IMMUNOLOGY OF CHAGAS' DISEASE

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This memorandum was drafted on the occasion of a Meeting of Investigators on the Immunology of Chagas' Disease, which took place in December 1973 at the Departamento de Inmunología, Instituto Nacional de la Nutrición, Mexico. Signatories are: Dr. S. ALARCON SEGOVIA, Departamento de Inmunología, Instituto Nacional de la Nutrición, Mexico City, Mexico; Dr. Z. A. ANDRADE, Department of Applied Pathology, Faculty of Medicine, Federal University of Bahia, Bahia, Brazil; Dr. B. R. BLOOM, Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, New York, USA; Dr. S. ESTRADA PARRA, Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Mexico City, Mexico; Dr. H. C. GOODMAN, Chief, Immunology, World Health Organization, Geneva, Switzerland; Dr. W. L. HANSON, Department of Parasitology, College of Veterinary Medicine, Athens, Georgia, USA; Dr. F. KIERSZENBAUM, Departamento de Microbiologia y Parasitologia, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina; Dr. P. H. LAMBERT, Department of Haematology, Hôpital Cantonal, Geneva, Switzerland; Professor W. H. R. LUMSDEN, Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, London, England; Dr. INES MALAVE, Departamento de Medicina Experimental, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela; Professor L. McINTYRE, Department of Veterinary Medicine, Veterinary Hospital, Glasgow, Scotland; Professor F. MILGROM, Department of Bacteriology and Immunology, State University of New York at Buffalo, Buffalo, New York, USA; Dr. M. MURRAY, Department of Veterinary Medicine, Veterinary Hospital, Glasgow, Scotland; Dr. L. ORTIZ ORTIZ, Departamento de Ecología Humana, Facultad de Medicina, Universidad Nacional Autónoma de Mexico, Mexico City, Mexico; Dr. R. PEREZ TAMAYO, Departamento de
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IMMUNOLOGY OF CHAGAS' DISEASE

1. INTRODUCTION

Chagas' disease is caused by infection with Trypanosoma (Schizotrypanum) cruzi, a flagellate of the Order Kinetoplastida, to which belong also the organisms causing African sleeping sickness (Trypanosoma Trypanozoom brucei spp.), kala-azar and cutaneous leishmaniasis (Leishmania spp.). T. cruzi is transmitted, mainly, by blood sucking bugs of the Reduviidae: Triatominae, commonly referred to as kissing bugs.

Chagas' disease is widely distributed in Central and South America and affects mainly rural populations living in poor housing. Its exact prevalence is difficult to discover as statistics for such populations are limited or non-existent. However, there is little doubt of its importance as clinical cases and deaths due to it are commonly recognized over huge areas of Central and South America - as far south as middle Argentina.

The disease exerts an influence beyond its actual incidence because it is known to be often lethal and virtually incurable - a widely applicable chemotherapeutic agent has not yet been discovered.

1.1 Morphology and multiplication

T. cruzi exists in three morphologically different forms related to three different environments in which it lives (Fig. 1):

- Amastigotes - without a flagellum; spherical or oval organisms about 2μm in diameter; a dividing form found intracellularly in mammalian hosts.
- Epimastigotes - with Kinetoplast located anterior to the nucleus; flagellum and short undulating membrane; spindle-shaped organisms about 20 μm long; a multiplying form found in the vector's digestive tract and in culture.

*Prepared by Dr. Giorgio Torrigiani, Medical Officer, Immunology Unit, World Health Organization, Geneva, Switzerland.
FIG. 1
DIAGRAMATIC ILLUSTRATION OF THE MORPHOLOGICAL FORMS
IN LIFE HISTORY OF TRYPANOSOMA CRUZI

FLAGELLM

KINETOPLAST

NUCLEUS

TRYPOMASTIGOTE
(TRYPANOSOMAL)

AMASTIGOTE
(LEISHMANIAL)

EPIMASTIGOTE
(CRITHIDIAL)
Trypomastigotes - with Kinetoplast posterior to nucleus; flagellum and undulating membrane along whole length of the organism; about 20 μm long; a non-multiplying infective form. It occurs in the lumen of the rectum of the reduviid bug and is infective to the mammal. Trypomastigotes also occur in the mammalian host where they transfer the infection from cell to cell or initiate infection in the bug when ingested with a blood meal.

1.2 Transmission

Bugs become infected by ingesting trypomastigotes (Figs. 1, 2) from the peripheral blood of infected mammals. In the lumen of the midgut of the bugs the organisms multiply as epimastigotes (Figs. 1, 2) and this multiplication leads, after a period from 15-30 days, to the development of the metacyclic trypomastigotes in the rectum of the bug. These infective forms are passed out with the bug faeces and the trypomastigotes initiate infection in new hosts entering via skin abrasions or mucous membranes. Such transmission is referred to as "posterior station" or "contaminative" and is distinct from the transmission mechanism of the trypanosomes of African sleeping sickness. In African trypanosomiasis the infective forms are associated with the mouth parts of the vector insect and are introduced into new hosts by the bite-"anterior station" or "inoculative" transmission.

1.3 Multiplication in the mammal host (Fig. 2)

Infective trypanosomes from the bug enter cells and multiply as amastigotes forming so called pseudocysts which are cells packed with amastigotes. The amastigotes then transform to trypomastigotes and upon rupture of the cells are set free - to invade other cells or be ingested by a bug (see above).
FIGURE 2. LIFE CYCLE OF TRYPANOSOMA (SCH. TRYPSANUM) CRUZI

Trypomastigotes transform to epimastigotes

Epimastigotes multiply by binary fission in hindgut

Epimastigotes transform to infective trypomastigotes in hindgut

Transmission by penetration of trypomastigotes through skin or mucous membranes (contaminative transmission)

Trypomastigotes in peripheral blood ingested by bug

INVERTEBRATE HOST Reduviid bug

VERTEBRATE HOST eg. man, opossums, armadillos, cats, dogs, rodents

Trypomastigote parasitaemia; trypomastigotes do not multiply, but infect new cells

Trypomastigotes transform to amastigotes and multiply, forming a pseudocyst

Amastigotes transform to epimastigotes and trypomastigotes in pseudocyst; pseudocyst ruptures

This cycle goes on continuously in the vertebrate host

Modified from a figure by Miss V.C.L.C.Wilson
1.4 Course of infection and pathology of the disease

There appears to be four distinct phases of Chagas' disease, both in man and experimental animals.

I. Incubation period, with proliferation of amastigotes within cells, cell-to-cell transfer via trypomastigotes and early entry of trypomastigotes into the blood stream. This phase lasts 1-3 weeks.

II. Acute disease, often characterized by chagoma, fever and hepatosplenomegaly. Chagoma is a nodular lesion of the skin at the portal of entry. When the portal of entry is the conjunctiva an oedematous lesion of the eyelid may follow (Romana's sign). Both are characterized by an almost exclusively mononuclear infiltrate. This phase is accompanied by a marked increase in organisms at the local site and lasts 2-3 weeks. The draining nodes are enlarged and show what is described as non-specific inflammation. Often acute disease reveals marked parasitaemia (tryomastigotes) resulting in invasion of, and proliferation in, the cells of many organs. There are strain differences in the parasite's ability to invade particular organ systems. Reticulotropic and myotropic strains represent extremes of this spectrum. The former being usually more virulent. Intracellular parasites seem not to excite inflammatory reactions. By immunofluorescence, their antigenic constituents have been shown to be contained within the pseudocysts. Rupture of these pseudocysts with extracellular release of antigen is accompanied by inflammation. There is massive infiltration with mononuclear cells. Severe lesions of this type in the heart or other organ systems may lead to death. Lesions can occur in ganglia of the autonomic plexuses in such viscera as the oesophagus, colon and heart. Parasitic invasion (mainly of satellite but also of ganglion cells), destruction and subsequent mononuclear infiltration has been described. Rarely is it possible to find meningoencephalitis. This phase may last for months.
III Indeterminate or latent form, in which there is no clinical disease and low parasitaemia may continue, apparently coming from continuing intracellular multiplication of parasites in various organs. This period may last indefinitely, or develop into chronic Chagas' disease. Spontaneous cure with elimination of the parasite has never been reported.

IV Chronic disease, usually appears 10 or more years after the initial infection. This phase involves either progressive myocarditis, irreversible dilation of hollow viscera, or both. The heart lesion is characterized by infiltration of mononuclear cells, destruction of myofibres and interstitial fibrosis. This is accompanied by perivascular and sometimes interstitial plasma-cell aggregation. Normally the conducting tissue is involved. Parasites are extremely difficult to demonstrate. Megaoesophagus and megacolon result from dysperistalsis, which may result from the loss of autonomic ganglion cells or other unidentified changes affecting the plexuses.

1.5 Animal models

Chagas' disease when experimentally induced in dogs reportedly closely resembles the human disease in all its phases (Johnson, 1938). In smaller laboratory animals the course of the disease varies widely depending upon the host and parasite strains used, the route of inoculation and the size of the inoculum. In the commonly used strains of laboratory mice, infection with trypomastigotes results in the lesion at the inoculation site and acute disease comparable with that in man. However, various mouse strains differ markedly in their resistance to T. cruzi (Marr & Pike, 1967). More-resistant strains might provide a good model for the chronic disease. The use of mice is particularly valuable, in view of the large amount of information available about their immunological systems.
The general pattern of Chagas' disease shows a formal similarity to that of syphilis, with its primary, secondary, latent and tertiary stages. This fact has not led to useful insights so far. Leishmanial diseases, characterized by intracellular proliferation of amastigotes, may provide insights about the intracellular phase of T. cruzi infection. Similarity might also be found between African trypanosomiasis and the trypomastigote phase of Chagas' disease. For instance, a striking resemblance exists in the clinical aspect and histopathology of the initial lesion at the portal of entry: the chagoma in T. cruzi infections and the chancre in T. rhodesiense infections. Other similarities between African and American trypanosomiasis are the cardiac lesions, particularly the fibrosis and infiltration of mononuclear cells. However, it should not be overlooked that marked differences between the two diseases occur, such as the high levels of IgM throughout African trypanosomiasis (Mattern, 1968) as opposed to the temporary rise in Chagas' disease (Lelchuk et al., 1970; Marsden et al., 1970). Furthermore, the predilection sites of lesions: the brain during the later stages of African trypanosomiasis as opposed to the viscera and heart in the American form. In addition, the acute cardiac involvement of the former differs from that of Chagas' disease in that the parasites are extra-cellular and elicit a violent inflammatory reaction. As for anaemia, haemolytic anaemia is a common complication in the African disease, while no manifest clinical signs have been reported in America. The occurrence of sub-clinical forms of anaemia in the latter, however, remains to be excluded. The nephritis and immune-complex deposits in the kidney as has been reported in experimental infections with trypanosomes of the brucei complex have not been observed in T. cruzi infections.

Diagnosis

Diagnosis in the acute stage of the disease is straightforward as it is usually possible to demonstrate trypomastigotes in the peripheral blood.
by microscopy. Diagnosis in the latent and chronic stage is, however, a different matter. During this stage, virtually the whole remaining life span of the infected mammalian host, parasitaemia is very low and difficult if not impossible to demonstrate. Direct methods, such as microscopy of thick blood films, concentration by centrifugation or other means, are considered less sensitive than multiplicative methods such as xenodiagnosis, culture or the inoculation of laboratory mice. Xenodiagnosis — the feeding of laboratory-bred, known uninfected bugs on suspected hosts and their subsequent examination some weeks later for the presence of parasites in the gut lumen — is generally accepted as the most sensitive method but it is laborious, slow to yield answers and somewhat dangerous to the personnel involved in examining the bugs.

Serological tests for anti-T. cruzi antibody (Maeckelt, 1965), such as complement fixation, indirect haemagglutination and indirect immunofluorescence, give broadly parallel results. However, standardization of materials and techniques utilized is a needed goal. These tests indicate past infection but not the present epidemiological infectivity of hosts.

1.8 Epidemiology

The general cycle of transmission of T. cruzi from vertebrate host to vertebrate host with cyclical development in triatomine bugs has been described. The most important situation as regards human infection is certainly that of houses of poor construction, which are infested with domiciliated bug species. These bugs shelter in crevices in the walls and roof and sally forth at night to feed on human and domestic animal inhabitants of the house. Intensities of infestation and infection vary widely but in bad examples there may be several thousand bugs, 70% of which may be infected. In such circumstances, the prevalence of infection in the human inhabitants may approach 100%.

T. cruzi, or at least organisms morphologically indistinguishable from T. cruzi, have also been reported from many species of triatomine bugs, many
of which do not commonly bite man, and from at least 92 species of
wild animals (C.F. Goble, 1970) - marsupials, rodents, primates, etc. -
many of which are sylvatic and do not live in close relation to man.
It seems inconceivable that these represent a single population of
organisms continuous with that infecting man. Many, surely, represent
separate cycles of transmission not impinging upon man. However, in
our present state of knowledge when we are unable to differentiate
these organisms, we can only guess at the relative importance of domestic
and wild hosts on circumstantial evidence as to the closeness of their
association with man and their overlapping with man in respect of
host-usage by likely vector species of bug. Thus, in order to work out
the natural epidemiological patterns which are of importance in understanding
the transmission of T. cruzi to man, we stand in need of methods for the
characterization of T. cruzi populations so that among the vast assemblage
of parasites that occur in nature we can distinguish those transmission
cycles which involve man. Apparently most T. cruzi-transmission of immediate
epidemiological significance is from man to man or from domiciliary animal
to man but for planning control and eradication programmes information is
needed about what sylvatic cycles might be expected to replenish the
domestic cycle involving man.

Recommendations

(1) Studies of the possible role of genetic factors in resistance to T. cruzi
infection, particularly the relation of HL-A and H2 histocompatibility
antigens, in man and in mice respectively, to the pattern of Chagas' disease
should be studied.

(2) The use of mouse strains of known genetic susceptibility to Leishmania
and comparison of strains highly susceptible to T. cruzi, such as C3H
or highly resistant, such as C57 black, could provide important information
as to predisposition.
(3) In order to study effectively both acute and chronic Chagas' disease, it is desirable to have animal models of both stages of the disease. Particularly, the usefulness of dogs and primates as models for chronic Chagas' disease should be explored. The potential complication of other widespread diseases with cardiac involvement, such as leptospirosis and heart-worm infections in dogs must be taken into account.

(4) More studies on lymphnode and spleen histopathology are desirable.

2. HOST-PARASITE RELATIONSHIPS

2.1 Variations in pathology of Chagas' disease

A basic problem in protozoology generally is the explanation of the wide range of pathological results which may be associated with infection of vertebrate hosts by organisms of identical morphology. *T. cruzi* in man is an outstanding example. The result of infection varies from clinically inevident to lethal in the short or long term. Clearly such differences in pathological outcome will be determined not solely by differences in one or other of the two components - host and parasite - but by different parasite populations interacting with host populations differing in manifold ways.

It is generally acknowledged that Chagas' disease varies in its clinical features between different areas of its geographical distribution. Acute cases are seen more frequently in Argentina than elsewhere and "mega" gut conditions are common in central Brazil, rare in Venezuela. There is need, however, for much more precise and detailed information on these matters;
a system of record and report of Chagas' disease should be established and an epidemiological investigation of a random choice of affected localities should be carried out.

It is important, therefore, to attempt to categorize one or other component, host and parasite, and, of the two, study of the parasite seems to offer the more immediate advantage. A further advantage that would probably accrue from the characterization of organism populations would be the ability to identify the sylvatic transmission cycles which are of importance for the maintenance of the human disease.

2.2 Parasite factors

One factor which may be responsible for the diversified clinical picture in Chagas' disease as it occurs in different geographical areas is the strain of parasite involved. It has been shown that different strains can behave quite differently in laboratory animals. Differences occur in respect of such characteristics as the course of infection, the degree of parasitaemia, tissue tropisms, and histopathological changes and mortalities induced. A few of these characteristics taken in isolation are of little value; however when several are taken together, *T. cruzi* strain patterns may be characterized and can provide baselines for the comparison of *T. cruzi* strains from different geographical areas. For instance, in one study 14 strains from human patients and two from infected vectors collected from houses in different parts of one region of Brazil showed essentially the same behaviour in laboratory animals. Strains of *T. cruzi* from Colombia and Peru, while differing from the Brazilian strains, remained stable in respect of such characters over years of laboratory observation.
Attempts to type strains immunologically have shown that they may be classified in serological groups (Nussenzweig et al., 1963; Gonzalez Cappa & Kagan, 1969). This grouping has so far shown no correlation with other factors such as pathogenicity, geographical distribution or epidemiology (Nussenzweig et al., 1963; Hauschka et al., 1950). With some other protozoa - *Plasmodium* and *Leishmania* - recent work on characterization by biochemical means is promising. This approach could be applied more extensively to *T. cruzi*.

2.3 *Host factors*

*Host factors* are less susceptible to standardization and so to systematic study, but it is clear that different strains of mice may vary in their response to infection with the same strain of parasite. As regards the human host, ethnic (genetic), nutritional, social and other factors have been suggested as possibly determinant.

2.4 *Strategy of study of parasite populations*

In any natural situation, be it in the vertebrate host or in the insect vector, it is quite possible that the trypanosome population is heterogeneous being the accumulation of several different infecting episodes in the past. It follows, therefore, that the procedures of introducing organisms from natural studies into the laboratory, in susceptible animals or in culture (isolation), carries with it the risk of selecting organisms other than those of particular pathological interest. Further, maintenance of organismal populations in the laboratory in serial passage either in susceptible animals or in culture, entails the danger of selecting from the isolated populations those most easily able to adapt to
the special conditions of the laboratory. These possibilities cannot be avoided entirely but may be minimized by cryopreservation of the parasite populations at the earliest possible passage level (Lumsden, 1973). The possibility that differences in "strain" behaviour in different studies are due to selection of different components of a heterogeneous population may be avoided by cloning. These procedures should, therefore, be followed wherever possible.

In considering the advantages of the study of the classification of protozoal populations, a distinction needs to be drawn between characterization and taxonomy. By the first is meant the procedures for the recognition of individual protozoal populations, by the second the interrelationships of the various types and species to one another. Drawing a parallel with the evolution of studies on the arboviruses, the procedures for the recognition of individual virus populations were immediately more pathologically and epidemiologically useful than was information on the inter-relationships of arboviruses.

2.5 Comparative value of characteristics presently available for the categorization of protozoal populations

These vary according to the degrees to which they can be related directly to the protozoal population under study or be subject to adventitious influences. A suggested classification of characteristics on this line of thought is as follows:
**Intrinsic characteristics, related directly to the organism:**

- Morphology by light microscopy, by electron microscopy;
- Chemical structure, DNA buoyant density, DNA hybridization, isoenzyme configuration;
- Antigenic characterization by humoral response (serotyping), cell-mediated response, protection.

**Extrinsic characteristics, related with the reaction to other components of the organism:**

- Behaviour in laboratory hosts, behaviour in insect vectors, behaviour in cultures, clinical outcome in man.

Antigenic characterization is included among intrinsic factors, even though it involves a component other than the organism, as it can be represented as a way of recognizing particular characteristics of the organism. As regards extrinsic characteristics, laboratory hosts, vectors and culture are susceptible to standardization to some extent and so behaviours in those contexts are regarded as more significant than is clinical outcome in man, where virtually no consistent parameters can be used.

**Recommendations**

1. Methods to induce trypanosomes to multiply in the laboratory should be improved. This could possibly be achieved by the use of particularly susceptible animals, such as particular strains of mice, or suckling mice, or different types of culture, or particular bug species or strains.

2. Systematic accumulation of low-passage, uniformly-treated cryopreserved stabilitates from defined situations is needed for various proposed study
tools, such as methods of immunological and biochemical characterization of organisms.

(3) Quantitative study on the survival of cryopreserved T. cruzi should be performed to assess the possibility of selection of populations.

(4) Methods of estimation of the infectivity of organisms should be developed. By these methods attempts should be made to quantitate the challenge to which the humans are exposed in field conditions.

(5) The characterization and taxonomy of T. cruzi should be pursued systematically on cryopreserved low-passage homogeneous materials. At this stage, characterization, i.e. the recognition of particular populations, is regarded as more immediately useful than taxonomy, i.e. the grouping of organisms by types and species.

3. IMMUNE RESPONSE IN CHAGAS' DISEASE

Circumstantial evidence strongly suggests that many of the features of Chagas' disease must have an immunological component. Among these are: acquired resistance following infection with strains of low virulence or following recovery from acute forms of the disease; proliferation of organisms in the absence of lesions followed by the sudden onset of inflammation of a type which resembles cell-mediated hypersensitivity, progressive tissue damage in the chronic phase of the disease associated with minimal numbers of organisms.

A number of findings have established the importance of the immune response in T. cruzi infections. Immunosuppression with x-irradiation, cyclophosphamide, and corticosteroids has been reported to exacerbate infection in mice, (Goble, 1970). Additionally, both neonatal thymectomy and (Schmunis et al., 1971; Roberson et al., 1973a)
treatment of antilymphocyte serum (Noberson et al., 1973b) have been reported to result in higher rates of parasitemia, greater density of tissue parasites and increased mortality. Although these experiments indicate that the infection is suppressed by immune mechanisms, the precise immunological mechanisms involved are only beginning to be understood.

3.1 Antigens

There is no evidence at present for antigenic variation in T. cruzi comparable to that in African trypanosomiasis and malaria, but this has been insufficiently investigated. Antigenic differences in T. cruzi strains have been encountered in serology (Nussenzweig, 1963; Gonzalez Cappa & Kagan, 1969). Significant progress in the isolation and characterization of individual antigens which stimulate antibody formation or cell-mediated immunity respectively (these need not be the same) is being made at present but no purified antigen, in any of these contexts, yet exists.

3.2 Specific Resistance

3.2.1 Humoral Immunity

a) Demonstration of specific antibodies

Antibodies to T. cruzi have been universally observed in patients or animals which suffer from Chagas' disease as well as in some individuals who have been exposed to T. cruzi but who are without clinical disease. These antibodies have been identified by complement fixation, direct and indirect immunofluorescent and haemagglutination techniques. The first antibody is IgM, which appears early in the disease. This is followed by IgG antibody which persists for the duration of the disease (Vat Uone et al., 1973; Marsden et al., 1970). It has been reported that, as the result of
chemotherapy, both IgM and IgG antibodies may disappear. It remains controversial whether there is generally a rise in serum IgM or IgG during acute disease, as well as a rise in IgG during chronic infections.

b) **Role of antibodies in protection**

At present the role of these antibodies in resistance is not clear. For example, antibodies have been detected in blood simultaneously with parasitemia, yet in vitro studies indicate that immune serum together with complement can lyse *T. cruzi* under appropriate conditions. In addition complement depletion in mice results in enhanced disease (Pizzimenti et al., 1977).

Most significantly, while not all reports are in agreement, a number of investigators have reported specific protection when experimental animals with early parasitaemia were treated with immune sera. In a recent study, sera obtained from mice at six weeks following recovery from acute *T. cruzi* infection appeared effective, while sera collected at earlier or later times were less effective. Conflicting reports in the literature may be explained by differences in the titre or specificity. In addition to the therapeutic effects of immune serum, prophylactic activity has also been reported (Kagan & Norman, 1961).

**3.2.2 Resistance to Chagas' disease mediated by cells***

(a) **Demonstration of specific cell-mediated immunity in Chagas' disease**

There is evidence that cell-mediated hypersensitivity reactions can be found in patients and animals with Chagas' disease. For example, delayed-type skin reactions to extracts of *T. cruzi* have been reported by some laboratories (Goble, 1970), and in vitro correlates such as blast cell transformation (Tschudi et al., 1972) and inhibition of macrophage migration have been observed (Seah, 1970).

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* The assistance of Dr A. Rowen in preparing the draft for this section is acknowledged
(b) Role of cell-mediated immunity in protection

In human leishmanial diseases, there is a spectrum of forms ranging from disease in which there is apparently no cell-mediated immunity and extensive proliferation of the organisms (kala-azar, disseminated cutaneous leishmaniasis (Convit et al., 1972) to disease where strong cell-mediated immunity occurs and the proliferation of the parasite and lesion formation are very localised (or to one single lesion), oriental sore, lupoid leishmaniasis (Maeckelt, 1972; Mauel & Behin, 1974). In both animals and man, a reciprocal relationship frequently exists between cell-mediated immunity and humoral antibody, possibly justifying the suspicion that the presence of antibody acts to inhibit the development or expression of cell-mediated immunity. No experiments have been made to establish whether these relationships hold as well for the intracellular amastigote phase of T. cruzi infection, though it is probable that they do.

While there is evidence indicating the presence of cellular immunity in Chagas' disease, it has not been demonstrated that it is involved in protection. In this regard, it has been shown that resistance in mice and rats is dependent upon the presence of thymus-derived lymphocytes which are required for cell-mediated immunity but these lymphocytes also act as helper cells in antibody formation. Further, recent studies have indicated that resistance in inbred rats can be transferred passively by means of spleen cells obtained from rats which have survived acute infections (Hanson & Roberson, 1972). It has not yet been established whether cellular transfer operates through cell-mediated immune mechanisms or is simply due to transfer of antibody-producing cells.
(c) Possible sites of action of cell-mediated immunity

Norma macrophages have the capacity of killing and degrading phagocytosed organisms and play an important role in non-specific resistance. The importance of macrophages in T. cruzi infections has long been appreciated (Pizzi, 1957; Goble & Boyd, 1962). This may be illustrated by recent observations that agents, such as silica, known to be specifically toxic to macrophages, cause marked exacerbation of the disease in mice. In contrast, non-specific stimulation of the reticuloendothelial system by diethylstilbestrol or complete Freund's adjuvant enhances resistance. (Kierszenbaum & Budzko, 1974).

Like other infectious disease systems and in some experiments with T. cruzi, virulent organisms enter macrophages, proliferate and even kill the cells. In the case of bacteria, specific immunization can provide enhanced resistance to infection by a number of unrelated microorganisms (WHO 197). In such systems it is clear that lymphoid cells carry the specificity for the response and that macrophages can be activated by them to kill the organisms. As mentioned above, in Chagas' disease it is not clear what role cell-mediated immunity plays in protection. Macrophages from specifically-immunized mice or mice non-specifically immunized and challenged with BCG have markedly enhanced capacity to kill T. cruzi in vitro.
There are at least three places in which this reaction might be involved. The first would involve localization and possibly destruction of the organisms at the inoculation site. The second level is the systemic removal and inactivation of infectious organisms by macrophages before they reach and multiply in various tissues. It should also be added that in other diseases, such as leprosy, macrophages may also play a role in spreading the infection into various organs and this must be considered. A third possible level at which cell-mediated immunity could be involved would be the destruction of infected somatic cells, such as cardiac and muscle tissue.

3.3 Role of immune response in lesion formation

There is no evidence that humoral antibody produces either cell lytic or anaphylactic lesions in Chagas' disease. With regard to immune complex lesions, while there theoretically exists an antigen excess in the early phase of the acute disease, characteristic renal lesions have not been found in an extensive series of Chagas' disease patients, in contrast to the striking immune-complex deposits demonstrated in both kidney and heart during experimental African trypanosomiasis. Further studies of the possibility that there may be such deposits in heart and kidney during the acute phase of Chagas' disease, and in the heart in the chronic phase, are needed.

In studies of human and experimental leishmaniasis it is known that infiltration of lymphocytes and histiocytes about host cells parasitized by amastigotes occurs, usually with destruction of the infected cells,
phagocytic uptake of the parasites, and their intracellular destruction in the resulting macrophages (Taliaferro & Pizzi, 1954; Scorza & Scorza, 1972). These changes are lacking in individuals without cell-mediated immunity. While no comparable studies exist for Chagas' disease, it is a reasonable presumption that the macrophage infiltrate of the chagoma and the inflammatory response to parasite antigen(s) released by the bursting of pseudocyst in the heart or other organs during the acute phase are of this type. It is less possible to be sure of the relationship of this mechanism to the occasional plasma-cell infiltrates and the extensive inflammatory and fibrotic myocarditis of chronic Chagas' disease. The ganglion and satellite cell destruction, in the ganglia, appears to be secondary to the inflammation in many cases.

3.4 Possible role of autoimmunity in Chagas' disease

There is little substantive evidence that autoimmunity plays any role in the pathogenesis of Chagas' disease (Kozma, 1962; Edjen & Lanari, 1967). The recent discovery of an antibody reactive against human heart muscles present in 95% of patients with chronic myocarditis, (Cossio et al., 1973) is of uncertain significance since this antibody reacts equally with trypomastigotes, amastigotes and guinea-pig erythrocytes. Similar heterophile antibodies are produced not infrequently in animals subjected to primary tissue damage of non-immunological origin, e.g. myocardial infarction. More thorough surveys, however, are needed in this area.
3.5 Possible role of immunologic non-reactivity on Chagas' disease

It has been suggested that specific immunological non-reactivity involving the cell-mediated component of the immune response may play a role in some forms of leishmaniasis, such as diffuse cutaneous leishmaniasis (Convit et al., 1972; Bryceson, 1970). It is appropriate to investigate the role of either of specific tolerance or of antibody-mediated inhibition of the response in Chagas' disease. While such a mechanism might be envisaged as contributing to intracellular survival and growth of the parasite, especially early in the process, existing evidence favours the view that there is an early and intense cell-mediated response.

There have been no studies in humans of possible suppressive effects of Chagas' disease lesions on general immunological reactivity. Acute Chagas' disease in mice is accompanied by lymphocyte depletion (Taliaferro & Pizzi, 1954) and a marked suppression of the plaque-forming cells' response to exogenous antigens (sheep red blood cells). The mechanism of this change is not yet clear.

An important problem for which no experimental point of attack now exists is the reason for development of severe chronic disease in a proportion of individuals after a long period of clinical inactivity. One can speculate on possible changes (brought about by age, nutrition, incidental disease or other causes) in the balance between number of parasites, humoral and cell-mediated responses, and specific or non-specific forms of non-reactivity. There could, for example, be a gradual loss of "tolerance" or of "enhancing" antibody which permits an increased level of cell-mediated immunity. Alternatively, other elements, such as autoimmunization or an antigenic change of parasite, may enter the picture.
Recommendations

1) Confirmation in different animal hosts and with different strains of *T. cruzi* that protection can be obtained by passive transfer of antibody or cells and elucidation of the mechanisms by which immune protection occurs.

2) Determination of the stage of infection where antibody or cells are most effective when transferred passively and whether they protect against the development of chronic as well as acute disease in appropriate animal models.

3) Isolation and characterization of immunoglobulin classes from antisera and elucidation of their function *in vivo* and *in vitro*.

4) Search for protective antibodies in human sera should be performed under better-defined conditions by *in vitro* neutralization methods and subsequent testing of the residual viability by animal inoculation.

5) Possible usefulness of the mouse protective models for assaying human neutralizing antibodies taken at various stages of the disease.

6) Clarification of whether the differences between effective and non-effective serum involves concentration of antibody, antibodies against determinants found in different stages of the parasite, or antibody produced against membrane versus cytoplasmic antigens.

7) It is important to confirm and extend cellular transfer studies of resistance to several strains of *T. cruzi* by means of immune cells in several animal species and to elucidate the mechanisms by:

   (a) treatment of immune cells with anti-Θ serum to ascertain whether T cells are required;

   (b) to study the effect of B cells depletion by passage through anti-immunoglobulin columns.
8) To determine if cell-mediated immunity destroys amastigote-infected tissue cells.
   (a) Are immune T cells capable of destroying tissue culture cells infected with *T. cruzi* in vitro?
   (b) Are normal lymphoid cells capable of destroying infected tissue culture cells in the presence or absence of antibodies to *T. cruzi*?

9) To clarify the role of macrophages in Chagas' disease by:
   (a) determining if there are differences in effectiveness of macrophages from normal animals in the presence or absence of antibodies to different strains of *T. cruzi*;
   (b) determining if macrophage activity is significantly enhanced by specific or non-specific activation in vivo and in vitro.

10) Characterization of the antigens involved in both humoral and cell-mediated immunity and whether they are specific for the amastigote, epimastigote or trypomastigote stages. Antibodies may be directed against determinants different from those operative in delayed-type hypersensitivity. For example, against carbohydrates, antibodies are easily produced but virtually no carbohydrate antigens can elicit cell-mediated immune reactions.

   For characterization of *T. cruzi* antigens, use of cell fractions as well as gently fixed organisms is recommended. Such characterized antigens could be used for instance in skin testing and for carrying out specific in vitro tests such as lymphocyte transformation and macrophage migration inhibition.

11) Investigation of the possibility that antigenic variation or modulation may occur under the influence of the immune response as it is assumed in African trypanosomiasis.
12) Continued study of the cell-mediated immunity status of patients in various stages of Chagas' disease, by skin testing and in vitro tests.

13) Attempts should be made to identify cells in lesions, e.g. of acute myocarditis, as T- or B-lymphocytes, macrophages or plasma cells by functional tests.

14) Similarly, the possible presence of immune complexes in lesions should be investigated using immunofluorescence.

15) A systematic search should be made for the occurrence of humoral antibodies or cell-mediated immunity against significant tissue antigens in various phases of Chagas' disease, particularly in the chronic phase.

16) To determine whether anergy to major antigens of T. cruzi is present in any phase of Chagas' disease.

17) To explore the role of antibodies or suppressor T cells in the possible suppression of cell-mediated immunity against the parasite.

4. IMMUNODIAGNOSIS

Serodiagnostic tests for Chagas' disease have been employed routinely since 1913. The first procedure to be developed was the complement fixation test with crude extract of T. cruzi as the antigen. Many modifications of this test have been employed. Up to now, most of the available data have been reported in qualitative rather than quantitative terms.

The Pan American Health Organization promoted efforts towards standardization of the procedure. A standard pooled positive Chagas' serum is being distributed by PAHO to interested laboratories through one
of their collaborating laboratories, Dr J.O. Almeida, Director, Department of Microbiology and Immunology, University of São Paulo, Ribeirão Preto, PO Box 301, São Paulo, Brazil.

Subsequently, other serodiagnostic procedures have been introduced, such as agglutination of antigen-coated erythrocytes, indirect fluorescent antibody test, agglutination of *T. cruzi* epimastigotes and precipitation of *T. cruzi* extracts. Of these tests, most experience has been obtained with the indirect fluorescent antibody test and agglutination of coated erythrocytes. They appear to be sensitive and to become positive at earlier stages of infection than the complement fixation test. On the other hand, the complement fixation test seems to be more specific (Maeckelt, 1960).

Serological tests have been used for confirmation of diagnosis in patients suspected of Chagas' disease. It has been generally experienced that in untreated patients serological positivity persists for the rest of their life.

In addition, these tests have also been used extensively for mass examinations in the framework of epidemiological studies. Recent studies by Maeckelt showed that the incidence of positive complement fixation tests in rural areas of Venezuela averaged 41%. Even though the vast majority of cases detected in mass examinations are symptomless, most investigators accept positivity in routine serodiagnostic tests as evidence of infection with *T. cruzi*. This conclusion appears to be justified since in control groups, consisting of apparently-healthy individuals living in non-endemic areas and of patients suffering from disorders other than Chagas' disease, positive results are exceptional (Freitas, 1951; Maeckelt, 1960).
The only well-documented cross-reactions with *T. cruzi* antigens were observed in complement fixation tests with sera of humans infected with *T. rangeli* and *Leishmania*. It is also noteworthy that in monkeys experimentally infected with *T. rhodesiense* positive CFT were obtained using *T. cruzi* antigen (Seah & Marsden, 1970).

Besides antibodies directed against *T. cruzi* antigens, patients suffering from Chagas' disease develop "heterophile" antibodies agglutinating sheep and rat erythrocytes (Muniz & Dos Santos, 1950; Neto & Da Silva, 1954).

**Recommendations**

1. Further comparative studies for specificity of the serological tests currently used are recommended. This could be achieved by collecting a panel of sera in various parts of the world. These sera should be tested by various laboratories involved in performing serodiagnostic tests for Chagas' disease. Sera from patients suffering from a variety of diseases, including active tuberculosis, leprosy, syphilis, neoplasia, systemic lupus erythematosus as well as sera from healthy individuals should be included in the study.

2. There is need for a simple standardized procedure which would be easily applicable in the field. The recently-developed technique using lyophilized sensitized erythrocytes (Boné, unpublished data) seems most promising. The application of chemically-characterized rather than crude extracts appears also desirable.

3. Sequential sera from individual patients should be titrated in simultaneous tests to investigate whether there is a correlation between the antibody titre and the clinical course of the disease. These studies should include tests
for heterophile antibodies, which are possibly formed in response to the release of altered autologous antigens. Therefore, this study may bring some insight into the extent of tissue damage.

(4) It is recommended that procedures should be developed for detection of soluble *T. cruzi* antigens in the patient's blood and possibly also urine. Immune sera from animals and/or patients should be used as reagents in serological procedures such as haemagglutination inhibition, gel precipitation tests including counter-immuno-electrophoresis. Should these procedures prove to be not sensitive enough, various radioimmunoassays may be explored.

(5) Detection of antigens may be helpful for diagnosis of Chagas' disease in those cases where antibodies are not detectable.

(6) There is no information available about presence of circulating antigen-antibody complexes in Chagas' disease. The demonstration of such complexes may be of value for unravelling pathogenic mechanisms.

(7) Information on the level of total haemolytic complement as well as various complement components may be of interest in view of reported C3 deficiency in monkeys infected with *T. rhodesiense*.

Studies on kininogen levels in *T. cruzi* infections also seem relevant, since decreased levels of kininogens were found in African trypanosomiasis.

(8) Tests for cell-mediated immunity. Various tests for cell-mediated immunity might prove to be of diagnostic value and this is another justification for pursuing this line of investigation.
5. VACCINATION

The fact that prior infection in experimental animals with either avirulent or virulent strains of *T. cruzi* generally leads to significant protection against acute Chagas' disease (Marr & Pike, 1967; Seah & Marsden, 1969; Yanovsky et al., 1969) is the foundation which supports the hope that protection against Chagas' disease by means of a vaccine may be feasible.

At present at least four different kinds of vaccine have been studied including: a) "avirulent" *T. cruzi*; b) killed *T. cruzi* or subcellular materials; c) irradiated *T. cruzi*; and d) other kinetoplastid flagellates.

a) "Avirulent" *T. cruzi*. "Avirulent" parasites, rendered avirulent by prolonged laboratory culture, have generally been found to be effective in stimulating protection against acute infections (Menezes, 1970). Unfortunately, evaluation of the potential use of such "avirulent" live vaccines is limited by lack of information regarding reversion to virulence and production of chronic infection which could lead to pathological changes characteristic of chronic Chagas' disease. Until the information is available, the use of such "avirulent" strains as vaccines in man cannot be recommended.

b) Killed *T. cruzi* and subcellular fractions. A great many efforts have been made to prepare non-infectious immunogens from killed organisms. These have included formalin- and phenol-killed epimastigotes, or epimastigotes disrupted by ultrasonication, mechanical disruption and disruption by freezing and thawing. In general, these preparations have
not been effective. From the standpoint of present knowledge of biochemistry, these procedures may have been too drastic and lead to denaturation of membrane and protein antigens. Recent approaches using more mild procedures are encouraging. Disruption of parasites by pressure in the Ribi apparatus have resulted in antigenic preparations that reduce parasitemia and mortality. Additionally, organisms killed by mild chemical means, e.g. chaotropic ions such as 2.5M perchlorate, have produced significant protection in mice.

c) **Irradiated T. cruzi.** Another approach which has been successful with a variety of infectious diseases is the use of organisms of virulent strains rendered avirulent by exposure to gamma or X-irradiation. T. cruzi trypomastigotes irradiated with 150 kr were rendered non-pathogenic for susceptible mice and non-cytopathogenic for cell cultures. Multiple immunizations with such irradiated trypomastigotes were found to produce protection in mice. It should also be mentioned that there is some evidence already to indicate that adjuvants may be useful in increasing the degree of protection.

d) **Other kinetoplastid flagellates.** Protection in mice following inoculation of living culture forms of plants flagellates have been reported.

While these procedures have been promising, none of the methods presently studied have resulted in absolute protection under conditions of challenge with large numbers of parasites. It is important that studies
of these and improved methods be continued and that approaches to an understanding of the basic immunological mechanisms which are operating in protection be made. This raises the general question of strategy and goals in immunization or vaccination against \textit{T. cruzi}. For example, antigenic preparations effective in inducing high antibody levels for the trypomastigote form might be expected to be protective at the level of preventing parasites from leaving the bloodstream and entering the tissues. Yet it is not clear whether, with an infective dose in the thousands or greater, antibodies can be totally effective in this, or further whether such antibodies could predispose to immune complex tissue damage if the infection became chronic. On the other hand, it is not yet clear to what extent cell-mediated immunity plays a role in protection. While it could be active in localizing invading parasites at the invasion site or later in the tissue cells by preventing large scale proliferation of the organism, it may be responsible for producing some of the cardiomyopathy and tissue damage associated with chronic disease.

Until the basic immune mechanisms involved in protection and in tissue pathology are better understood, it will not be clear whether the objective of an "ideal" vaccine for Chagas' disease should be a) to increase the level of humoral antibodies with little cell-mediated immunity; b) to increase both cell-mediated immunity and antibodies; c) to employ an antigen which persists only a short time to serve as an immunological "primer" in endemic areas; or d) to employ a long-persisting antigen source.
Recommendations

(1) Evaluate in experimental animals presently-available vaccines for effectiveness in protecting against acute disease and also in preventing development of chronic disease, particularly in dogs and monkeys.

(2) To study the possible augmentation of the effectiveness of present vaccines by simultaneous administration with adjuvants such as alum, pertussis vaccine, BCG, Freund's adjuvant or levamisol.

(3) To study the effects of repeated exposure to T. cruzi in vaccinated dogs or monkeys.

(4) To study the possible immunopathogenic effects of vaccination in animals with chronic infection, as models for possible cases of humans with unsuspected chronic infection.

(5) Development of improved vaccines, including purified subcellular fractions of specific forms, e.g. amastigote or trypomastigote, and non-pathogenic strains unlikely to induce disease in humans, such as plant trypanomatids.

(6) To consider whether immunological methods by themselves may be adequate to confer full protection against Chagas' disease or whether immunization to reduce acute disease could be coupled with chemotherapy to reduce or cure the chronic form.

(7) For the proper assessment of the protection afforded by different vaccination procedures, it is important that the methods should be developed to quantitate the challenge used in terms of infectivity rather than simply as numbers of organisms, as these two parameters may not be directly related.
6. GENERAL RECOMMENDATIONS

(1) No introduction to any aspect of the study of T. cruzi which involves the actual handling of the organisms, would be complete without a word of warning about the dangers involved. T. cruzi is an organism of high infective potentiality in both its bloodstream and its culture forms, and it may cause a dangerous disease for whose treatment only unsatisfactory chemotherapeutic agents are as yet available. Some recent work indicates that spread of infection by aerosols, via the nasal mucosa is possible. Nevertheless, with due attention to the usual precautions followed in microbiological laboratories for organisms of comparable danger, T. cruzi may be handled with confidence.

(2) The point of the advantage of an up-to-date circulation of information on Chagas' disease via the WHO Trypanosomiasis Information Service has already been made. There is, as well, a large accumulation of information in the literature since early in the century which is largely inaccessible because of language or journal distribution problems. Some preliminary moves have been made in Europe and the USA towards producing a computerized bibliography of the subject which would aid the systematic assessment and retrieval of this information. It is recommended that the production of such a computerized bibliography should be supported.

(3) Too frequently critical comparison of the results of different laboratories with a given Trypanosoma "species" is precluded by questions of the identity, relationship, homogeneity, etc. of the materials under discussion. The procedures for the elimination of these drawbacks have
been indicated above — special attempts to establish "difficult" materials in the laboratory, cloning, cryopreservation, etc. are inevitably laborious. It is, however, impossible to evade a special effort in this sphere so as to provide critically defined material for exchange between laboratories for special studies.

(4) Comparison of the immunological effects of _T. cruzi_ either as whole organisms, living or dead, or as any of its constituent antigens, will be reliable only in so far as the materials used in the different situations, perhaps in different laboratories, are homogeneous and identical. The whole range of _T. cruzi_ possibly concerned in the natural disease must be included in the experimentation.

(5) There is a need for development and characterization of additional animal models for chronic disease as well as acute disease for use in immunological studies.

(6) There is a great need for more basic research on the fundamental immunological mechanisms involved in protection against _T. cruzi_ infection. Particularly the effectiveness and rate of action of immune sera must be established. Additionally, the role of cell-mediated immunity in protection must be clarified.

(7) Identification, isolation and characterization of antigens involved in the immune response to Chagas' disease is essential to further immunological studies. Specifically, attempts must be made to define antigens which distinguish different forms of the parasite and different strains, as well as to determine which are involved in generating humoral antibodies or delayed-type hypersensitivity. This is also required for better immunodiagnostic tests.
(8) The role of humoral antibodies and cell-mediated immunity in the development of pathological changes in chronic as well as acute forms of the Chagas' disease must be further clarified.

(9) More basic knowledge of the immunological mechanisms involved in protection and in immunopathology, both in natural infection and following vaccination in experimental animals, will be required to achieve an effective and safe human vaccine.
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