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CHAGASIC CARDIOPATHY: A DISEASE REFLECTING IMBALANCE IN THE HOST-PARASITE RELATIONSHIP

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It is now recognized that *T. cruzi* infections offer superb models for the study of host-parasite relationship. In many cases, both host and parasite survive in a balanced fashion with each doing little harm to the other. But, exceptionally, ill-directed immune response can severely damage the host and may lead to death.

It is the study of these aberrant immune responses that forms the discipline of immunopathology.

Until recently the immunopathology of Chagas' disease has been largely unexplored: immunological findings have not been correlate with pathology and pathologists and immunologists have failed to identify common interests.

The situation has now changed. We have acquired a lot of data but we still have difficulties in fitting it into an overall pattern. Particularly difficult is the correlation of *in vitro* findings with the disease pattern seen in animal models and even more so with the patterns of human disease. Our present condition is best described as "immunoconfusion" but perhaps some light is appearing at the end of the tunnel.

Our group at Ribeirão Preto has developed a major interest in the immunological mechanisms involved in the cardiac and neuronal damage seen in Chagas' disease and we have been attempeting to develop animals models which mimic the human disease. During an acute infection with the "Y" or "Colombian" strains of *T. cruzi*, mice develop a high parasitaemia and many amastigotes are present in the myocardium. They appear to stimulate an intense and widespread inflammatory mononuclear infiltrate which is associated with damage not only to parasitized myofibers but also to apparently normal myofibers. In this context, it has been shown by immunofluorescence and radiolabelling techniques that *T. cruzi* antigens adsorb to the surface of normal heart cells as to several mammalian cell lines (Ribeiro-dos-Santos & Hudson, 1980a; Williams et al., 1984).

We have also carried out histopathological studies in athymic BALB/c mice and found very large numbers of amastigotes in the heart but without any associated inflammation. The normal inflammatory reaction was restored when the athymic mice were given thymocytes taken from their "nu/+" littermates. Immunofluorescence and electron microscopy studies were undertaken in order to determine the cell types in the lesion. More than 95% of the cells were macrophages and less than 2% were T cells as judge by the presence of the Thy-1 antigenic surface marker.

We can conclude from this data that most of the heart tissue damage occurring in the acute phase of mouse infection is due to immune response against not only *T. cruzi* infected cells but also against cells which have adsorbed *T. cruzi* antigens on their surface. *In vitro* studies support these conclusions (Ribeiro-dos-Santos & Hudson, 1980b).

The inflammation appears to be T cell-dependent but the effector cells are macrophages. If mice are treated with levels of cyclophosphamide known to knock out the precursors of T suppressor cells, the heart damage is increased and shown a cutaneous delayed type hypersensitivity (DTH) to *T. cruzi* antigens.

We interpret these data to mean that the T cells in the lesion are of the T*DTH* type and that their activity is modulate by T suppressor cells. But we must also appreciate that suppression may be mediated by antibody. We have unpublished findings that treatment of mice from birth with anti-u serum, which abolishes B cell activity, also potentiates the inflammatory lesion.

We must, however, recognize that acute infections of *T. cruzi* in mice differ importantly from most human acute infections. For example, in Brazil and Argentine this phase is usually accompanied by few symptoms and the mortality has been reported from other countries, as for example, Bolivia, so that regional differences in pathology exist. At this time we can only speculate on the reasons for these. Differences in the parasite strain virulence, genetic differences in the host, or the action of "modulating" antibodies transmitted by infected mother to their babies may all be involved.

Once the acute infection is brought under control, clinical symptoms are much reduced or absent. But the parasite remain, although at very low levels for the rest of the life of the host. These seems to be a homeostatic balance between host and parasite and this balance remains stable in most (about 75%) of human chronic infections and there is no disease. In a minority (about 25%) the balance is upset and disease, cardiomyopathy and/or megasyndrome, occurs.

Till now studies on the chronic phase of the disease have been hampered by the lack of a suitable experimental model, but we have now developed a mouse model which provides interesting parallels with the human chronic disease (Rossi et al., 1984).

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Mice are "vaccinated" with a single dose of 10 million epimastigote of the avirulent "PF" strain of *T. cruzi* (Menezes, 1969) and are challenged 30 days later (1000 parasites per gr. body weight) with the virulent "Colombian" strain. These mice present an acute phase with low parasitaemia, few amastigotes in the myocardium, little focal inflammatory infiltration and practically no mortality. On 30 days following challenge and for at least a year, parasitaemia is negative by direct examination, but positive by xenodiagnosis. Carditis is not present and this model correlates well with the majority of human infection.

Mice "vaccinated" five times with the "PF" strain and challenged with the "Colombian" strain behave at first in the same way as that described above. At 30 days after challenge parasites cannot be detected directly and there is little inflammation. However, three months after infection, focal inflammation increases and after six months we find polifocal inflammatory reactions with mononuclear cells surrounding many fibers which present different degrees of degeneration. There is myocytolitic necrosis with areas of fibrosis, and platelet aggregation is seen in the microcirculation of the myocardium. On macroscopic examination, about half of the hearts showed apical aneurism. Thus this experimental model correlates well with the human chronic chagasic cardiopathy.

We have explored the basic mechanisms underlying this pathology using similar methods to those used to study the acute phase. We are convinced that T*DTH* cells are also involved in the chronic phase but in this instance they appear to be autoreactive. If purified T cells from these mice are transferred to normal syngeneic recipients, multifocal carditis with mononuclear infiltration appears within 48 hours. *In vitro* studies also support the conclusion that autoreactive T*DTH* are involved. Spleen cells from chronic phase mice, but not the acute phase mice, are cytotoxic for myocardial cells in culture. If *T. cruzi* antigens are added to the culture, the myocardial cells are killed by both chronic and acute phase cells.

If macrophages are removed by filtering the chronic phase spleen cells through G10 Sephadex in nylon wool the remaining population, enriched for T cells, cannot kill the heart cells. Killing ability is restored if splenic or peritoneal macrophages from normal syngeneic mice are then added to the culture.

We now have evidence to support that a small population of autoreactive T*DTH* cells develops during the acute phase, although these cells are difficult to demonstrate, and certainly most of the inflammation is mediated by T*DTH* cells specific for *T. cruzi* antigens. We also believe that in the chronic mouse model without pathology, and in 75% chronic human cases, these autoreactive T*DTH* cells are in a state of balanced suppression, cellular and/or humoral. In the pathological mouse model and in about 25% of human chronic cases, the balance is disturbed, suppression fails, the autoreactive T*DTH* clones expand, and heart damage occurs.

By studying "infection with disease" and "infection without disease" we hope that some day we might be able to modulate the one to became the other. Although we might not be awarded the Nobel Prize for Physiology and Medicine for our discovery, we feel that understanding the ways in which *T. cruzi* can live at peace with its host, might encourage all mankind to do likewise, and then we might be awarded the Nobel Prize for Peace!