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Immunological Consequences of Infection and Vaccination in South American Trypanosomiasis [and Discussion]

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Immunological consequences of infection and vaccination in South American trypanosomiasis

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[Plate 1]

Trypanosoma cruzi infection provokes a vigorous immune response that terminates the parasitaemia associated with the acute stage within two to three months of initial infection. Even so, a variable proportion of patients may develop severe Chagas' disease, often decades after initial infection.

Recent experimental findings suggest that trypomastigotes of *T. cruzi* possess a surface bound neuraminidase and sugar binding protein by means of which they invade host cells – a mechanism very reminiscent of influenza virus. Studies of the antibody response to trypomastigotes in patients or murine models have identified a series of antibodies able to mediate lysis of live parasites in a complement mediated lysis (c.m.l.) assay. These antibodies have also been linked to resistance to infection *in vivo* and disappear following successful parasitological 'cure' in drug-treated animals and human patients. Immunochemical studies have shown that sera from infected patients or mice lacking this c.m.l. activity also lack those antibodies able to bind trypomastigote surface components of 85 and 160 kDa relative molecular mass.

The availability of rabbit and mouse models of Chagas' disease have produced data that suggest that chronic stage pathology may have an immunological basis dependent on the known cross reactivity between host and parasite cells. Delivery of the lethal hit leading to host cell destruction is probably facilitated by the ability of parasite antigens to bind to host cells thus exposing them to the host's own anti-parasite immune response. If Chagas' disease does indeed have an immunological basis, then this might be controlled in turn by immunoregulation, in a manner analogous to that achieved in experimental allergic encephalomyelitis.

1. Introduction

South American trypanosomiasis or Chagas' disease is caused by infection with the parasitic protozoan *Trypanosoma cruzi* and affects a wide variety of human and animal populations throughout Latin America. Though epidemiological data are incomplete, recent estimates indicate about 12 million cases of human infection. This level of infection is likely to rise owing to population migration from endemic rural areas into cities or into previously unaffected areas, for example, the Amazonian basin in Brazil (Miles *et al.* 1978).

The initial, acute phase of *T. cruzi* infection can be totally asymptomatic and goes unrecognized in approximately 70% of infected children and infants, even in areas with reasonable medical cover. In chronic phase infection, even though approximately 20% of patients may develop electrocardiographic (e.c.g.) changes consistent with myocarditis, only about 1% of the total infected population show sufficient symptoms to attract the attention of the physician (Rosenbaum & Cerisola 1961).

The currently available chemotherapy reduces parasitaemia but does not cure the acute stage infection (Cançado 1979). Moreover, it is of little value once the chronic stage is attained, which suggests a mode of drug action that is ineffective against the persistent intracellular forms of *T. cruzi* that typify the chronic stage. Given that the rate of introduction of new drugs is close to zero, it is not surprising that much interest has been generated in the potential for disease control by immunoprophylaxis. However, the description of cross reactive antigens, shared by parasite and host cells, and the known autoimmune phenomena associated with Chagas' disease, mitigate against the use of ill-defined vaccines based on whole organisms or homogenates. Unless the components of a candidate vaccine are well characterized, one is faced with the real possibility that immunization could cure or prevent infection but then itself precipitate or exacerbate disease.

The route to an effective vaccine against Chagas' disease is unlikely to be either easy or rapid, particularly as it seems that the only acceptable goal might be complete protection or sterilizing immunity (Brener 1980): a goal not yet attained for any parasitic disease.

2. Transmission and life cycle

The classical pattern of *T. cruzi* transmission in rural areas of Latin America is via a haematophagus triatomine bug which becomes infected after ingestion of a blood meal from a human carrier or animal reservoir host. As the parasite moves along the insect's gut with the blood meal it transforms to a physiologically adapted epimastigote stage which undergoes extensive proliferation. The final phase of the life cycle in the insect results in motile, non-dividing metacyclic trypomastigotes which are voided with the insect's faeces during a subsequent blood meal. Thus onward transmission is by contamination, rather than inoculation, and the parasite may enter a new vertebrate host via mucous membranes or any site where the natural barriers of the skin are broken. Although insect-related transmission is usually limited to rural areas with low socio-economic conditions, there is growing awareness of transmission via blood transfusion or congenital infection which may also involve urban, socially developed populations (Pinto Dias 1979; Soubihe *et al.* 1983).

Upon gaining access to an immunologically naive host, trypomastigotes invade cells at the portal of entry and transform into non-motile, dividing amastigote forms, which re-differentiate into trypomastigotes just before host cell rupture. This first wave of trypomastigote production serves to disseminate the infection via the bloodstream and initially involves cells of the mononuclear phagocyte system. Subsequent waves of trypomatigote production can produce intracellular infections in virtually any organ system of the body.

The natural history of *T. cruzi* infection follows the course of any infectious disease (figure 1). In the initial acute stage, the parasite divides without immune control and may be recovered from the blood and detected histologically in the tissues. With the emergence of an adaptive immune response the number of free parasites in the blood falls dramatically to a level where they may be detected only by the sensitive techniques of xenodiagnosis, using a long multiplicative phase in uninfected invertebrate hosts, or haemoculture. Once gained, immunity is life-long and, although a sterile state is never attained, parasite numbers are held at such low levels that direct pathological effects of the parasite must be considered as insignificant. Even so, the cardiac and digestive manifestations characteristic of chronic phase Chagas' disease emerge in a variable number of infected patients and sudden death is a frequent occurrence in the course of the disease.

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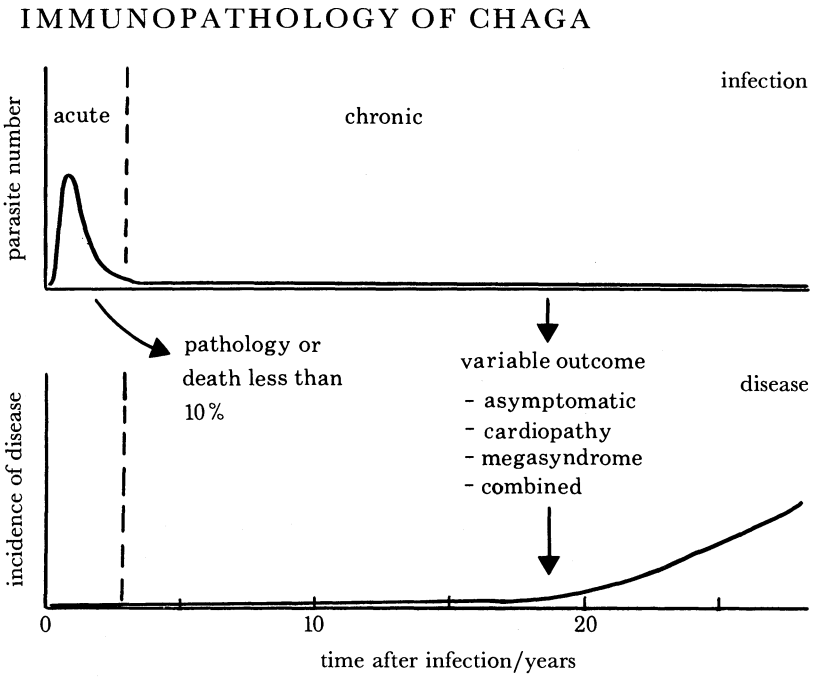

FIGURE 1. Temporal relationship between parasitaemia and onset of disease in *Trypanosoma cruzi* infection. Uniform infection of the population in an endemic area is often seen by the third or fourth year of life, but parasitaemia soon resolves to leave a latent state with positive serology but virtually no parasites. The outcome of infection is very variable, and overt disease is often not expressed until many years after initial infection. The sudden appearance of disease presumably reflects the collapse of the host's homoeostatic mechanisms which are no longer able to provide adequate compensation for muscle and nerve cell destruction.

Surprisingly, clinicians and pathologists have repeatedly observed a negative relation between the degree of parasitaemia and the severity of disease. If, as seems reasonable, this former parameter is linked to the quality and quantity of the anti-parasite immune response, then one is forced to the disturbing conclusion that patients who mount a vigorous immune response, and so clear their parasites, are especially vulnerable to developing severe Chagas' disease.

3. Clinical manifestations and pathology

During the acute stage of Chagas' disease the dynamic and morphological alterations seen in the heart are related to the intensity of the inflammatory response. If the inflammatory infiltrate is severe then cardiac structure and function may be modified but, as parasite numbers decline at the end of the acute stage, the cellular infiltrate decreases and normal structure and function usually return. Acute stage disease usually occurs in infants or children most of whom survive to enter a latent phase with positive serology but no clinical signs.

The outcome of the latent phase of Chagas' disease is very variable and as yet we have no reliable prognostic criteria. The geographical distribution of parasite types (zymodemes, Miles 1979) shows a general correlation with the geographical distribution of disease. This ranges from the relatively 'mild' form of Chagas' disease seen in Colombia (with a high rate of asymptomatic infection, few cases of cardiopathy and no reported cases of digestive manifestations) to a much more severe form seen in regions of Brazil (with a high rate of severe cardiopathy (Ribeirão Preto) either alone or associated with megaformations of the digestive system (Goiania)). The precise basis for this disease pleomorphism is unknown, but is presumably related to particular characteristics of the host and parasite.

Intriguingly, heart involvement in the chronic phase of this disease is charactized by cellular infiltration, myofibrillar degeneration and fibrosis in the virtual *absence* of circulating or intracellular parasites.

4. Experimental studies of parasite and disease

4.1. *In vitro studies*

In common with other parasitic infections, biochemical and immunological studies of *T. cruzi* rely on a convenient and reproducible source of well characterized parasites. Although techniques have been described for the isolation of trypomastigotes and amastigotes from infected rodents (Carvalho *et al.* 1981), they give relatively low yields (about 3×10^6 parasites per mouse) and have the attendant risk of parasite population selection by imune pressure *in vivo*. Fortunately, techniques are now available for the cultivation of each life-cycle stage *in vitro*.

The epimastigote stage may be cultured in simple monophasic medium (Avila *et al.* 1979) and has been adapted to grow in continuous flow chemostat culture (Williams & Hudson 1982) with an equilibrium density of 2×10^7 organisms per millilitre and uniform viability.

Amastigote organisms have also been cultured to high density *in vitro* using either monophasic media (Villalta *et al.* 1982) or with murine cell lines as feeder layers (Hudson *et al.* 1984). In the latter system, the normally intracellular amastigote parasites were cultured continuously as extracellular forms for periods up to two years and yet retained an absolute metabolic dependence on their host cells for survival and proliferation. Insect cells have been shown to play a similar, but equally ill-defined, role in the *in vitro* transformations of epimastigotes to metacyclic trypomastigotes (Lanar 1979).

The recent use of chemical antagonists of the nuclear enzyme ADP-ribosyl transferase to block *T. cruzi* stage-specific differentiation, without reducing parasite proliferation, provides an exquisite method of fine tuning for the routine control of this parasite's life cycle *in vitro* (Williams 1983).

Studies of host cell invasion in these *in vitro* systems have shown that trypomastigotes become intracellular by two basic mechanisms.

(i) Passive uptake into phagocytic cells. This process may be enhanced by antibody and complement mediated opsonization via F_c and C3b receptors, but only results in parasite killing in activated macrophages (Nogueira *et al.* 1982). It seems that *T. cruzi* can pass through the internalized membrane of the phagosome in unactivated macrophages or macrophages defective in oxygen metabolism and proliferate undisturbed in the cytoplasm (Tanaka *et al.* 1983).

(ii) Active penetration of non-phagocytic cells. Cell invasion by this route may be inihibited by *N*-acetyl-d-glucosamine (Crane & Dvorak 1981) suggesting that the parasite–host cell interaction might be mediated by a parasite protein which binds to sugar on the host cell. This possibility is particularly intriguing as it was rapidly followed by the description of a surface-bound neuraminidase on *T. cruzi* which might act in concert with the parasite sugar-binding protein to produce a molecular mechanism for invasion very reminiscent of host cell invasion by influenza virus (Csete *et al.* 1984). It seems we may now have the first clues to an important mechanism that might provide a target for specific immunization for the prevention of infection.

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4.2. *In vivo studies*

4.2.1. *Resistance to infection*

There is ample evidence to show that *T. cruzi* provokes a vigorous immune response which not only checks the infection within two or three months (for review see Brener 1980) but also keeps it under control throughout the life of the patient. Once the acute phase has passed, a recrudescence of parasitaemia is never seen in spite of repeated reinfection of patients in the endemic area. Significantly, if the infected patient is given immunosuppressive therapy for a concomitant illness, there may be a recrudescence of parasitaemia, but chronic stage pathology is neither reactivated nor exacerbated (Barousse *et al.* 1980).

Extensive studies on the immunological basis of resistance to experimental *T. cruzi* infection have yielded disappointing and confusing results (reviewed by Hudson 1981). Lymphocyte sub-populations or antisera from immune donors were transferred to syngeneic recipients, which were challenged by infection. Only partial protection was conferred by immune transfer and no clear mechanism for adaptive immunity emerged. Krettli & Brener (1982) performed similar transfer experiments but compared the degree of protection conferred by sera from mice immunized by infection to that given by sera from mice immunized with killed parasites or sub-fractions thereof. It is intriguing that antibodies raised by infection were able to confer significant protection in passive transfer experiments, far in excess of that gained by passive transfer of sera from mice immunized by 'non-living' antigen preparations. This *in vivo* protection was found to correlate with a complement mediated lysis (c.m.l.) assay using living trypomastigotes as target cells. In parallel studies of sera from infected human patients (Krettli *et al.* 1982) or mice (Krettli & Brener 1982) undergoing nifurtimox or benznidazol anti-parasite therapy, it was found that c.m.l. antibody activity was lost upon parasitological cure, even though conventional serology remained uniformly positive.

Ongoing work from this laboratory has examined the c.m.l. assay in relation to the range of antigens recognized by mouse sera raised as described by Krettli & Brener (1982). In brief, sera were mixed with detergent-solubilized material from ^{125}I surface iodinated *T. cruzi* trypomastigotes, immune complexes isolated by adsorption to protein A-Sepharose and bound material analysed by one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis. As expected a range of radioactively labelled polypeptides was found to be common to the immunoprecipitates prepared either with sera from chronically infected mice or from mice immunized with glutaraldehyde-fixed trypomastigotes. However, the latter sera consistently failed to precipitate a major component of 160 kDa recognized by the sera from infected mice (S. M. Martins and L. Hudson, to be published). Further studies were then conducted with coded serum samples from infected patients in the following groups: (i) untreated, c.m.l. positive; (ii) drug-treated, c.m.l. positive and (iii) drug-treated, c.m.l. negative. Immunoprecipitation profiles with detergent-solubilized material from [^{125}I]- or [^{35}S]methionine-labelled epimastigotes or amastigotes showed a precisely similar range of labelled polypeptides recognized by antibodies from all patient groups. Significantly, however, two patients, later found to be in the drug-treated, c.m.l. negative group, failed to precipitate antigens with apparent molecular masses of approximately 85 and 160 kDa which were major components in the immunoprecipitation profiles of the other groups (Martins *et al.* 1984). Work is now in progress to attempt to define these components using monoclonal antibodies from infected mice to provide a direct means for antigen isolation and characterization.

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4.2.2. *Experimental models of Chagas' disease*

The availability of purified parasite antigens, any of which could be possible components of a candidate vaccine (for review, see Scott, this symposium), has produced a demand for acceptable animal models of clinical *T. cruzi* infection and Chagas' disease. If one were to accept complete protection as the sole valid goal for efficacy of vaccination, then one need look no further than the acute, uniformly lethal parasitaemias produced by laboratory strains of *T. cruzi* in inbred mice.

Although such screening experiments could be conducted with rapid efficiency, their outcome could almost certainly be predicted with depressing accuracy. On present evidence, it is probable that an effective vaccine will have to have multiple components each of which would confer only partial protection when tested alone. Clearly a model of chronic disease is required in which to determine the effect of a partial reduction of parasitaemia on amelioration of disease.

The ideal experimental model of human chronic disease would develop a life-long sub-patent infection, controlled by an appropriate immune response. The typical cardiac or digestive manifestations, or both would be developed, but preferably on a more abbreviated time scale (weeks or months rather than years or decades) than in the human disease.

Several large animal models, for example, in monkeys and dogs, have been examined but the development of the pathological sequels of infection have either been unpredictable or absent. Rabbits have been shown to develop many features similar to human chronic disease, namely, myocardiopathy (see figure 2, plate 1), severe e.c.g. disturbances, megacolon, sudden death and a decrease in the number of neuronal ganglia (Teixeira 1979). Although these original studies using outbred rabbits have been confirmed using inbred rabbits and cloned *T. cruzi* parasites of the Ernestina strain, few laboratories have attempted to use this model system.

The outcome of *T. cruzi* infection in inbred mice is crucially dependent upon parasite and mouse strain, host sex and age, and size of the infecting dose of parasites. With the appropriate combination of parameters it is possible to produce a murine model with uniform survival to chronic sub-patent infection with associated inflammatory or fibrotic cardiac lesions, myositis and necrotizing arteritis (Laguens *et al.* 1980). Although e.c.g. measurements provide a continuous and non-invasive means of monitoring the evolution of cardiac pathology, considerable care and patience is required to obtain reliable results in infected mice (Laguens *et al.* 1981).

Table 1 shows data from a typical set of e.c.g. tracings obtained in this laboratory (Hindmarsh & Hudson 1984). The increase in the duration of the QRS complex was significantly different to controls in both the acute and chronic stage of infection and is consistent with the right branch bundle block frequently seen in human disease. The increased P–R interval was only seen late after infection and occurs at a similar time to the second degree atrio-ventricular block noted by Laguens *et al.* (1981).

Although individual infected mice showed marked left axis deviation during acute infection, the average value for the group failed to achieve statistical significance. It seems likely that the usefulness of the mouse model of chronic Chagas' disease might be enhanced by the use of one of the several mouse strains with genetically determined immunological defects which regulate spontaneous autoimmune disease (Boyer *et al.* 1983).

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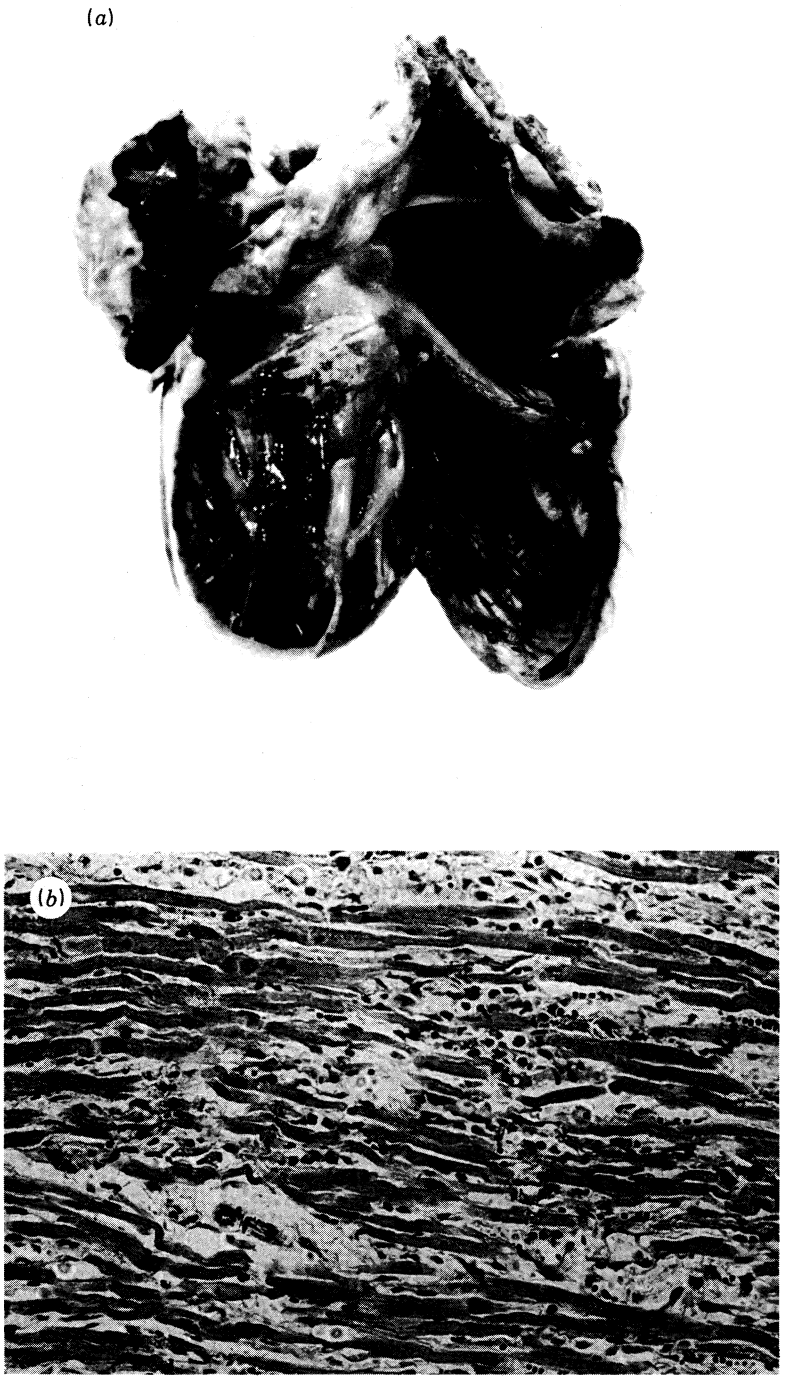

FIGURE 2. Cardiac pathology in rabbit infected with *Trypanosoma cruzi*. (*a*) Macroscopic view of heart from *T. cruzi* infected rabbit which died of chronic chagasic myocarditis. The heart is dilated and a thrombus is seen in the right atrium occluding the vena cava. (*b*) Histology of destructive myocarditis. Lymphocyte infiltration, adherence to heart cells and heart cell lysis are seen in the absence of parasites. Photographs by courtesy of Dr Antonio Teixeira.

(*Facing p.* 56)

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TABLE 1. CHANGES IN E.C.G. PARAMETERS IN *T. CRUZI* INFECTED BALB/C MICE

	time after *T. cruzi* infection/days			
	22	control	240	control
width of QRS complex	5.3±0.7	7.6±1.5	6.0±0.4	7.6±0.8
P–R interval	no significant change		10.6±0.8	12.4±0.9
cardiac axis deviation	no significant change		no significant change	

Table shows mean±s.d. for five mice per group in a typical experiment to measure e.c.g. changes following *T. cruzi* infection. Units are length (in millimetres) of the particular segments measured. E.c.g. traces were recorded under halothane anaesthesia at a chart speed of 250 mm s^{-1}, tracings were taken from all mice before the experiment to ensure that parameters were within limits established from a large batch of normal mice. Mice were infected with 10^3 Y strain trypomastigotes inoculated intraperitoneally to produce uniform survival through to the chronic phase. All data shown from infected groups differ from that of age- and sex-matched controls at a significance level of at least $P < 0.02$.

4.2.3. *Evidence for immune involvement in pathogenesis*

Autoantibodies reacting with endothelial cells, vascular structures, cardiac and striated muscle cells (e.v.i. antibodies) and peripheral nerves have been reported in a large proportion of chagasic patients, but present evidence shows no correlation with the development of the disease (Peralta *et al.* 1982). Similarly, inhibition of leucocyte migration in the presence of uninfected heart antigens or cell mediated lympholysis of heart target cells may be demonstrated with lymphocytes from patients with chronic myocarditis, but this only provides evidence of enhanced autoreactivity not necessarily connected with pathogenesis.

Texeira *et al.* (1975) provided the first good evidence for a direct involvement of the immune system in the genesis of chagasic lesions. Rabbits given intravenous injections of subcellular fractions of *T. cruzi* trypomastigotes once per week for six to nine months developed microscopic lesions in the heart and digestive tract similar to those seen in rabbits with chronic *T. cruzi* infection (see figure 2). An even more direct demonstration of immune involvement is provided by the demonstration that lymphocytes from *T. cruzi* infected mice may induce e.c.g. alterations and histopathological lesions in syngeneic recipients (Laguens *et al.* 1981).

5. PATHOGENESIS

Although in the acute stage many of the observed lesions might be due to the presence of parasites, the suggestion made by Chagas and his contemporaries that chagasic heart disease is a 'chronic parasitic myocarditis' is not tenable: there are two few parasites to produce direct host cell damage. When seen by microscopic examination, the rare nests of amastigote parasites observed in the chronic phase do not coincide with muscle and nerve tissue lesions. Any pathogenic mechanism for Chagas' disease must explain the inverse relation between the degree of infection and severity of disease, and take account of the observations that neuronal cells seem especially vulnerable and yet are rarely parasitized.

5.1. *Mechanisms for host cell destruction*

The suspicion that *T. cruzi* shared certain common antigens with vertebrate muscle and neuronal cells first came from cross absorption studies using sera from chagasic patients. More recently monoclonal antibody technology has been used to reinforce this notion. Monoclonal antibodies specific for human Purkinje neurons react with *T. cruzi* (Wood *et al.* 1982), and

similar antibodies raised against *T. cruzi* bind to a range of mammalian neural elements (Snary *et al.* 1983).

Studies have also been published that show that *T. cruzi* antigens are released, both *in vivo* and *in vitro*, in a form that allows them to bind both to infected and uninfected host cells, thus rendering these parasite-modified cells susceptible to the host's own anti-parasite immune response (Ribeiro dos Santos & Hudson 1980). How might these apparently unrelated phenomena be involved in the pathogenesis of Chagas' disease?

We recently proposed that the intracellular disposition of cross reactive antigens seen by monoclonal antibody labelling might be a general phenomenon, such that cross reactive antibodies raised early in infection might be unable to gain access to their intracellular target without prior permeabilization or death of the host cell (Hudson 1983). The essential trigger

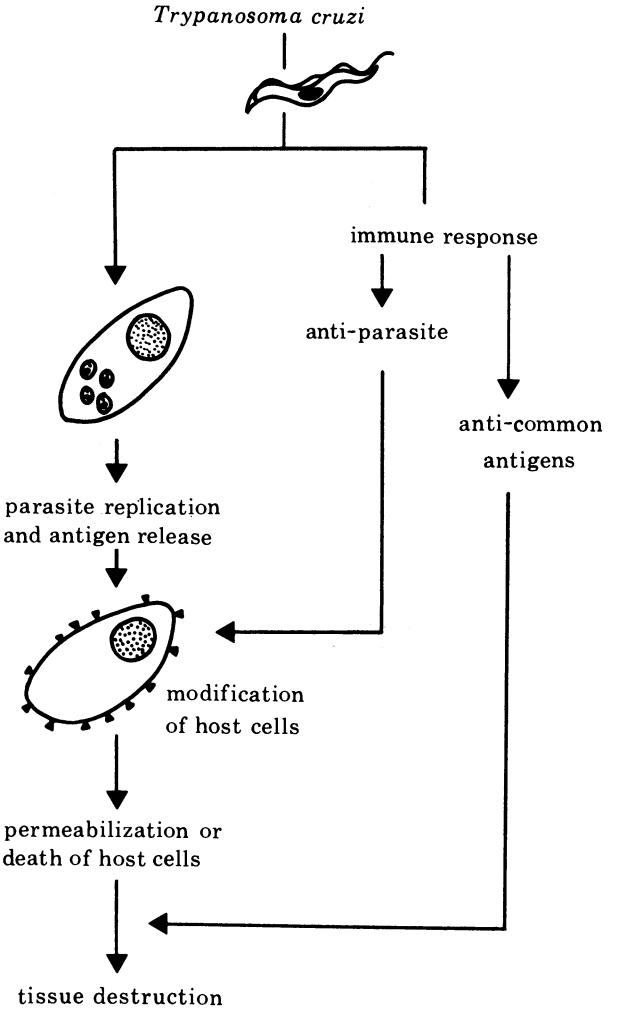

FIGURE 3. Possible relation between cross reactive antigens and host cell modification in the generation of chagasic pathology. In a manner analogous to other autoimmune states, for example type I diabetes mellitus, it is suggested that the autoantibodies provoked by *T. cruzi* infection are unable to gain access to their target antigens because of their intercellular disposition (Wood *et al.* 1982). The high avidity of parasite antigens for host cell surfaces, and the consequent intervention of the host's own anti-parasite immune response, might provide the essential step by which the membrane of the vunerable cell could be made permeable and their destruction facilitated.

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for permeabilization of host cell death might be supplied during the acute phase by the parasite specific components of the immune response reacting against parasite modified host cells, as shown in schematic form in figure 3. Subsequent interaction between cross reactive antibody and antigen would then either precipitate or facilitate host cell death leading to the release of self antigens which could serve to stimulate a secondary immune response and so maintain a self perpetuating cycle of host cell destruction and autoimmune stimulation, now free of the need for parasite antigens.

Although entirely speculative, this proposed pathogenic mechanism is strikingly similar to that envisaged for the pathogenesis of type I diabetes mellitus: a 'real' autoimmune disease (Mirakian *et al.* 1982). In a prospective study of first degree relatives of diabetic patients, almost 40% were found to have anti-endocrine cell antibodies for one to three years before the onset of disease. The onset of the disease state in seven patients was linked to concomitant viral infection which was postulated to act by giving access to antigens known to be intracellular on membranes.

The mechanisms discussed in this section are entirely antibody based, not because they are considered to be the only or even the most important immune component in the autoreactivity seen in chronic stage Chagas' disease, but rather because of an absence of similar observations with cloned T cell lines or hybridomas. Experiments to establish and characterize T cell lines from *T. cruzi* infected human patients and mice are on-going in this and other laboratories. Except to state the immunological dogma that T lymphocyte-mediated immune mechanisms must be important in intracellular infections such as that seen in *T. cruzi* chronic phase, it would be unrewarding to speculate as to the likely outcome of these experiments.

6. Prospects for disease control

It is certain that the most effective control of Chagas' disease will come from the prevention of *T. cruzi* infection either by immuno- or chemoprophylaxis. However, there are a huge number of already established cases of *T. cruzi* infection and Chagas' disease that might benefit from studies designed to understand and modify disease pathogenesis.

The description of a highly purified parasite component of 25 kDa which reacts specifically with sera from patients with chronic disease is exciting (Scharfstein *et al.* 1983) and suggests that prognostic criteria might soon be available for patient assessment. It remains to be seen whether variation in the type of parasite antigens recognized by the host underlie the progression to an asymptomatic or diseased chronic state. Host cross-reactive or parasite-specific components of *T. cruzi* would be immediate candidates for this type of prospective study.

Chagas' disease has yet to benefit from the potential offered by T lymphocyte lines. In this context, the approach used by Ben-Nun & Cohen (1982) to gain immunological control of those lymphocytes mediating experimental allergic encephalomyelitis (e.a.e.) might be of value in combating the evolving disease process in an already infected chagasic patient. There is little doubt that immunological control of e.a.e. was facilitated by the existence of only a single major epitope on myelin basic protein. Although a similar approach is likely to be feasible in experimental models, its wider application would require that a similar restricted range of important epitopes should also exist in *T. cruzi*. Experience from other parasite systems suggests that important epitopes might indeed be restricted, perhaps not only for immuno-prophylaxis (cf. malaria epitope: Cochrane *et al.* 1982) but also for immunopathogenesis.

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Discussion

M. J. Turner (*M.R.C. Biochemical Parasitology Unit, Molteno Institute, Downing Street, Cambridge CB2 3EE, U.K.*). Given that any candidate vaccine for *T. cruzi* is going to require at least a 40-year field trial, in areas where the logistics of following-up immunized individuals are formidable, what does Dr Hudson think are the real prospects for *cruzi* vaccines?

L. Hudson. Even though one may undoubtedly reduce the time scale for clinical trials implied in the question and certainly obtain stable patient groups in several areas of Latin America, the difficulties are still formidable. The parasite is antigenically invariant and, even in the natural infection, is susceptible to control by the immune response – this much, at least, is good news for the immunologist. However, it seems likely that the possibility of cross-reactive immunization will remain a central problem even when one has identified and eliminated all the potentially harmful antigens by screening in experimental animal models of disease. One can never be sure that the human immune system will react in the same way.

Consider instead the alternatives. We might halt transmission by eradicating the vector but, because of the enormous range of reservoir hosts for *T. cruzi* and the dynamics of the insect populations, it would rquire a substantial financial commitment to achieve control by repeated applications of insecticide. Even though it is not directly relevant, I suspect the failure of the malaria eradication programme would stop most governments attempting control on the required scale. Chemotherapy or chemoprophylaxis is also a possibility but, to my knowledge, *T cruzi* infection and Chagas' disease is not a significant component in the research and development programmes of any of the major pharmaceutical companies. It seems we have few alternatives, a vaccine against *T. cruzi* infection might be expensive in development but, with modern technologies, should be cheap in its application. Perhaps 40 years is not so long after all: a successful conclusion would certainly merit the effort.

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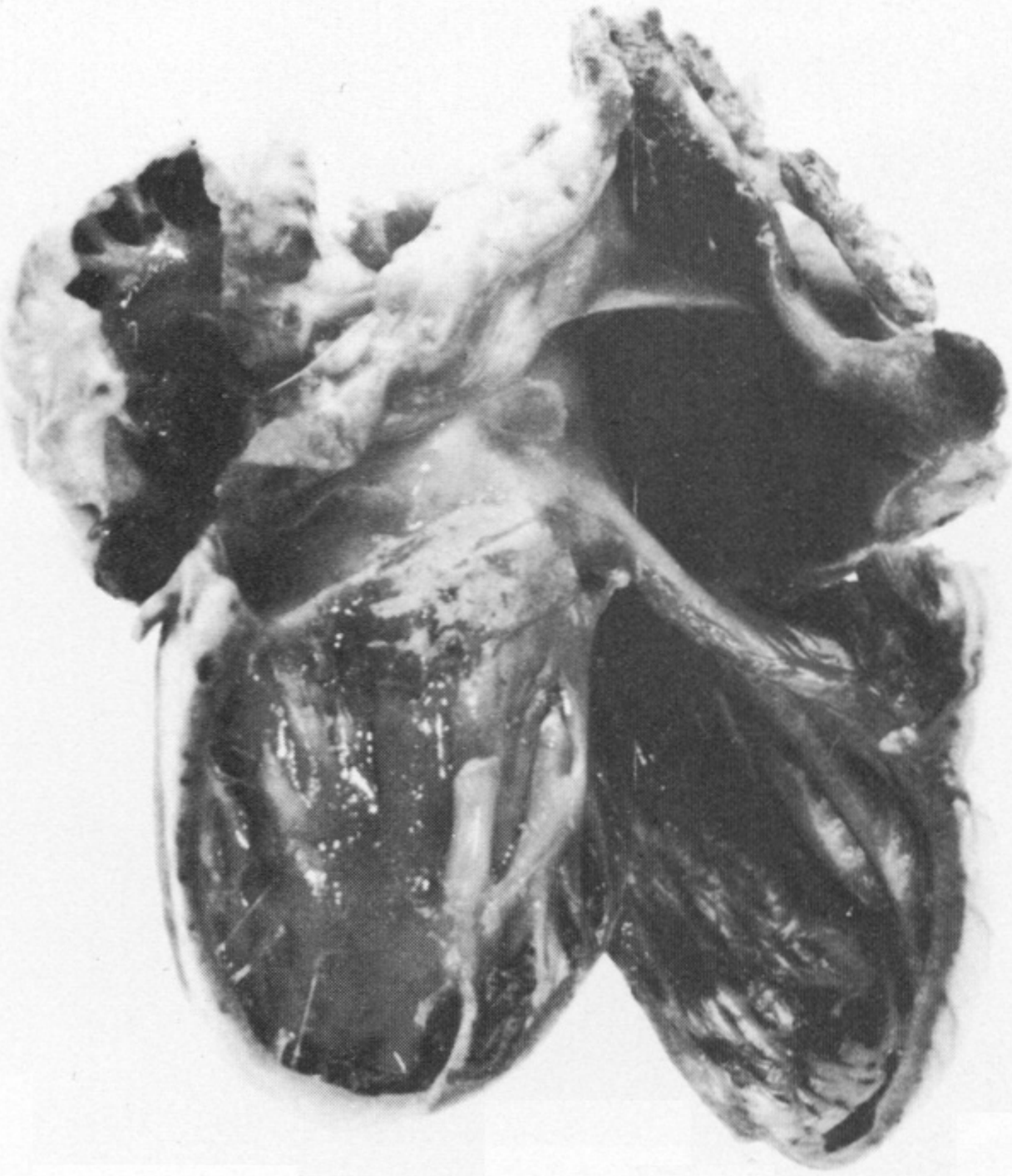

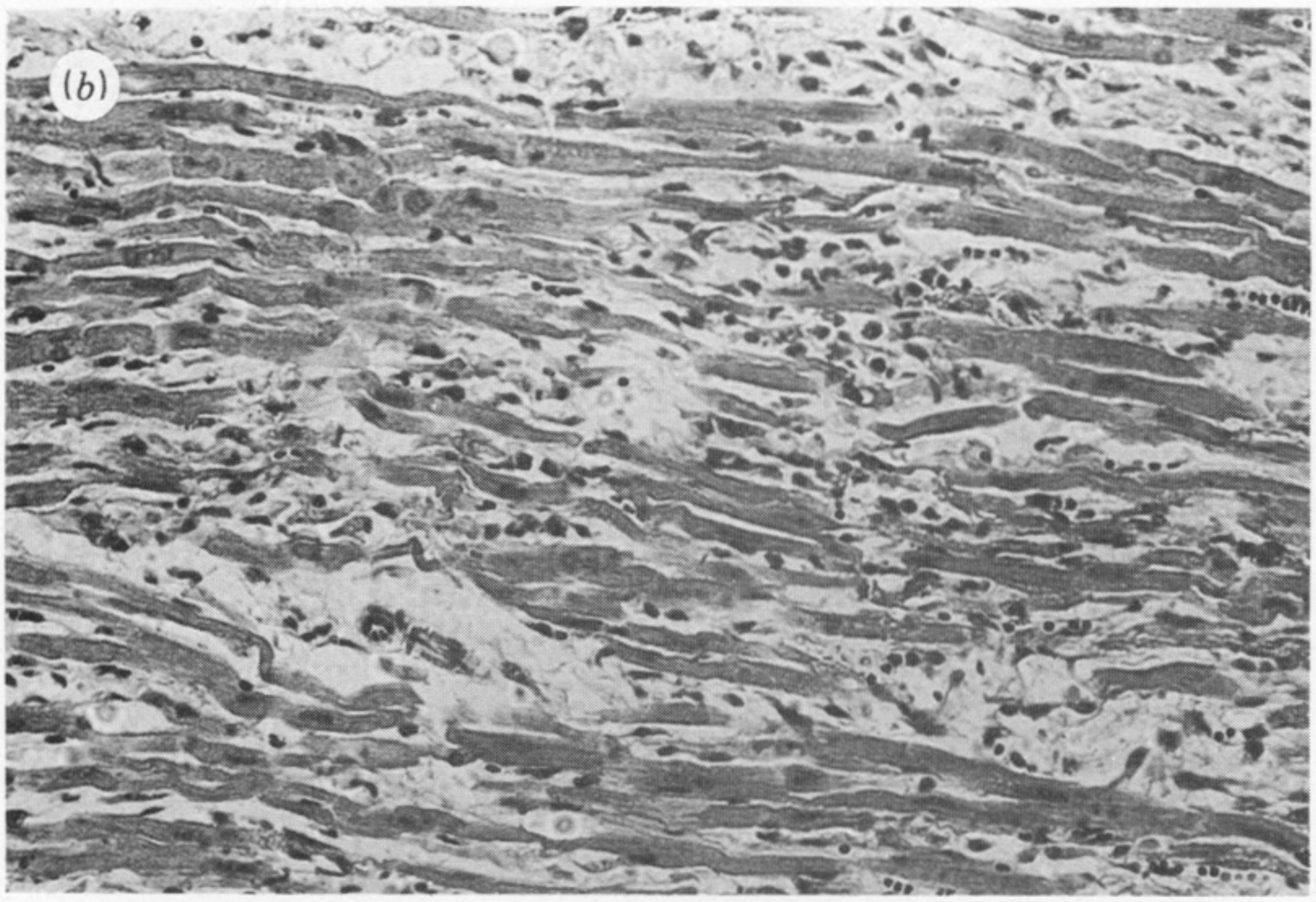

FIGURE 2. Cardiac pathology in rabbit infected with *Trypanosoma cruzi*. (*a*) Macroscopic view of heart from *T. cruzi* infected rabbit which died of chronic chagasic myocarditis. The heart is dilated and a thrombus is seen in the right atrium occluding the vena cava. (*b*) Histology of destructive myocarditis. Lymphocyte infiltration, adherence to heart cells and heart cell lysis are seen in the absence of parasites. Photographs by courtesy of Dr Antonio Teixeira.