the animals receiving unfractionated T cells or CD4+ cells but not in those given CD8+ cells. Controls injected with similar cell preparations from either normal mice or mice hyperimmunized with avirulent T. cruzi did not reject the transplants. Completing these observations was the finding of in vitro proliferative responses by unfractionated T or CD4+ cells from chronically infected mice but not by the CD8+ cell population upon stimulation with a myocardial antigen preparation. Anti-CD4 antibody inhibited lymphoproliferation in these assays. From these results, the authors concluded that cellular autoimmunity had to be the major mechanism involved in rejection of the grafted syngeneic hearts, tacitly implying that the native hearts of chronically infected mice might be damaged by a similar mechanism. If there was a key piece of information that was missing in this work (102), it was data about the presence of T. cruzi in the rejected hearts. Also, if there is an important implication from the observation that heart transplant rejection failed to occur in mice hyperimmunized with T. cruzi, it was that an immune response to the parasite could not affect the grafted hearts. More light on the role of T. cruzi on the rejection was to be shed by a paper by Tarleton et al. (121) showing that it was not just closely linked to but was actually very heavily dependent on graft invasion by T. cruzi.

Tarleton’s group (121) also used the heterotopic syngeneic newborn-heart model system in various combinations of mouse strains (C57BL/6 and C3H/HeSnJ) and T. cruzi strains (Brazil and Y isolates and the Sylvio X10/4 clone). In stark contrast to the results of Ribeiro dos Santos et al. (102), Tarleton’s group was able to demonstrate that in mice with cCHD, the grafts survived for prolonged periods, did not show signs of either autoimmune rejection or pathologic inflammatory cell infiltration, and yielded negative results for the presence of T. cruzi (tested by a highly sensitive and specific in situ PCR test). The lack of rejection was not a fortuitous event characteristic of a particular combination of mouse strain and T. cruzi isolate, since the vast majority of C3H/HeSnJ mice chronically infected with Sylvio X10/4 T. cruzi and C57BL/6J mice chronically infected with Brazil or Y organisms also failed to reject their grafts. The occasional rejections seen in both normal and chronically infected mice were traced back to contamination or graft damage during excision. Because infection of C3H/HeSnJ mice with the Sylvio X10/4 clone of T. cruzi causes an intense heart tissue inflammation and injury with very low, generally undetectable parasitemia, not unlike what is seen in human cases of cCHD (100, 101), it represented a useful model in which the chances of graft infection were apparently greatly diminished. This was a major difference from the model used by Ribeiro dos Santos et al. (102), who used the Y and Columbian T. cruzi isolates, generally known to be quite invasive (100, 101).

When Tarleton et al. (121) transplanted newborn hearts to normal mice on the day the latter were infected or during the acute phase (on day 30 p.i.), the grafts sustained heavy functional and physical damage, displaying the same pattern of inflammation as seen in the native hearts. These results provided clear evidence for access of both circulating parasites and inflammatory cells to the transplants under these conditions. Straight injection of T. cruzi into grafts that had been well established in chronically infected mice caused electrocardiogram anomalies and mechanical failure in all 21 mice tested in this manner. All monitored immunological parameters (expression of cytokines, major histocompatibility complex, and adhesion molecules, and infiltrating inflammatory cell types, including a predominance of CD8+ cells) were similar in the grafted and native hearts of these animals (121). Injection of parasites into hearts transplanted into normal, noninfected mice also caused loss of function, whereas injection of killed T. cruzi induced only a moderate inflammation with loss of function of only one of three heart grafts. Taken together, these results indicate that in relation to T. cruzi infection, damage to transplanted hearts requires tissue invasion by viable organisms and not merely the presence or immunologic recognition of parasite antigens. In this context, the involvement of CD4+ lymphocytes in rejection of grafted hearts described in the work of Ribeiro dos Santos et al. (102) would appear to have been a response to tissue antigens exposed after parasitemia-inflicted injury.

Containment of parasite dissemination to the heart grafts was impressive in the study by Tarleton et al. (121), since T. cruzi kinetoplastic DNA was still undetectable 8 months after implantation into the ears of chronically infected mice. This may have been due to effective immunological control. Clear examples of such control include the lack of reestablishment of acute-disease symptoms or resurgence of parasitemia in chronically infected mammalian hosts injected with fairly high doses of virulent T. cruzi (reviewed in reference 13) and the exacerbation of disease with reappearance of parasites in the blood shortly after administration of an immunosuppres-
tant, demonstrated in mice (15, 21, 49) and humans (75, 90, 112). If immunologic control is what prevented the Brazil, Y, and Sylvio X10/4 organisms from straying into the heart grafts inserted into mice of two different strains (121), the question arises why this was not the case for the Y and Columbian parasites in the study of chronically infected BALB/c mice (102).

Silva-Barbosa et al. (111) reported the formation of a thick laminin matrix during rejection of grafted hearts in the BALB/c-Columbian T. cruzi model system, with donor-derived CD4+ lymphocytes accumulating in the laminin-rich areas. Antibodies to laminin or to the VLA-6 laminin receptor were found to inhibit the rejection of the implanted hearts in mice with cCHD, suggesting that recruitment of the implicated CD4+ cells or their function was somehow regulated by a mechanism involving laminin–VLA-6 interaction. The interpretation, meaning, and implications of these observations should become clearer once it is established to what extent the inflammatory reaction is due to reactivity with intact heart tissue as opposed to tissue damaged by the parasite.

Gattass et al. (42) compared the anti-heart cellular reactivity present in lymph nodes from normal mice immunized with T. cruzi epimastigote and trypomastigote antigens with that in lymph nodes from chronically infected mice boosted with T. cruzi trypomastigote antigens. Specific autoimmune reactivity to self heart antigen was undetectable in the immunized mice, whereas a small but detectable level of anti-heart reactivity was found in a proportion of the chronically infected animals. These particular results show that a possible cross-reactivity between heart tissue antigens and those of epimastigotes and trypomastigotes is unlikely and suggest that the anti-heart reactivity seen in some mice may occur in response
to antigens exposed following tissue damage resulting from infection.

Bottasso et al. (11) carried out a simple and interesting experiment to establish the effect of adult thymectomy on the development of myocardial lesions in chronically infected rats. Three months after infection, the proportions of thymectomized and sham-thymectomized rats presenting chronic focal myocarditis were found to be comparable but the foci of myocarditis were larger in the former group. Although adult thymectomy is not as drastic as neonatal thymectomy in depleting T-cell compartments, it does, nevertheless, lead to generally decreased T-cell function, in spite of which myocardial lesions developed.

Mosca and Plaja (95) used a lymphoproliferation assay to compare cellular reactivity for heart antigens in 27 patients with cCHD and 52 patients with aCD. The proportions of reactive patients, 28.6% and 25.0%, respectively, were found to be statistically comparable and not supportive of a correlation between cellular antiheart reactivity and the extent of heart disease. However, because no antiheart reactivity was seen in the control group consisting of healthy individuals and patients with nonchagasic heart conditions, there was an apparent link between the presence of the antiheart antibodies and CD in general.

This section cannot be closed without making reference to practical information about the impact of cCHD on transplanted human hearts. A paper appeared in 1995 (6) describing two recipients of heart transplants monitored for 12 and 72 months after surgery. Neither of them presented signs of myocarditis during the observation period. It should be noted that these patients had been subjected to immunosuppressive therapy which would have curtailed T-cell function to a certain extent but would not have altered any effect mediated by preexisting antibodies. Another study, in 1996, described the follow-up of 10 heart transplant recipients for 73 to 124 months (29). At the time the paper was submitted for publication, seven heart recipients were alive, fitting into functional class I defined by the New York Heart Association guidelines. Interestingly, the rate of heart rejection was lower among the chagasic patients than among age- and sex-matched control patients. Moreover, when rejection occurred it was less severe in the chagasic patients. Examination of myocardial biopsy specimens revealed no signs of disease recurrence in the transplanted hearts. The patients in this group had also received immunosuppressants and chemotherapy to control the parasite. A third publication, in 1996, reported on the follow-up of 22 patients who had undergone orthotopic heart transplantation (7). Nine of these patients had received hearts between 1985 and 1991; the remaining 13 had been operated on between 1991 and 1995. The two groups had received similar postoperative treatments, except for the administration of smaller doses of cyclosporin A to the second group in an attempt to minimize the resurgence of parasitemia. Reactivation of CD occurred in 5 of the initial 9 patients but in only 1 of the next 13 patients. Neoplasia developed in 5 of the initial patients but in only 1 of the next 13 and contributed to death in 3 of these patients. No information about the presence of inflammatory infiltration in biopsy specimens was provided, but the surviving patients, particularly those in the second group, were deemed to have progressed satisfactorily after transplantation, at least from a clinical standpoint. A review of these communications raises the following question: how is it that an immune system that, according to the autoimmunity hypothesis, should have contributed in large measure to the deterioration of the natural hearts spared a large proportion of the transplanted hearts for as long as 10 years? Regardless of dosage, the immunosuppressant treatment is unlikely to have kept autoimmunity effectively in check for such long periods. It is also difficult to assess to what extent T. cruzi, whose presence is so often and readily evidenced after cyclosporin A treatment, was responsible for the cases of CD reactivation, but the higher incidence of resurgent disease among patients in the first group (5 of 9) than in the second group (1 out of 13) suggests that higher doses of the immunosuppressant might have had something to do with it.

ARE PARASITES REALLY ABSENT AT CHRONIC CHAGASIC TISSUE LESIONS?

It is too rare a paper on autoimmunity and CD that does not underscore the fact that the parasite is often absent at tissue lesions. This absence has been construed by a number of investigators as being indicative of a mechanism of pathogenesis independent of parasite invasion and destruction of host tissues. This assumption, based on negative histologic examinations, raises an important question: is microscopic detection of parasites at chronic tissue lesions reliable? It has been amply documented that mammalian forms of T. cruzi are highly sensitive to immunological mechanisms of cell destruction such as, for example, antibody-mediated complement-dependent lysis (18, 28, 60, 72) and phagocytosis by monocytes, macrophages, eosinophils, and neutrophils (67, 69, 98, 130–133). The molecules and cells involved in these processes are plentiful in the body fluids and tissues of hosts with cCHD, making it sensible to expect efficient containment of T. cruzi. This expectation has a massive evidentiary basis not only in the resurgence of parasitemia in animals with cCHD and patients receiving immunosuppressive therapy for various reasons (15, 21, 49, 75, 90, 112) but also numerous studies on host resistance against T. cruzi infection (16, 17, 66, 68, 71, 72). Viewed against this background, searching for intact parasites in damaged tissues of chronically infected hosts would seem to be a difficult undertaking, as has proven to be the case so often. However, in the majority of instances in which evidence for past T. cruzi infection has been sought at lesion sites presenting inflammatory cell infiltration by using sensitive, indirect methods, it has been readily found. This has been the case when the PCR technique for parasite DNA amplification has been used (12, 52, 125, 126). Although less sensitive than PCR, immunohistochemistry has also been used successfully to track down vestiges of parasites at tissue lesions of patients with cCHD. Therefore, Bellotti et al. (5) used serum from a rabbit hyperimmunized with T. cruzi to probe regions of endomyocardial and surgical biopsy specimens and found evidence of T. cruzi antigen in 69% of 16 chronically infected patients and parasite antigen detection in 71% of 14 regions with moderate or severe myocarditis but in only 3 (16.6%) of 18 regions with mild or absent myocarditis. It should also be noted that although it is widely believed that parasites are rare or absent in the body fluids of chronically infected hosts, the PCR method has detected T. cruzi DNA in their blood or serum samples without major difficulty (53, 134).

NONAUTOIMMUNE HYPOTHESES FOR THE PRODUCTION OF CHRONIC CHAGASIC TISSUE LESIONS

Tissue Damage Caused by T. cruzi

An indisputable mechanism of cell and tissue destruction in CD is that which results from T. cruzi invasion. Intracellular replication of the parasite leads to cell bursting. Released
organisms can then penetrate neighboring cells or be swept into the circulation and carried to other organs. Although few investigators would challenge this mechanism, some believe that it is not sufficient to justify the damage that occurs in various tissues, particularly when intact parasites or recognizable parasite debris are often not detectable at chronic tissue lesions by microscopic examination.

**Neurogenic Hypothesis**

Although it is among the oldest notions attempting to explain cardiac and digestive (e.g., megacolon and megasopha-gus) chagasic lesions, the neurogenic hypothesis continues to be debated as much as ever. Enlargement and dilatation of an organ are believed to be the consequence of selective parasite destruction of postganglionic neurons charged with autonomic control of the affected organ. Destruction of parasympathetic innervation would lead to uncompensated sympathomimetic dominance, rendering cardiac cells hypersensitive to catecholamine effects. However, since cardiac denervation is also seen in non- chagasic heart diseases, a clear link with *T. cruzi* has not been established and some investigators believe that nerve cell damage may be a consequence rather than the cause of cCHD. A succinct but incisive article on this controversy was published by Davila et al. in 1989 (27).

**Microvascular Disturbance Hypothesis**

It has been postulated that alterations in the coronary microcirculation, leading to ischemia, could result in heart tissue damage. The evidence supporting this view is largely experimental and includes the observation of hypoperfusion in regions with normal or mildly impaired wall motion, suggesting that microcirculatory restriction may precede and result in heart tissue damage (81, 104, 105, 120). Collagen-associated platelet-fibrin aggregates subjacent to damaged endothelial tissue have been seen in the thoracic and abdominal aorta from acutely infected rats (105) and might conceivably occur in heart blood vessels. In the general population of chagasic patients, epicardial coronary arteries are usually clear of obstruction. However, atherosclerotic coronary artery disease has been seen in patients after an episode of acute myocardial infarction (81). Because the patterns of atherosclerotic coronary artery disease in these patients are similar to those seen in the nonchagasic study population (81), it is not clear what link, if any, exists between *T. cruzi* infection and these changes. The precise mechanism restricting circulation in the heart microvasculature has not been defined.

**Continual Granulocytic Cell Activation Hypothesis**

The notion that eosinophils and possibly neutrophils (both minority components of chagasic inflammatory cell infiltrates) can lead to slow development of tissue damage emerged with the observation that the number of eosinophils in these infiltrates, although small, correlates with the severity of human heart chagasic lesions, reaching maximal levels at sites having myocarditis with degeneration and necrosis (91). Immunohis-tochemical tests have demonstrated the presence of activated eosinophils (i.e., in the secretory stage) and deposits of the highly toxic eosinophil granule components at those sites (92). Moreover, in vitro studies have shown that coculture of human eosinophils and *T. cruzi* amastigotes results in the release of molecules causing lysis and detachment of bystander heart myoblasts (93). Neither the eosinophils nor the amastigotes could reproduce this effect by themselves. The in vitro bystander cell damage was inhibited by reagents that specifically neu-tralize or inhibit the toxicity of eosinophil granule components. Similar results were seen when neutrophils substituted for eosinophils in similar experiments and inhibitors of neutrophil granule components were used (93). The possibility was raised that granulocytes initially recruited to clear tissue debris and parasites could degranulate (as they do when they take up microorganisms or particles), causing further tissue damage mediated by polycationic granule components. This new damage would necessitate the recruitment of additional granulocytes for clearance, and so on (65). Clearly, the initiator of such a vicious circle would be *T. cruzi* but, if this proposed model paralleled reality in host tissues, cell destruction could continue to occur after parasite removal.

It should be emphasized that there is no claim or evidence that any of these mechanisms, even if conclusively documented, would be exclusively responsible for tissue damage in CD.

**PERSPECTIVE**

There are reasons for the skepticism with which some investigators have received the autoimmunity hypothesis of pathogenesis in CD. One is that although several auto- or cross-reactive antibodies have been identified in chagasic patients or laboratory animals and some of these antibodies have been reported to cause some functional in vitro (10, 115) or in vivo (79) changes reminiscent of those seen in infected mammalian hosts, no convincing evidence exists for their ability to induce lesions as seen in infected patients. Attempts to cause nerve damage by direct endoneural injection of chagasic sera into normal mice have failed in this regard (107), and, as noted above, hyperimmunization with the recombinant *T. cruzi* protein K1-7 did not cause chagasic damage either (57). Moreover, anti-mouse cardiocyte antibodies capable of mediating antibody-dependent cellular cytotoxicity in vitro failed to cause in vivo lesions like those seen in chronically infected mice (74).

With regard to lymphocyte reactivities or cross-reactivities, some evidence has been reported for the occurrence of tissue lesions after transfer of sensitized T cells in murine model systems (25, 50, 102). However, some investigators have questioned these results because of reproducibility problems (76a), insufficient data (121), disagreement about whether *T. cruzi* has anything to do with lesion production in transfer experiments (121), or the fuzzy specificity of some injected T-cell clones (see “Cellular aspects of autoimmunity” above). In addition, some workers find it difficult to accept that results obtained with mice are readily applicable to human CD because of the marked differences in some aspects of the pathologic findings that develops in these hosts (103, 107). In fact, the lack of model systems that closely reproduce the various aspects of the pathologic findings associated with human CD has remained a problem.

The merits of the autoimmunity hypothesis for the pathogenesis of CD have been argued for and against for nearly a quarter century. In the meantime, and while good progress has been made in some geographical areas through persistent vector control efforts (30, 80, 94), large segments of the populations of the areas of endemic infection have continued to endure the consequences of CD because of the lack of effective chemotherapy or vaccination. The question in front of scientists and health authorities alike is whether we should continue to argue and confine all of our hopes to continued and permanent success of vector control measures or start actively pursuing the alternatives.

**REFERENCES**

1. Avila, J., M. Rojas, and H. Towsin. 1988. Serological activity against galactosyl α(1-3)galactose in sera from patients with several kinetoplastida in-


