Pathophysiologica Insights Into the Cardiomyopathy of Chagas’ Disease

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Chagas’ disease, or American trypanosomiasis, is caused by the hemoflagellate Trypanosoma cruzi and is the leading cause of cardiac disease in South and Central America. The insect vector occupied the attention of Charles Darwin during a voyage to Argentina in 1888, when he described in detail its peculiar nocturnal blood-sucking habits. Indeed, Darwin’s own subsequent clinical symptoms have been ascribed to chronic Chagas’ cardiomyopathy (CCM), presumably acquired as a consequence of exposure to the Reduviida insect.1 Twenty years after Darwin’s voyage, in a remarkable piece of investigative analysis, Carlos Chagas described the salient features of the disease, identified the causative parasite, and characterized its life cycle.2 Chagas’ disease is an extraordinarily complex process with a poorly understood pathophysiologic. In this review, we focus on more recent studies that permit the formulation of new hypotheses that explain the pathology and clinical course of myocardial involvement in chagasic cardiomyopathy. Comprehensive reviews of earlier studies are available.3,4

Details of the clinical course of Chagas’ disease and the life cycle of the T. cruzi can be found in several texts.5,6 Only the salient features are summarized here. The clinical course of Chagas’ disease includes an acute and a chronic phase, which are separated by an indeterminate period during which the patient is relatively asymptomatic. The acute disease, which follows parasite infection, occurs along with parasitemia. Although no tissue is spared from infection, striated muscle and the nervous system, especially autonomic ganglia, are most severely affected. The acute disease is characterized by an active infection, inflammation, and myocardial damage. Clinical symptoms in conjunction with the appearance of parasitemia may include fever, malaise, lymphadenopathy, hepatosplenomegaly, vomiting, diarrhea, and meningeal irritation.5 Symptoms spontaneously resolve within 3–4 months in more than 85% of infected individuals. In a small minority, an acute infective myocarditis may occur, which is characterized by tachycardia, prolongation of the P–R interval, low-voltage QRS complexes, nonspecific T wave changes, cardiomegaly, and heart failure. A significant minority of individuals afflicted with acute myocarditis will die. In necropsy studies, abundant amastigote forms of the parasite are found in cardiac, skeletal, and smooth muscle and glial cells. Tissue pathology appears to be directly correlated with the presence of the parasite and may follow as a consequence of direct parasite-associated cytolysis, including a cytotoxic role for eosinophils recruited by parasites7 or amastigote-associated interference with host cell metabolism without cytolysis. In patients surviving acute infection, a period of clinical quiescence ensues that is remarkable for seropositivity in the absence of both obvious parasitemia and clinical symptomatology. However, a majority of otherwise similar seropositive individuals have no history of an antecedent acute illness. In a small proportion of seropositive individuals, clinically apparent disease (chronic Chagas’ disease) appears approximately 10–50 years after the initial infection. Progression from the acute to the chronic form of Chagas’ disease may coincide with clearance of the parasite from the bloodstream and infected tissues.

Chronic Chagas’ Cardiomyopathy in Human Subjects

Myocardial involvement in chronic Chagas’ disease includes arrhythmias, ventricular ectopy, conduction defects, cardiomegaly, congestive heart failure, thromboembolic phenomena, or sudden death.8 Alternatively, autonomic neuronal dysfunction may predominate with resulting “megasyndromes” that usually involve the large bowel or esophagus but possibly any other tubular organ. Whether manifest as cardiac syndromes, megasyndromes, or a combination, a noteworthy clinical aspect of CCM is the paucity of parasites in the myocardium. Indeed, hemoculture and xenodiagnosis are the only techniques available to demonstrate the presence of the very few circulating trypomastigotes. In the congestive cardiomyopathic form of CCM, necropsy studies

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reveal marked dilatation of all four chambers. In contrast, in patients dying of sudden death, the heart may be normal or only slightly increased in weight. Characteristics common to both conditions include an absence of significant large- or small-vessel coronary artery disease and apical thinning of the myocardium or aneurysm formation with or without thrombosis, that is frequently although not exclusively found in the left ventricle.

In CCM, depending on the time of examination, micropathology reveals focal but widespread areas of cellular infiltrates comprising plasma cells, eosinophils, mast cells, and macrophages. Focal and diffuse areas of myocellular hypertrophy may be seen with or without inflammatory infiltrates. In other areas, extensive fibrosis occurs, replacing previously damaged myocardial tissue. All areas of the heart, including the conduction pathways, may be involved. Microvasculature involvement, manifest by basement membrane thickening, has recently been demonstrated. In contrast to the situation observed in acute disease, even when present, the rare parasites bear little relation to myocardial pathology. Thus, postmortem examination of the heart in CCM is remarkable for the focality of pathology as well as the paucity of parasites. In contrast, in the hearts of patients who die from sudden death, the most remarkable pathology appears to be that of the conduction system, with inflammatory infiltrates along the bundle of His, atrioventricular node, and associated pathways.

Many controversies characterize theories regarding the pathogenesis of CCM. One theory attributes an autoimmune basis to CCM, whereas another implicates a primary role for autonomic nervous dysfunction. However, more recent clinical and animal studies suggest an alternative view of CCM, namely, a disease characterized by a progressive, focal, and relentless course, in which cardiac manifestations occur at an earlier stage than once appreciated. The implication of these findings is that the infected individual may remain clinically asymptomatic until a critical mass of impaired tissue has accumulated, resulting in clinical symptomatology. We propose, as have others, that the consequences of infection converge on the microvasculature of the myocardium, resulting in focal areas of hypoperfusion. In the remainder of this review, we present clinical and experimental data in support of this hypothesis.

**Contraction Abnormalities in Chagas’ Cardiomyopathy**

Cineangiographic studies first suggested left ventricular abnormalities in otherwise asymptomatic seropositive individuals. Forty-one percent of seropositive individuals with no clinical, electrocardiographic (ECG), or radiologic evidence of heart disease (group 1) demonstrated apical or anterior apical asynergy, whereas 98% of seropositive individuals with only abnormal ECG findings (group 2) demonstrated extensive asynergy, left ventricular dilatation, decreased distensibility, and depressed contractility. In those with both ECG abnormalities and heart failure (group 3), patients were older and the left ventricular chamber was greatly dilated, hypokinetic with large apical aneurysms (40%) and thrombosis within the left ventricle (20%). The study established that a substantial proportion of clinically asymptomatic individuals (group 1) had myocardial dysfunction, and the stratification of patients with symptomatology suggested that duration of disease correlated with compromise in myocardial function as well as with apical aneurysm. In contrast, an earlier autopsy study of more than 5,000 chagasic patients demonstrated no relation between age, duration of disease, and the apical aneurysm. It must be noted that establishing the progressive nature of CCM requires a longitudinal study in which a set of patients are individually followed throughout the duration of disease, with frequent myocardial biopsies. Such a clinical study has not been reported. Hence, interpretation of stratification studies within a longitudinal context is necessarily limited. Nonetheless, in lieu of prospective studies, several more recent reports continue to support the progressive nature of this disease.

Using similar patient stratification, with the exception of patients in group 3 being younger, it was found that 30% of individuals in group 1 had completely normal biopsies, whereas the incidence of abnormal biopsies increased dramatically in groups 2 and 3. Of the histopathological parameters examined, myocardial hypertrophy and fibrosis correlated best with the severity of cardiac symptoms such as cardiomegaly and congestive heart failure. The incidence of inflammation did not follow patient stratification. The histological findings in clinically compromised seropositive individuals were qualitatively similar to those observed in necropsy studies, differing only in magnitude and extent and consistent with a progressive course of the disease.

More recently, Guerra et al stratified patients in group 1 as group 1A (asymptomatic, normal ECG findings, and no hemodynamic or cineangiographic findings of heart disease) or group 1B (asymptomatic, normal ECG findings, but evidence of segmental myocardial damage demonstrated by cineangiography). In contrast to a previous study, a somewhat higher incidence (60%) of microscopic abnormalities was seen in group 1A, whereas alterations in the contractile system were more prominent in group 1B. No cellular infiltrates were found in biopsies from either group 1A or 1B patients. Fibrosis and inflammatory infiltrates were observed in group 2 and increased in group 3. Abnormalities in endothelial cells and capillaries were noted, consistent with microvascular involvement. More recently, biopsy studies of group 3 chagasic patients revealed extensive basement membrane thickening involving a majority of myocytes and capillaries, again consistent with microvascular disease.
Echocardiographic studies are also consistent with a progressive course for CCM. A study of group 1 and 2 patients revealed significant abnormalities in diastolic and systolic function, including delayed closure of the aortic valve, delayed mitral valve opening, and prolonged isovolemic relaxation. Abnormalities during isovolemic contraction and the early relaxation phase were ascribed to asynchronous onset of contraction, which is similar to patients with more conventional ischemic heart disease.

**Electrocardiographic and Electrophysiological Abnormalities in Chagas’ Cardiomyopathy**

The progressive incidence of ECG abnormalities, notably right bundle branch block (RBBB) and left anterior hemiblock (LAHB), in CCM was recently demonstrated in a prospective study of 1,051 persons in an endemic area of Brazil. The study also established that seropositive individuals with RBBB, especially in combination with anterior fascicular block or ventricular extrasystoles, had significantly higher mortality rates than seropositive persons with normal electrocardiograms.

Prospective studies comparing pathological lesions in conduction pathways with electrophysiological abnormalities have not been reported. However, electrophysiological alterations occur in chagasic patients even in the absence of detectable ECG changes. Sinus node dysfunction was demonstrated in 18% of 44 otherwise asymptomatic seropositive individuals with normal electrocardiograms. During incremental or programmed atrial pacing, 14 patients demonstrated atioventricular nodal conduction disturbances. It is of interest to note that 41% of patients demonstrated repetitive ventricular responses to single ventricular premature beats, showing an early predisposition to ventricular arrhythmias in this asymptomatic group, consistent with the clinical impression that CCM patients are prone to ventricular arrhythmias. Unfortunately, in these as well as other studies, the data do not permit a prediction of which patients with the earliest electrophysiological abnormalities will ultimately progress to CCM.

**Autonomic Abnormalities in CCM**

The early appearance of ECG and electrophysiological abnormalities as well as the prominence of autonomic nervous system dysfunction have led many investigators to propose that CCM is initiated by autonomic neuronal damage to the heart. Extreme variability in the density of myocardial vagal ganglia of chagasic hearts has been reported, in part reflecting the limits of the histochemical analysis and rendering the results inconclusive at this time. Hence, in the absence of a sensitive method of analysis, the precise relation between aganglionosis and CCM remains to be determined. No studies of ganglion populations have conclusively distinguished chagasic patients who die suddenly with a grossly normal heart from those with CCM. Only in tissue obtained from the latter individuals have there been consistent reductions in the number of vagal cardiac neurons. Moreover, vagal denervation has been observed in dilated cardiomyopathies of other etiologies, so its association with advanced Chagas’ disease does not necessarily imply a primary role.

More recent data on autonomic dysfunction in Chagas’ disease fail to implicate its causative role in CCM. One such study used heart rate response to exercise. The earliest increase in heart rate after exercise occurs as a consequence of the withdrawal of inhibitory parasympathetic influence. In an analysis of group 1 and 2 seropositive individuals, a marked decrease in the rate of heart rate increase was seen during the initial 10 seconds of dynamic exercise. The results were consistent with a compromise in the influence of vagus tone on sinus node function. In contrast, no alterations were observed during the longer periods of workload, suggesting that the sympathetic component of the heart rate response to exercise was unchanged. More important, the compromise in parasympathetic activity occurred equally in all seropositive chagasic patients independent of cardiac dysfunction, as have been reported in other studies of parasympathetic dysfunction in seropositive individuals. Abnormalities in baroreflex-mediated responses are known to be characteristic of congestive heart failure of any etiology. Nonetheless, the fact that alterations in parasympathetic function can be demonstrated in seropositive chagasic patients before the onset of clinically significant myocardial dysfunction may prove to be a unique aspect of the disease. Abnormalities in the sympathetic component of the autonomic nervous system in chagasic patients, as reflected in the apparent reduction in levels of plasma norepinephrine, were also found, in contrast to increased levels in nonchagasic cardiomyopathic patients. Furthermore, in addition to their lower resting heart rate, only chagasic patients had a reduction in diastolic blood pressure in the upright position.

The capacitance of the coronary circulation in excised human hearts obtained from chagasic patients was found to be markedly increased compared with hearts obtained from patients who died from cardiomyopathic disease of nonchagasic etiology. The unique increase in coronary capacitance associated with CCM was ascribed to the loss of parasympathetic innervation leading to a predominant dilating effect of the sympathetic nervous system on coronary arteries. However, coronary artery tone in vivo reflects a dynamic relation between sympathetic, parasympathetic, and a host of local unknown factors. An isolated, nonperfused heart would not be expected to demonstrate this dynamic interplay of multiple inputs; hence, it is difficult to ascribe the increase in coronary artery capacitance solely to a loss of parasympathetic input. Moreover, coronary angiography, compared with postmortem studies, has not demonstrated enhanced patency of coronary arteries in chagasic cardiomyopathy.
In summary, recent clinical studies of CCM suggest a progressive, focal compromise in the integrity of both the myocardium and the conduction system. The association of RBBB, LAHB, and left ventricular apical aneurysm suggests that focal pathology may be regionally selective. The pathology appears to demonstrate a progression of ventricular dysfunction due to myocellular necrosis, fibrotic replacement, and reactive hypertrophy of the unaffected cells. Although inflammatory cells are usually associated with myocytolysis, their variable presence and poor correlation in biopsy studies of chagasic patients place their potential pathological role in question at this time. Inflammatory cells may contribute to a state of microvascular hypoperfusion by secretion of cytokines and other factors known to influence platelets and endothelial cells. At this time, no pathophysiological mechanism can be assigned to the associated ventricular aneurysm, which seems to be relatively unique, particularly considering the presence of patent coronary arteries. In addition, the autonomic dysfunction in Chagas’ patients may represent yet another unique aspect of the disease.

Animal Models of Chagas’ Cardiomyopathy

Experimental models of Chagas’ disease include the mouse, rabbit, dog, and monkey and demonstrate many but not all features consistent with both acute and chronic disease. Host factors, parasite strain, and route and number of organisms influence survival and progression from acute symptoms to chronic disease. The experimental models of Chagas’ disease share myocardial pathology analogous to that observed in human subjects and thus are useful models in which to study issues related to the pathophysiology of chagasic cardiomyopathy, which are addressed below.

Altered Immune Responses in Chagas’ Disease and Role of Autoimmunity

The pathology of CCM in the absence of parasites inspired the concept that CCM is an example of autoimmune myocarditis. An additional observation was that although almost all tissues are initially infected by the parasite, clinical disease was localized to myocardial and skeletal muscle and the autonomic nervous system. Several excellent reviews in favor and against the autoimmune hypothesis have recently appeared. The autoimmune-type antibodies first observed in chagasic sera have now been identified in sera of patients with other infectious and noninfectious diseases without cardiac involvement. In addition, the demonstration of immune deposits in the targeted tissues has not been universal. More recent studies in favor of autoimmunity in CCM use uninfected animals inoculated with components of parasite preparations or sensitized T lymphocytes from previously infected syngeneic animals. Interpretation of the former studies is complicated by observations that all forms of the parasite have been grown in media containing host antigenic material or that the parasites themselves retain host cell tissue antigens when obtained directly from their animal source. Accordingly, determining whether parasite-specific antigens or host contaminants are responsible for an immune-type myocarditis is difficult. The presence of a contaminating host antigen on T. cruzi membrane preparations has been circumvented by the use of adoptive transfer studies, which use activated lymphocytes obtained from infected animals that are inoculated into syngeneic recipients. In this manner, a chagasic-type myocarditis has been reported in mice and rabbits in the absence of humoral evidence for infection. However, the reports have been subject to criticism, and it remains to be determined whether cell-mediated pathology can be demonstrated by other investigators.

Defining the role of autoimmunity in the expression of Chagas’ disease will continue to be extremely complicated, sharing similar difficulties encountered in other forms of myocarditis (i.e., Does primary myocardial injury lead to release of modified tissue antigens, triggering an immune response in the host with formation of antiheart antibodies?). The application of pharmacological agents to modify the host’s immune response as well as molecular biological approaches to establish the presence of cross-reacting epitopes will yield a more definitive answer. We believe that immune mechanisms, not related to autoimmunity per se, may play a significant role in the ultimate expression of the disease.

Contribution of Parasites to the Expression of Disease

If CCM can occur after adoptive transfer experiments or vaccination with parasite material, then the continued presence of the parasites may not be necessary for disease. The implications of such a finding are of great importance with regard to the development of a vaccine. Thus, vaccination may protect the host against acute infection, but the associated immune response may eventually result in CCM. Nonetheless, despite the difficulty in finding parasites in tissues from chronically infected individuals, no person dying of chronic Chagas’ disease has been antibody-negative. Indeed, it is important to recognize that many investigators believe the difference between acute and chronic infection is not necessarily the presence or absence of parasitemia or of parasites in the tissue, but rather the number of these parasites. Moreover, because necropsy studies rely on visual identification of the parasite in tissue specimens, the development of more sensitive and quantitative methods with parasite specific antisera or DNA probes may conclusively establish the precise relation between the presence of parasites and myocardial pathology in the chronic state. Studies of this nature are of critical importance to understanding the role of parasites in the expression of CCM.
Relationship Between Acute Complications of Infection and Development of Chronic Disease

Although healing of necrotic lesions in conducting tissue in acute disease has been reported in rats and dogs, there are data to suggest that pathological events occurring during the acute disease could conceivably contribute to the chronic disease. To substantiate this notion, it must be demonstrated that CCM exists in the absence of such early changes or that they persist throughout the duration of the asymptomatic period of the disease. However, several biochemical and structural abnormalities have been documented to occur during acute infection, and they could play an important role in the expression of chronic disease. Thus, early depletion of choline acetyltransferase in the heart was reported after 7 days of infection in C3H/HeJ mice, before parasitemia or tissue pathology was demonstrable.41 Choline acetyltransferase catalyzes the production of acetylcholine by cholinergic neurons, and its depletion could adversely affect parasympathetic nervous function. In this regard, it was recently reported that depolarization-induced acetylcholine overflow was significantly reduced in atria from T cruzi-infected rats with electrocardiographically characterized cardiopathy.42 Alternatively, hearts from A/J mice infected for 15 days with the Brazil strain revealed numerous areas of focal vascular constriction, microaneurysm formation, and dilatation and proliferation of microvessels.43 The lesions were identical to those found in the cardiomyopathic Syrian hamster.44 Moreover, the presence of inflammatory infiltrates established during acute disease could also negatively influence microvascular perfusion by virtue of their secretion of intermediaries in the synthesis of thromboxane and cytokines.25 In our laboratory, we demonstrated that platelets derived from acutely infected mice demonstrate a hyperreactive response to pharmacological stimulants of aggregation.45 It remains to be determined whether infection-associated alterations in choline acetyltransferase and microvasculature are retained throughout chronic infection.

Parasite-Associated Factors or Parasite-Induced Host Cell Factors Associated With Infection and the Expression of Cardiac Disease

Evidence in favor of a circulating chagasic “factor,” or “toxin,” first attracted attention when the disparity between the paucity of parasites and tissue pathology in chronic disease was noted. Such a putative toxin has eluded identification. However, infection-associated antibodies identified in chagasic sera have been demonstrated to express pharmacological activity and could function in the role of toxin, or factor. Thus, ouabain failed to evoke any significant positive contractile effect on atria beating in seropositive human chagasic sera and produced only toxic reactions.46 Sera obtained from normal individuals did not interfere with the classic dose-dependent positive inotropic effect associated with ouabain. Verapamil, with or without propranolol, overcame the inhibitory influence of chagasic sera on ouabain activity. This is particularly intriguing considering the reported palliative influence of verapamil on the expression of acute chagasic cardiomyopathy.47 Similarly, an IgG component was isolated from the sera of infected mice and humans, and early characterization suggested the presence of β-adrenergic–like activity, including adenylyl cyclase activity as well as positive inotropic activity inhibitable by β-antagonists.48–50

The possible toxin of T. cruzi infection may not necessarily circulate as a free moiety. In this regard, we have recently demonstrated an influence of infection on the extracellular matrix (ECM). The ECM is an insoluble mixture of molecules between and around cells.51 All matrices consist of collagen scaffolding, to which cells are bound by adhesion proteins. Associated with collagen and adhesion proteins there are polymers of sulfated polysaccharides, glycosaminoglycans, and proteoglycans. The ECM has been demonstrated to be critically important in developmental expression of cells and, more recently, in the cardiomyopathic state. In Swiss mice infected in a sublethal manner with T. cruzi, histopathological studies showed the presence of abundant collagen matrix with predominance of types III and IV collagen distinct from normal tissue.52 It remains to be determined whether these alterations are primary in the course of initial infection or are secondary to an earlier event.

Parasite-Related Changes in Cell Physiology in the Absence of Cytolysis

As previously noted, studies in cultured cells suggest that under currently undefined circumstances, intracellular amastigotes can exist for prolonged periods of time and alter host cell metabolism, thus interfering with the capacity to respond to external stimuli. For example, infection of L_E9 myoblasts inhibits production of messenger RNAs for muscle-specific β-actin and myosin heavy chain.53

Infection of endothelial cells was found to influence several important cell functions that may play a role in microvascular physiology. First, infection increased resting levels of cytosolic Ca2+54 and altered the kinetics and magnitude of response to agents known to stimulate the accumulation of cytosolic Ca2+, particularly bradykinin. In a complementary study, infection was found to have increased the basal activity of phospholipase C,55 the products of which include inositol triphosphate 3 (IP3), the presumed second messenger for release of cytosolic Ca2+. Common to both reports was the finding that this influence of infection on second-messenger generation was independent of the magnitude of infection. Second, in addition to influencing cytosolic second-messenger generation, infection also altered the synthetic capabilities of endothelial cells. Thus, sulfation of the glycosaminoglycan side chains of the heparan sulfate proteoglycans deposited in the ECM of endothelial cells was markedly increased after infection.56 Third, when uninfected endothelial cells were grown...
on the ECM deposited by infected endothelial cells, the uninfected endothelial cells produced a new ECM identical to the ECM deposited by the infected endothelial cells, suggesting that the signals responsible for directing the synthesis of this altered synthetic process resided in the ECM itself. Indeed, the infection-associated changes in Ca" and phospholipase C regulation were found to be independent of the number of infected cells present. Thus, the studies with ECM provide a plausible basis whereby a few infected cells can influence larger numbers of uninfected cells in a coordinated response. Finally, infected endothelial cells were shown to bind platelets more avidly than uninfected endothelial cells. This may lead to a compromise in microvascular perfusion. The enhanced platelet and endothelial cell interaction could also be reproduced in uninfected endothelial cells grown on the ECM of infected cells. Evidence for enhanced platelet-endothelial cell interactions during chronic infection have been reported.

Enzymatic activities of the parasite per se may influence host cell function without causing cytolysis. Thus, trypomastigotes elaborate a neuraminidase that removes sialic acid from the surfaces of cardiac and endothelial cells. In other studies, it has been shown that loss of sialic acid from cardiocytes alters intracellular Ca" homeostasis and ultimately myocardial function. The activity profile of the parasite-associated neuraminidase was shown to correlate with the myotropism of the parasite strain. Moreover, neuraminidase elaborated by a few circulating trypomastigotes in the chronic disease could destroy platelet prostacyclin receptors, causing increased platelet aggregation and thereby increasing the likelihood of decreased perfusion. Finally, sera obtained from cultures of infected (but not uninfected) fibroblasts, vascular smooth muscle cells, and myocardial cells were found to stimulate fibroblast DNA and protein synthesis as well as proliferation, suggesting a pathway by which fibrosis occurs in CCM. It remains to be determined whether this activity can be demonstrated in vivo.

Several important issues must be considered in the interpretation of a parasite influence on host cell or tissue function. Although infection-associated changes may be demonstrable in almost all tissues examined, only a discrete number of organ systems appear to be functionally compromised. Hence, although an infection-associated alteration in host enzyme or protein activity may be ubiquitous, its apparent contribution to organ dysfunction may be greater in a tissue in which adequate compensatory responses are lacking.

**Infection-Associated Events and Development of Microvascular Disease**

The evidence for microvascular disease in both acute and chronic Chagas' disease has been noted previously. In summary, clinical studies, including functional and pathological analyses, suggest focal, progressive myocardial involvement with extensive microvascular involvement. Similarly, microvascular pathology can be demonstrated in acute and chronic murine Chagas' disease. Finally, in vitro studies of infection demonstrate altered endothelial cell function, enhanced platelet reactivity, and enhanced platelet-endothelial cell interactions, which again are consistent with microvascular disease. We propose that microvascular disease in acute and CCM follows as a coordinated response of multiple infection-associated events, including 1) secondary immune responses; 2) infection of endothelial cells and cardiocytes that results in alterations in the synthetic patterns of the ECM and/or other structural proteins as well as biochemical alterations in signal transduction and other systems that influence cell response to physiological stimuli; 3) inflammatory response, which potentiates platelet, granulocyte, and endothelial cell interactions; and 4) parasite-associated neuraminidase, fibroblast stimulating, or as-yet-unidentified activity. In myocardial and neuronal cells, the slow cell turnover ensures that the changes will be prolonged. Individually, these infection-associated events are not sufficient to result in significant dysfunction. However, under unidentified conditions, these various "sublethal" events converge to precipitate a climate conducive to microvascular hypoperfusion, which results in profound changes in the surrounding myocardial cells. Specific tissues (cardiac) are targeted because the collective influence of these events is more damaging because of either the absence of compensatory mechanisms or the slow turnover of cells. The distinction between acute and chronic myocardial involvement may reflect the relative influence of each of these events over time.

Once established, damage to the microcirculation culminates in consequences that are essentially identical to those of other disease processes that involve the microcirculation of the myocardium. Ischemia results in myocytolysis, after which nonviable tissue is replaced by fibrosis and surrounding cells hypertrophy. The focal pathology results in diffuse dysynchronous behavior of the myocardium, conditions more favorable to those that resulted in the initial hypoperfusion, and greatly accelerates the frequency with which microvascular disease occurs.

**Biochemical Consequences of Microvascular Disease in Chagasic Cardiomyopathy**

An outstanding characteristic of myocardial dysfunction frequently observed in states of ischemia and microvascular hypoperfusion is an alteration in the β-adrenergic receptor complex, most often a decrease in adenylate cyclase activity in response to stimulation. It seems reasonable to suggest that if cardiac involvement in Chagas' disease includes microvascular pathology with a compromise in vascular perfusion, similar alterations in the myocardial β-adrenergic adenylate cyclase complex may be expected. This has been the case.
Six days after infection of mice, cardiac β-adrenergic receptor density was found to increase, whereas the affinity of the receptor for its agonist decreased.26 The $V_{\text{max}}$ (maximal rate of cyclic AMP generation) but not the apparent $K_{\text{act}}$ for isoproterenol-dependent adenylate cyclase activity decreased. At 21 days of infection, when peak parasitemia is evident, additional changes in the adenylate cyclase complex occurred. Specifically, adenylate cyclase activity that was stimulated or inhibited by agents that affect the guanine nucleotide–binding proteins $G_i$ or $G_o$ also decreased, whereas β-adrenergic receptor density increased. In addition, changes in cholera toxin–dependent ADP ribosylation of membranes were also associated with infection. At 6 and 21 days after infection, cholera toxin–dependent ADP ribosylation of protein $G_i$ was decreased. Moreover, the influence of NADP, a nonspecific inhibitor of NAD glycohydrolase activity that increases cholera toxin–dependent ADP ribosylation, was markedly increased when examined in myocardial membranes obtained from infected animals in contrast to its relatively small influence on membranes obtained from uninfected animals.

Considering the amelioration of the clinical expression of cardiomyopathy in the Syrian hamster by verapamil and the known palliative influence of the calcium channel blocker on microvascular perfusion in general,44 the effect of chronic verapamil administration on the consequences of $T. cruzi$ infection in mice was studied. Verapamil administration dramatically decreased myocardial inflammation and fibrosis, producing a decrease in mortality rates from 60% to 6% during a 70-day infection period.47 Verapamil treatment also increased myocardial isoproterenol-dependent adenylate cyclase activity in uninfected animals and “restored” myocardial β-adrenergic adenylate cyclase activity in infected animals to control levels. In myocardial membranes prepared from infected animals treated with verapamil, cholera toxin–dependent ADP ribosylation was increased to levels more than that determined in infected untreated animals. However, despite the influence of verapamil treatment on reversing infection-associated changes in morbidity, mortality, pathology, and β-adrenergic adenylate cyclase activity, the calcium channel blocker did not alter the influence of infection on cholera toxin–dependent ADP ribosylation in the presence of NADP.

We next sought to determine whether the observations on the β-adrenergic receptor complex in the acute murine model of chagasic cardiomyopathy related to a chronic experimental model of Chagas’ disease in dogs (S.A. Morris, S. Barr, L. Weiss, H.B. Tanowitz, M. Wittner, and J.P. Bilezikian; unpublished observations). To this end, pure-bred beagles of the same litter were infected with either an opossum-derived or a dog-derived strain of $T. cruzi$. Infection with the opossum-derived strain of $T. cruzi$ (symptomatic) resulted in parasitemia and obvious symptoms of Chagas’ disease, whereas infection with the dog-derived strain of $T. cruzi$ (asymptomatic) resulted in parasitemia but no symptoms of Chagas’ disease, hence providing an opportunity for distinguishing the effects of parasitism per se from those of parasite-associated myotropism.

Twenty days after infection with the symptomatic strain of $T. cruzi$, lengthening of the P-R interval occurred as well as episodes of transient multifocal ventricular premature contractions. After parasitism decreased to undetectable levels, animals infected with the symptomatic strain continued to demonstrate ventricular premature contractions and frequent runs of ventricular tachycardia. In animals infected with the symptomatic strain of $T. cruzi$ for 60 days, runs of ventricular tachycardia could be induced by sudden movement. In contrast, in uninfected animals and animals infected with the asymptomatic strain (dog strain) of $T. cruzi$, no obvious changes in ECG measurements occurred during the 230-day period of observation. In addition to these ECG changes in animals infected with the symptomatic strain, changes in echocardiographic analysis were observed. There was a progressive and substantial decrease in ejection fraction of animals infected with the symptomatic strain of $T. cruzi$ but little or no change in ejection fraction of uninfected animals or animals infected with the asymptomatic strain of $T. cruzi$. Despite echocardiographic and ECG evidence for compromised myocardial function in the infected symptomatic animals, at the time of necropsy (230 days), all animals were of similar weight, activity, and eating habits and had no evidence of clinically detectable heart failure.

Biochemical studies on the β-adrenergic receptor complex were performed on myocardial membranes of the animals killed at 230 days. In contrast to uninfected controls and dogs infected with the asymptomatic strain of $T. cruzi$, animals infected with the symptomatic strain of $T. cruzi$ demonstrated a substantial decrease in $V_{\text{max}}$ for isoproterenol-dependent adenylate cyclase activity measured alone or in the presence of Gpp(NH)p. Similarly, infected symptomatic animals demonstrated a marked decrease in sensitivity to isoproterenol measured alone or in the presence of Gpp(NH)p. When analysis of the β-adrenergic receptor density was performed, there was a slight decrease in β-receptor density in infected symptomatic dogs compared with uninfected dogs or dogs infected with the asymptomatic strain of $T. cruzi$. There was no difference in receptor affinity for the β-adrenergic ligand between uninfected and infected animals. Evidence that the coupling between the receptor and the guanine nucleotide–binding protein may be involved in symptomatically infected animals was obtained using agonist displacement curves. In infected symptomatic animals, displacement of 50% of the tracer β-adrenergic ligand required a twofold greater concentration of isoproterenol than that required in membranes obtained from uninfected animals or infected asymptomatic animals, suggesting a decreased sensitivity to the ago-
nist. Moreover, the shape of the isoproterenol displacement curve in membranes prepared from symptomatically infected animals was considerably steeper, consistent with loss of high-affinity coupled receptors. In the presence of Gpp(NH)p, agonist affinity substantially decreased in both uninfected and infected asymptomatic animals, although the magnitude of the rightward shift was considerably less in infected symptomatic animals.

To directly address G proteins in this cardiomyopathic model, we examined cholera toxin–dependent ADP ribosylation of cardiac membranes prepared from uninfected and infected dogs. In membranes prepared from symptomatic infected animals, there was a greater than 50% reduction in cholera toxin–dependent ADP ribosylation compared with levels observed in uninfected or infected asymptomatic animals. Similar to results obtained in vitro and in the acute murine model, the presence of NADP in infected symptomatic animals more than doubled cholera toxin–dependent ADP ribosylation compared with the insignificant changes in ADP ribosylation observed under otherwise identical reaction conditions in membranes from uninfected animals or asymptomatic infected animals.

In addition to the loss of high-affinity binding β-adrenergic receptor sites and the decrease in cholera toxin–dependent ADP ribosylation, examination of myocardial tissue in the chronic dog model revealed additional evidence for altered protein Gs. Western blots of myocardial membranes probed with rabbit antisera directed against specific amino acid sequences of protein Gs revealed substantial decreases in the levels of antibody binding in 36-, 42-, and 45-kDa regions compared with uninfected animals and asymptomatic infected animals. Although the identity of the 36-kDa band remains to be determined, the results are consistent with a quantitative reduction in the levels of the α-subunit of protein Gα. The observation that protein Gs levels may be decreased was also suggested in cys-reconstitution experiments. Cys-cells lack a functional protein Gα, but have β-adrenergic receptors and catalytic units. Detergent-extracted protein Gs obtained from dog heart membranes can reconstitute cys-mutant S49 lymphoma cells and thereby restore isoproterenol-dependent adenylate cyclase activity. In this assay, adenylate cyclase activity determined in cys-cells using donor protein Gs from infected symptomatic dogs was substantially less active than adenylate cyclase activity determined using donor protein Gs from uninfected dogs or asymptomatic infected dogs under otherwise identical reaction conditions. Collectively, the data suggest that the infection-associated decrease in isoproterenol-dependent adenylate cyclase activity appears to be a consequence of an alteration in both the quantity and quality of the Gs protein. Hence, as in the acute murine model of Chagas’ disease, we observed a striking compromise in the activity of the β-adrenergic receptor complex in the chronic model of CCM. That all of these changes in the β-adrenergic receptor complex occurred only in the symptomatic infected animal and not in the asymptomatic infected animal implies that parasitism alone does not result in these biochemical changes.

It must be noted that the acute murine model of chagasic cardiomyopathy, unlike the dog model of CCM, occurs in the setting of parasitemia and parasitic invasion of myocardial tissue. In this regard, the differences between the two models in β-adrenergic receptor density, affinity, and protein Gs amount may reflect unique responses of the animals to infection as well as differences in the magnitude of microvascular involvement in acute or chronic stages of infection. However, to more fully explore the direct consequences of parasite invasion of host tissue on the β-adrenergic receptor complex, a series of preliminary in vitro studies were performed. Cultured cardiocytes (S.A. Morris, D. Spray, H.B. Tanowitz, M. Wittner, and J.P. Bilezikian; unpublished observations) were infected with T. cruzi. In contrast to the situation obtained in vivo, infection of cultured cardiocytes altered neither β-adrenergic receptor density nor adenylate cyclase activity in response to isoproterenol, forskolin, or Gpp(NH)p. However, infection of cultured cardiocytes specifically enhanced the ability of NADP to magnify cholera toxin–dependent ADP ribosylation. Thus, the in vitro model could distinguish between the influence of infection on the β-adrenergic receptor complex and its effect on NAD-glycohydrolase activity. Collectively, our in vitro and in vivo studies are consistent with the hypothesis that infection with T. cruzi secondarily, presumably by virtue of alterations in microvascular perfusion, has profound effects on individual elements of the β-receptor complex at the level of the β-receptor itself and its associated guanine nucleotide–binding proteins. In contrast, the infection-associated influence on NAD-glycohydrolase activity may follow as a direct consequence of the parasite. Nonetheless, that these changes in the β-adrenergic receptor complex in Chagas’ cardiomyopathy are also observed in other models of ischemic cardiomyopathy strongly supports the notion that microvascular disease subserves these various states of myocardial dysfunction.

Summary

The evidence gained from both human and animal studies of chronic chagasic cardiomyopathy suggests that the disease occurs as a consequence of several discrete and progressive pathophysiological processes occurring after infection, the ultimate expression of which depends on a host of unidentified factors. Collectively, the infection-associated events compromise microvasculature function and result in hypoperfusion, with consequences indistinguishable from those observed in other, nonparasitological cardiomyopathic diseases secondary to hypoperfusion. Therefore, chronic chagasic cardiomyopathy...
may share similar pathophysiological abnormalities with other chronic congestive cardiomyopathic states.

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