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Trypanosoma cruzi: The Immunological Consequences of Infection

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Trypanosoma cruzi, the causative agent of Chagas' disease, infects an estimated 12 million people in Latin America and may induce cardiopathy and megaformation of the oesophagus and colon. During the early, acute stage of the infection, parasite-induced inflammatory infiltrates may cause transitory disease which terminates with the emergence of an immune response sufficient to reduce the parasite to insignificant levels. Even so, severe disease may develop many years after the original infection. It has been suggested that this might result from an autoimmune process triggered by the parasite and mediated either 1) by the adsorption of parasite antigens to host cells, thus rendering these cells susceptible to the host's own antiparasite immune response, or 2) via cross-reactive antigens shared by the host and parasite. In common with many parasitic diseases, there is an urgent need for studies on the T-cell response to T cruzi infection, as this might not only hold the key to the immunopathology but also serve as a means of clearing this lifelong infection which survives by sequestering into an intracellular site.

Key words: antigens, Chagas' disease, autoimmunity, immune response, Trypanosoma cruzi, immunopathogenesis, immunoprophylaxis, monoclonal antibodies

Trypanosoma cruzi is a protozoan parasite that may infect virtually any mammalian species. As a medical problem, this parasitic infection is confined to Latin America where it affects 10–12 million patients according to current WHO estimates. Sylvatic cycles of T cruzi transmission in wild animals do not overlap into human populations for ecological and social, rather than physiological or immunological, reasons. Thus, human invasion of virgin forest areas combined with the adoption of a peridomiciliary life-style by previously sylvatic mammals has brought additional transmission cycles whereby new parasite strains may be introduced into human communities. Classically, T cruzi infection follows prolonged contact with the triatomine insect vector and is associated with socioeconomic deprivation and rural areas. However, transmission in urban, socially developed populations is increasingly recognised following blood transfusion. There are very few drugs available for the treat-

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ment of this infection, and none is universally effective, particularly during the so-called chronic stage.

INFECTION AND DISEASE

The course of T cruzi infection in man and experimental animals follows that of any infectious agent susceptible to control via the immune response.

The parasite multiplies at the site of introduction as intracellular, nonmotile amastigotes and then transforms into the motile trypomastigote stage. Trypomastigotes circulate in the bloodstream and serve to disseminate the infection, to involve any organ system in the body. The acute stage, wherein trypomastigotes may be detected by microscopic examination of a blood sample, is terminated by the emergence of an immune response. In the human patient, the acute stage lasts for only a few months and is fatal in only about 10% of cases. Once gained, the immune state is sufficient to maintain the parasite at very low numbers throughout the life of the infected individual: sterile immunity (complete clearance of the parasite) has never been reported.

The chronic stage of infection is associated with overt disease in a small but variable proportion of infected patients. Although cardiopathy and the digestive megaformations that characterise Chagas' disease may be a significant feature of disease in some Latin American communities, T cruzi infection is usually inapparent and, in the absence of good medical surveillance, often goes unrecognised.

Although control of T cruzi infection via immunoprophylaxis is a valid goal for the immunologist, immunological interest goes deeper to involve the pathogenesis of Chagas' disease. It has long been recognised that the severity of Chagas' disease is not directly related to the degree of T cruzi infection; indeed many of the disease manifestations are seen only during the chronic phase when the parasite is present at an insignificant level. This is illustrated by the findings of Barousse and colleagues [1], who studied patients undergoing immunosuppressive therapy unrelated to concomitant chronic stage T cruzi infection. The disease status of these patients was assessed before and after recrudescence of parasitaemia; there was no recognisable exacerbation of disease. Several workers [reviewed in 2] have suggested that the uncoupling of the degree of infection and resultant disease might be due to an autoimmune reaction triggered, but not necessarily maintained, by parasite infection.

PATHOGENESIS

Clinical Relevance of Experimental Findings

The lack of a valid animal model for human Chagas' disease is undoubtedly one of the major rate-limiting steps in research toward understanding the mechanism of this disease. To date, T cruzi-infected mice have been the most frequently used experimental model but these animals reproduce only some aspects of the human disease. For example, chronically infected mice frequently show ECG abnormalities [3,4] but rarely show intestinal pathology. Study of human postmortem material led Köberle and his colleagues [5] to suggest that Chagas' disease is of neurogeneic origin, arising as a result of the intense neuronal destruction occurring mainly in the acute phase of T cruzi infection. Infected mice are known to undergo a similar phase of destruction of cardiac neurons such that 50% denervation may occur within 20

days of infection [2]. Recent work from Antunes Barreira et al [6] has suggested that the destruction of peripheral neurons may continue into the chronic phase of infection associated with a parasite-induced inflammatory infiltrate.

The need for an animal model of disease is especially crucial for immunoprophylactic studies aimed at the control of infection because of the implied association between parasite antigens and autoimmunity. In vitro experimental evidence has suggested at least two mechanisms whereby parasite antigens might mediate the destruction of otherwise uninvolved host cells. T cruzi antigens have been shown to adsorb to the surface of a range of host cell types, to involve both infected and uninfected cells. In this way, parasite-modified host cells may be destroyed by the host's own antiparasite immune response in vitro assays. To investigate the composition of these antigens T cruzi parasites were radiolabeled with ^{125}-I and disrupted by freezing and thawing. Treatment of a muscle cell line with this antigen preparation resulted in the binding of ten major radiolabeled bands, detected after detergent solubilisation, SDS-PAGE analysis, and autoradiography (Fielder and Williams, unpublished observations). Although a similar process has been shown to occur in the vicinity of intracellular amastigote in infected mice [7], the importance of this potential mechanism in the human disease remains to be determined.

Cross-reactive Antigens

Serological studies in man and animals have shown that some components of anti–T cruzi sera may be absorbed by uninfected muscle or neuronal cells. Conversely, EVI (endocardium, vessels, and interstitium) and NP (peripheral nerve) titres of sera may be reduced by cross absorption with T cruzi. On this basis, the concept of antigenic cross reactivity between parasite and host has remained one of the favoured mechanisms to account for the chronic stage pathology of Chagas' disease. Confirmation of this antigenic cross reactivity has been obtained recently using monoclonal antibodies either raised against parasite and tested on host cells or vice versa. In one such study [8], a monoclonal antibody, CE5, raised against dorsal root ganglia of normal rats, was shown to bind to the surface of viable T cruzi amastigotes, as well as to human and rat central and peripheral neurons of subpopulations known to degenerate in Chagas' disease. CE5 antibody from mouse ascitic fluid has been shown to immunoprecipitate a 87.5 kD polypeptide from the in vitro translation products of T cruzi epimastigote mRNA (Hudson and Eisen, unpublished observations) demonstrating that the CE5-defined epitope is specified by the parasite genome and is not merely a passively acquired host cell component. Again the significance of T- or B-lymphocyte responses to this antigen can only be determined by reference to an animal model. Experiments are under way to produce this polypeptide by recombinant DNA techniques with the eventual aim of in vivo immunisation. If this is an important antigen in the pathogenesis of Chagas' disease, then chronic immunisation should produce pathology.

DETERMINATION OF DISEASE OUTCOME

Although the variable outcome of infection must be determined by host- and parasite-related factors, only a few of these factors have been tested. Parasite diversity, demonstrated by strain variation in morphology, drug resistance, pathogenicity isoenzyme analysis, and restriction endonuclease fingerprinting [9], is great, but no

particular attributes of this diversity have been shown to be of prognostic value. In similar studies, patients developing the various manifestations of Chagas' disease showed no particular association with HLA haplotype. Studies from Segura et al [10] have suggested that immunisation of mice with the flagellar fraction of T cruzi can protect against lethal challenge, whereas immunisation with an internal, microsomal fraction not only confers no immunity but also can mediate pathology. On this basis it seemed reasonable to suppose that one of the deciding factors in the outcome of infection might be the range of antigens recognised by the infected patient. Arguing by analogy, one might suggest that patients recognising only "surface" (flagellar) determinants might develop protective immunity and reduce parasitaemia without disease, whereas patients recognising "internal" components in addition to external protective determinants might set up an autoimmune response by virtue of their reaction to cross-reactive antigens. Accordingly, sera from patients with T cruzi infection, with or without disease, were used to immunoprecipitate antigens from detergent solubilised extracts of T cruzi parasites radiolabeled either with ^{125}-I- (surface) or ^{35}-S-methionine (surface and internal antigens). Table I shows the results of our preliminary findings (Hudson, Hindmarsh, and Ribeiro dos Santos, unpublished observations). Within the constraints of the assay system, there is a single major polypeptide in the region of 74–77 kD recognised by all sera thus far tested. This antigen is also precipitated from amastigotes and trypomastigotes labeled under similar conditions (data not shown). Although it is clear that additional major antigens are precipitated from extracts of metabolically labeled parasites compared to surface-labeled parasites (for example, antigens of 94 kD in the cardiopathy groups and 68 and 41 kD in the asymptomatic group), there is no simple relationship between disease state and the molecular class of antigens recognised. The true significance of this type of analysis can only be obtained by a longitudinal prospective study of patients, starting early in infection. In the group of acute patients shown in Table I two of the patients have unique bands: 1) at 116.5 kD with trypomastigotes, but not amastigotes or epimastigotes; and 2) at 43 kD with amastigote antigens but not epimastigotes or trypomastigotes. We are now waiting the 30 years required to see what will happen to these patients.

TABLE I. T cruzi Internal and Surface Membrane Antigens Recognised by Sera From Patients With Chagas Disease

Patient group					
		Chronic			
Acute		Cardiopathy		Asymptomatic	
Total	Surface	Total	Surface	Total	Surface
—	—	94	—	—	—
—	—	—	87	82	88
75	74	74/75	77/75	—	77/79
69	—	—	—	68	—
—	—	—	—	41	—

The table shows the major polypeptide bands (apparent molecular weight in kD) precipitated from Renex 30 extracts of ^{35}-S-methionine (total) or^{125}-I- (surface) labeled T cruzi epimastigotes using sera of patients with T cruzi infection. Data are derived from ten patients per group. Control patients from the same geographical region gave no major immunoprecipitation bands.

CRITICAL QUESTIONS

To date, studies on the interaction of T cruzi with its host have yielded more critical questions than definitive data. Although some of the unanswered questions apply to virtually all host-parasite systems, some are unquestionably T cruzi-specific.

Association Between Infection and Disease

Köberle suggested that neuronal destruction was the key to the pathogenesis of Chagas' disease and that reduction in neuron numbers occurred in the acute phase of infection [5]. If this is so then one can be optimistic about the value of immunoprophylactic regimes sufficient to reduce acute-stage parasitaemia, but not sufficient to eliminate the parasite (for example, the finding of Scott and Snary [11]). In this way a reduction in acute-stage parasitaemia should ameliorate chronic-stage pathology.

Importance of Autoimmunity in Pathogenesis of Chagas' Disease

Although there is no room to doubt the existence of autoimmunity in human or experimental T cruzi infection, its importance in pathogenesis remains to be established. Two predictions can be tested in inbred mice: (1) Can chronic immunisation with defined parasite antigens lead to any of the features of disease following mouse infection with T cruzi? (2) Can the disease be transferred with immune lymphocyte populations, with or without antigenic stimulation, in the absence of viable T cruzi organisms? Although these may seem simple experiments, the lack of a valid animal model has thus far precluded their definitive execution. Laguens and his colleagues have claimed to be able to induce first-degree auriculoventricular block, ventricular extrasystolia, and bradycardia within 4 days of transfer of lymphocytes from T cruzi injected into normal mice [12]. Mice showed a lymphocytic infiltration of the myocardium within 2 weeks. It is doubtful whether this alone may be considered unequivocal evidence of the transfer of Chagas' disease; evidence for disease transfer must be based upon more stringent and generally accepted criteria.

Role of T Lymphocytes in Immunity and Disease

In common with other parasitic diseases, the protective and etiologic role of antibody in T cruzi infection and Chagas' disease has been extensively studied, particularly with the advent of hybridoma-derived antibodies. T-cell cloning is now a practical possibility and can be used with the reproducibility of monoclonal antibody. The ability of T cruzi to sequester to an intracellular site, and thus produce a lifelong infection, highlights infection-associated parasite antigen on the surface of host cells as a prime target for studies on cloned T-lymphocytes.

The involvement of T-lymphocytes in the immunopathology of Chagas' disease has long been suspected [13]. It now seems that this process might be amenable to immunological manipulation. These studies may usefully simulate the intriguing work of Ben-Nun and his colleagues [14], who showed not only that T-lymphocyte clones against myelin basic protein could produce experimental autoimmune encephalomyelitis (EAE) when injected into normal rats, but also that prior immunisation with X-irradiated T-clones could generate T-suppressor cells with sufficient activity to control both the lymphocyte-mediated and myelin basic protein plus adjuvant-mediated disease. It is considered that suppressor-cell control was particularly potent in this system because of the single myelin basic protein epitope recognised by the T-cyto-

toxic cells. The recent description of single, immunodominant epitopes in malarial sporozoites and merozoites [15] suggests that a similar situation might pertain for T cruzi. In this way one might not only prevent infection in an unexposed animal but also halt the disease process in individuals already infected.

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