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Review

THE FUNDAMENTAL NATURE OF CHAGAS' DISEASE: A VIEW PROVIDED BY IMMUNE RESPONSES AND IDIOTYPES

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Abstract: Chagas' disease is caused by the protozoan *Trypanosoma cruzi* which infects 10-15 million people in endemic areas throughout Latin America and is naturally transmitted by insect vectors of the family Reduviidae. Infection can also occur by congenital passage, oral ingestion, laboratory accident, and in organ transplants and blood transfusions. There are 3 life-cycle forms of *T. cruzi*. Epimastigotes multiply in the midgut of the insect vector, differentiate in the hind-gut into infectious trypomastigotes, and are excreted with the feces or urine after a blood meal. They enter the body by mucous membranes or abraded skin, enter mammalian host cells, escape the phagolysosomal vacuole, and differentiate into amastigotes. Intracellular amastigotes multiply in host cells, redifferentiate to trypomastigotes, and are released upon cell rupture.

Acute Chagas' disease can be asymptomatic, or a mild to severe illness. Morbidity can be localized and/or systemic and is usually accompanied by general immunosuppression with blood and tissue parasitemia. It can be fatal. Usually, symptoms and parasitemia decrease within months, followed by a life-long, chronic infection with little morbidity and few apparent parasites. Serologically-positive, chronic asymptomatic patients are termed indeterminate (I). Morbidity develops in 20-30% of chronic patients after 10-30 years and often involves myocardiopathy (Cardiac disease; C), ranging from minor electrocardiographic changes to sudden death by heart failure. Severe "digestive" megasyndromes can also develop. Chagasic myocardial inflammatory infiltrates are associated with cardiac muscle and neuron destruction, followed by progressive fibrotic replacement. Asymptomatic I-patients who die of unrelated causes have identical (but less intense) lesions.

Responses against *T. cruzi* antigens, their immunoregulation, and responses against related idiotypes (Ids) correlate with the presence of the different clinical forms of the infection. Although there are numerous findings of autoimmune lymphocyte and antibody reactivities in chagasic patients, a causal relationship is always difficult to prove. Chagasic patients' peripheral blood mononuclear cells (PBMC) respond to *T. cruzi* epimastigote antigens (EPI) with varying vigor. Almost all low responders are C-patients, and their responses are usually augmented by removal of adherent macrophage suppressor cells or the addition of indomethacin. Chronic I-patients are medium or high responders, and exhibit little adherent cell-mediated immunoregulation. Western blotting

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studies indicate patients' Ab and cell-mediated responses to EPI show differences between C- and I-patients. PBMC from I-patients responded more often to high molecular weight components (100-150 kD), while both I- and C-patients responded well to moieties between 28-32 and 48-57 kD. All chagasic patients' sera had Abs and PBMC responses to *T. cruzi* GP57/51 antigen.

Chronic Chagas' patients have peripheral blood anti-Id T cells that respond to anti-EPI Abs purified from patients' sera. Some patients' PBMC anti-Id responses to anti-EPI Ids from C-patients (Id-C) are inhibited by chloroquine (Group 1), but some are not inhibited by chloroquine, anti-HLA Class II antigens, or sodium azide (Group 2). Most patients in Group 1 are asymptomatic, but all Group 2 patients have severe disease. Direct (non-processed; non-MHC-presented) stimulation of anti-Id T cells from C-patients by Ids expressed on anti-EPI Abs from C-patients could be immunopathogenic. Anti-Id specific rabbit sera detect Id differences in the anti-EPI Abs from pooled or individual C *vs.* I-cases. Competitive ELISA assays and Western blot analyses of Abs show that Ids on I-patients' anti-EPI Abs are primary structure expressions, while Ids on C-patients' anti-EPI Abs are defined by intact Ab molecules.

The chronic, endemic nature of Chagas' disease allows maternal/neonatal Id interactions that might influence later immune response and immunoregulatory abilities of children born of infected mothers. Such interactions occur because cord blood mononuclear cells from chagasic mothers' children respond to Ids on anti-EPI Abs. A hypothesis based on Id-induced pathology and immunoregulation will be described that could account for certain aspects of the immunology and pathology of Chagas' disease.

History and prevalence

Chagas' disease (South American Trypanosomiasis) is an endemic zoonosis produced by *Trypanosoma cruzi* which is found presently only in the Americas. The disease is named for the 29 year old Brazilian physician, Carlos Chagas, who in 1909 identified and described the parasite in the hind-gut of insects from the family *Reduviidae*. Dr. Chagas later observed its pathogenicity in mammals and located and described many of the domestic (dogs, cats, goats, etc.) and wild (armadillo, opossum, etc.) reservoirs. He went on to demonstrate this infection in humans and described its acute and chronic stages. Recent epidemiologic studies in South America suggest there may be 10 to 15 million people serologically positive for *T. cruzi* (Brener, 1982; Chagas, 1988). Reports on the incidence of infection crudely estimate it to be over 800,000 new cases each year with an annual mortality of around 60,000 (Dias, 1987).

Like many parasitic infections, Chagas' disease is most common in, but not confined to, people occupying the lower socioeconomic classes. Contact with the vector and transmission have been correlated most strongly to poor housing construction and inadequate vector control (Marsden, 1984). These factors play a major role in transmission of Chagas' disease as the vector and trypanosome are much more widely distributed than is human infection (Grogl *et al.*, 1984; Beard *et al.*, 1988; Yaeger, 1988).

Transmission in endemic areas is largely vector-related and thus limited to the Americas, but recent reports have shown that congenital passage (Azogue *et al.*, 1985), laboratory accidents (Brener, 1987), organ transplants and blood transfusions (Kirchhoff, 1989) play a considerable role in transmission in endemic countries and may become a serious problem for non-endemic countries as well. Two recent reports of transfusion related *T. cruzi* infection in North American hospitals have stressed the danger which *T. cruzi*

contaminated blood could pose for non-endemic countries (Grant *et al.*, 1989; Nickerson *et al.*, 1989). These cases involved two patients undergoing chemotherapeutic treatments for leukemias. Each of these patients had received platelets from Latin American immigrants. These blood products were screened for conventional infectious contaminants and deemed fit for use, but there are no protocols in the United States of America or Canada which consider *T. cruzi* contamination. Screening of donors within endemic areas of Latin America is becoming more common and there are highly sensitive and specific serological assays available (complement fixation, indirect immunofluorescence and ELISA) which should help decrease the risk of transfusion or transplant related infection. Presently, Brazil reports 10,000 to 20,000 transfusion related *T. cruzi* infections a year (Brener and Camargo, 1982), and 5-51% of the blood units recently tested in Bolivia were serologically positive (Carrasco *et al.*, 1990). With the recent increase in immigration of people of Japanese heritage from Brazil to Japan, transfusion related Chagas' disease may become something that the Japanese medical community will need to consider in the future.

Biology of *Trypanosoma cruzi*

Trypanosoma cruzi has a complex life cycle involving both vertebrate and invertebrate hosts (Pereira, 1990). Under natural conditions, the trypanosome is transmitted to its definitive mammalian host via the feces or urine of an infected reduviid bug of the genera *Triatominae, Rhodnius,* or *Panstrongylus* (Ghauri, 1973). Those strictly hematophagous reduviids which are the most efficient vectors of human infections defecate shortly after taking a blood meal. The feces and urine contain the infectious trypomastigote stage which develops from the non-infectious, asexually dividing epimastigote form found in the hind-gut of the vector. Trypomastigotes cannot penetrate intact epidermis but enter through breaks in the skin or through intact mucous membranes. Therefore, infection is not due to a direct inoculation by the insect vector, but rather indirectly through the victim wiping and scratching at the bite and subsequently contaminating the wound, their eyes or mouth. Evidence of primary infection is sometimes seen at the site of entry as localized inflammatory reaction resulting in an indurated nodule at the bite (chagoma) or by unilateral periorbital swelling and conjunctivitis (Romana's sign).

The non-dividing blood stage trypomastigote form has the well described ability to avoid complement lysis (Joiner *et al.*, 1988; Rimoldi *et al.*, 1988) and phagocyte killing (Osuna *et al.*, 1986; Reed, 1988; Reed *et al.*, 1989) and is capable of receptor mediated entry of a wide variety of host cell types (Velge *et al.*, 1988; Davis and Kuhn, 1990). Once inside the cell the trypomastigote produces a recently described perforin-like molecule, analogous to C9 of the complement cascade, and passes from the parasitoferous vacuole into the cytoplasm of the cell (Andrews *et al.*, 1990). The trypomastigote differentiates into the amastigote form in the cytoplasm. Amastigotes multiply asexually and develop into trypomastigotes while liberating antigens into the host cell cytoplasm (Andrews *et al.*, 1988). These antigens theoretically would be expressed in a Class I restricted manner on the surface of infected cells. Whether acute and chronic lesions are related to host cell lysis by either immune cytotoxicity or passive rupture due to parasite load is not clear. The release of parasites results in trypomastigotes and amastigotes in the circulation. These organisms are capable of disseminating and parasitizing diverse tissues throughout the host or can be taken up by a feeding reduviid to complete the cycle (Andrews *et al.*, 1987).

The intracellular, dividing amastigote stage is readily identified in histologic sections of cardiac, intestinal and cerebral tissues from infected individuals early in the course of infection and is localized predominantly to neuronal or conductive fibers within the parenchyma of these organs (Andrade *et al.*, 1978, 1984; Said *et al.*, 1985). Sequestration of the parasite in these intracellular sites confers a significant survival advantage to *T. cruzi*. The intracytoplasmic residence of these organisms means that effective sterilizing chemotherapy regimens would need to achieve trypanocidal concentrations of drugs inside host cells. This is not thought to occur with the current drugs of choice (nifurtimox and benzinidazole) and poses a major challenge in the area of drug development and chemotherapy (Filardi and Brener, 1987; McCabe, 1988). The intracellular location of the parasite also allows it to largely avoid the immune response. The host's early immune responses induce a resolution of blood parasitemia but they are unable to clear the persistent, intracellular infection. It is commonly assumed that intact host cells with resident intracellular amastigotes do not elicit inflammatory responses early in infection. The intense focal inflammation typical of acute infection is believed to occur only after the rupture of parasitized cells which exposes the host to concentrations of parasite antigen. However, during chronic infection parasites are scarce in blood and are usually not found within the observed diffuse inflammatory responses in host tissue. This has lead to the widely proposed, but still controversial, theory of an autoimmune basis for Chagas' disease (Takle and Hudson, 1989; Hudson and Hindmarsh, 1985).

Clinical aspects of infection

Chagas' disease, like many chronic endemic parasitic infections, demonstrates a spectrum of clinical presentations. The initial infection can go completely unnoticed or result in lethal cardiac infarctions. Chronic patients range in presentation from asymptomatic but serologically-positive to severe debilitation and death. This heterogeneity is no doubt due to a multitude of parasite and host factors but recent reports have begun to link specific host immune responses during infection to the various clinical stages (Gazzinelli *et al.*, 1988a, b; Reis *et al.*, 1989; Gazzinelli *et al.*, 1990; Colley *et al.*, 1990). Interestingly, although the acute phase of primary infection does occur in endemic human populations it is rarely seen by health care workers. Oftentimes, acute cases of parasitic diseases in non-endemic people present as severe infections in contrast to those of endemic peoples (von Lichtenberg, 1987; Nash *et al.*, 1982; Ottesen, 1984). These findings suggest that there are many individuals within endemic populations who are somehow able to better modulate the severity of disease in the acute, and possibly chronic, phases of infection with parasites.

When acute chagasic infection is clinically apparent it is usually defined by a marked parasitemia with symptoms ranging from mild fatigue and fever to severe myocarditis and death (5-10% of acute patients) (Nogueira, 1986). Diagnosis is made by demonstrating parasites in stained blood smears or live parasite isolation (xenodiagnosis or hemoculture) and is occasionally aided by description of a chagoma or Romana's sign. Diagnosis of acute Chagas' disease by serological testing is generally of little use possibly due to the rapid and severe immunosuppression seen in these patients (Brener, 1980; Kuhn, 1981; Beltz *et al.*, 1989).

Because the acute phase is not often recognized, data on cellular and humoral events during acute Chagas' disease are generally not available. A few reports discuss the humoral response progression during acute accidental infection of laboratory workers from sero-

negativity through to seroconversion and frank parasitemia (Brener, 1984, 1987; Hofflin *et al.*, 1987). But little is known about cellular reactivities during this phase of human infection. The acute phase of experimental Chagas' disease has been widely studied (Teixeira *et al.*, 1975; Tarleton and Scott, 1987). Acute murine infection with *T. cruzi* demonstrates a tremendous polyclonal stimulation involving both B and T cells. Nearly half of all spleen cells appear to be blast cells undergoing mitosis. This response is truly polyclonal: all Vh-gene families studied (covering more than 95% of the repertoire of the entire locus) are stimulated (Minoprio *et al.*, 1989). The majority of these activated splenic and lymph node plaque forming cells are not specific for the parasite (Minoprio *et al.*, 1988). There are no reports in the literature concerning the existence or the extent of this polyclonal activation in humans but its presence has been proposed to play a role in the marked immunosuppression and possible autoimmunity seen in human trypanosomiasis (Petry and Eisen, 1989). Similar panlymphocytic, polyclonal proliferation has been reported in viral infections, bacterial (leprosy) infections, and *Leishmania* infections (Petry and Eisen, 1989). All of these organisms develop in macrophages. During the acute phase of infection *T. cruzi* also largely infects macrophages (McCabe *et al.*, 1984; Villalta and Kierszenbaum, 1984). Because of this unifying observation, several researchers have proposed roles for cytokines produced by the infected macrophages as being responsible for the immunological disturbances seen in *T. cruzi* infections (Kierszenbaum and Wirth, 1987; Reed, 1988; Wirth and Kierszenbaum, 1988; Tarleton 1988; Reed *et al.*, 1989).

The chronic phase of the disease usually presents 20-30 years after initial infection and often without history of acute disease (Nogueira, 1986). Intracellular parasites are believed to maintain a lifelong tissue infection but trypomastigotes are typically scarce or absent in the blood as parasitemia is tightly controlled by the host's immune response. This becomes very apparent in those chronic patients who, for reasons unrelated to their Chagas' disease, receive immunosuppressive therapy and subsequently develop a new "acute" infection complete with patent parasitemia (Brener, 1980; Kierszenbaum *et al.*, 1983; Hudson and Britten, 1985). In the chronic phase, demonstration of parasitemia is generally very difficult because of the low numbers seen in the peripheral circulation. Simple blood smears are completely useless for diagnostic purposes. This requires very sensitive techniques which rely on a long multiplicative phase either in uninfected reduviid bugs allowed to feed on patients' blood (xenodiagnosis) or *in vitro* culture (hemoculture) of parasites from patient blood directly. For this reason serology is usually the method of choice for positive diagnosis of chronic Chagas' disease (Brener, 1982). New approaches using molecular biological techniques such as the polymerase chain reaction (PCR) for highly sensitive and specific detection of organisms are now being explored (Moser *et al.*, 1989; Sturm *et al.*, 1989).

Most chronic patients are asymptomatic and typically present with positive serology and sub-patent parasitemia. Between 60% and 80% of people infected with *T. cruzi* fall into this category and are termed "indeterminate" (Brener and Camargo, 1982). By definition, these people do not suffer from disease related morbidity and mortality, but this does not mean that they are free from pathology caused by their infections. A series of autopsies performed on "sudden death" cases in an endemic area indicate varying degrees of sub-patent chagasic cardiac pathology are present in indeterminate individuals (Brener and Camargo, 1982; Pereira-Barretto *et al.*, 1986). This has lead to the hypothesis that the clinical forms of Chagas' disease represent a progression rather than distinct and unrelated disease states.

Why certain infected individuals progress more rapidly to morbidity while others regulate the infection their entire lives without clinically apparent disease is not known.

The minority of chronic patients (20-30%) demonstrate a more severe chronic course (Brener and Camargo, 1982). Patients with the "cardiac" clinical form demonstrate severe lesions to the conducting and muscular structures of the heart which commonly lead to a dilative cardiomyopathy and congestive heart failure. Lesions of the conducting system of the heart may lead to right bundle branch block and life threatening arrhythmias. This is evident in electrocardiographic changes, and rich inflammatory infiltrates can be demonstrated in post-mortem sections of the heart— often in the absence of demonstrable parasites or parasite antigens (Andrade *et al.*, 1978). The clinical finding of chagasic cardiopathy include recurrent palpitations and ECG changes combined with cardiac insufficiency and heart enlargement. The electrocardiographic changes commonly involve right bundle branch block with occasional complete atrio-ventricular block. Fibrosis and chronic inflammation are prominent features of the histology of chronic chagasic cardiac pathology (Ribeiro dos Santos and Rossi, 1985; Carrasco-Guerra *et al.*, 1987; Brener and Krettli, 1990). It is this cardiopathology which contributes to Chagas' disease being among the leading cause of death in adults in some endemic areas.

A further subgroup of chronic patients with severe Chagas' disease present with aperistalsis and dilation of the esophagus, large bowel or both. These patients have "megadisease" or the "digestive" clinical form. Histology of lesions typically shows mononuclear infiltrates in the myenteric plexus of the gut wall with demyelination and sclerosis of surrounding conductive fibers. The loss of autonomic control and subsequent dilatation are believed due to the inflammatory destruction of the ganglionic plexus and nerve fibers. However, direct destruction by intracellular parasitism no longer observed at the site cannot be ruled out (Hudson and Hindmarsh, 1985; Hudson and Britten, 1985). A small minority of patients demonstrate both cardiac and digestive involvement.

Lesions and their possible immune etiology

The etiology of chronic chagasic pathology has been examined in a number of experimental animal models and in human infection. In 1974, Cossio and co-workers (Cossio *et al.*, 1974) reported a series of experiments concerning antibodies reacting with endocardium, vascular structures, and interstitium of striated muscle (EVI antibodies) present in the sera of patients with chronic chagasic cardiopathy. This was one of the first findings which suggested a mechanism for the lesions to heart and digestive tract tissues and implicated an autoimmune etiology. These EVI antibodies reacted with human, mouse, bovine and guinea pig heart tissue from non-infected subjects. In these studies, similar cross-reactive antibodies were found in over 90% of patients with chronic chagasic cardiopathology. Later findings that these antibodies could be absorbed by epimastigotes and by laminin lent further support to this theory (Szarfman *et al.*, 1982; Brener *et al.*, 1983; Gazzinelli *et al.*, 1988d). However, these results have been difficult to reproduce, and it is now known that EVI-autoantibodies are also found in the sera of over 40% of indeterminate patients and in patients with other parasitic infections (malaria, leishmaniasis, African trypanosomiasis and *Trypanosoma rangeli*) (Khoury *et al.*, 1983; Avila *et al.*, 1984; Kierszenbaum, 1986; Avila *et al.*, 1987; Towbin *et al.*, 1987). These findings have fueled debate over the significance of these antibodies in the pathogenesis of *T. cruzi* (Kierszenbaum and Hudson, 1985).

Many mouse and rat monoclonal antibodies exist which are reported to cross-react with *T. cruzi* and host tissue. Some of these antibodies and the parasite and host epitopes recognized have been defined in detail. For example, Eisen and colleagues have described two anti-*T. cruzi* monoclonals, VESP 6.2 and VESP 8.2, which were shown by indirect immunofluorescence to recognize different glycolipids on the surface of fixed, CL-strain trypomastigotes and also epitopes on cerebellar neurons in culture and frozen sections of heart, digestive tract and peripheral nervous tissues (Petry and Eisen, 1989). Another mouse monoclonal antibody (CE5) raised by Wood and colleagues against membranes of rat dorsal root ganglia also recognizes epitopes on the surface of fixed Y-strain epimastigotes (Wood *et al.*, 1982). Western blots using CE5 demonstrate a complex banding pattern of proteins (50-100 kD) in both epimastigote and amastigote antigen preparations which may reflect a common amino acid sequence in the distinct proteins of these two life cycle stages. CE5 also recognizes several bands on Western blots of preparations from rat heart, intestine and brain but not from tissues known not to be affected in Chagas' disease. Snary and his co-workers have used monoclonal antibodies derived from immunized and infected mice to demonstrate cross-reactivity between epitopes on the parasite surface and those found on mouse central and peripheral neurons and glia (Snary *et al.*, 1983). All of the monoclonal antibodies discussed were shown to have no activity against the culture medium or fetal bovine serum used to raise the parasites, making it unlikely that these cross-reactivities are due to antigen scavenging by *T. cruzi.*

The existence of cross-reactive clones such as those described above is not unexpected in situations of intense lymphocyte polyclonal activation. This has been shown in, and is correlated with, pathogenesis in a number of autoimmune diseases (Klinman and Steinberg, 1987). If Chagas' disease does have an autoimmune component, its etiology may be related to polyclonal stimulation of lymphocytes such as that observed in experimental *T. cruzi* infection of mice. It is interesting to note that some systemic autoimmune disease in both humans and mice are characterized by hypergammaglobulinemia and abnormally high levels of serum autoantibodies, two things known to occur in acute human and experimental Chagas' disease (Minoprio *et al.*, 1989). How these observations correlate with pathology and what is responsible for the breakdown of normal control of humoral autoreactivity in these diseases are not clear at present.

Cell transfer studies by Laguens and colleagues gave the first evidence suggesting a T cell-mediated autoimmune etiology in experimental Chagas' disease (Laguens *et al.*, 1981). These studies used "parasite-free" non-adherent spleen cells isolated from infected mice to create lesions in non-infected syngeneic recipient mice only 4 days after cell transfer. In similar studies, some recipient animals later died of *T. cruzi* infections so that parasite generated pathology could not be ruled out. Recently, Hontebeyrie-Joskowicz and co-workers have used CD4+ T cell clones to create truly parasite free pathology in an MHC-restricted manner in healthy mice. These CD4+ clones were shown to have specificity for both epimastigote antigen and syngeneic murine sciatic nerve antigen giving strong support for a cell-mediated autoimmune mechanism in chagasic cardiopathy (Hontebeyrie-Joskowicz *et al.*, 1987; Ben Younes-Chennoufi *et al.*, 1988).

The above observations have lead to several theories on the development of the pathogenesis associated with *T. cruzi* infection. The most widely tested hypothesis involves generation of autoreactivity due to infection. The mechanisms involved in the development

of this auto-reactivity are unknown but several models are commonly addressed in the literature. A few of these include: 1. Antigen mimicry; 2. Polyclonal blastogenesis of both B and T cells, stimulating a B cell repertoire already naturally expressing a large number of anti-self reactivities; 3. Development of autologous responses to the idiotypes or the isotypes of the original antigen specific clones. How these possibilities might occur and how they would relate to specific lesion development in chronic infection with *T. cruzi* is only beginning to be examined.

The spectrum of immunologic responses

The clinical spectrum observed in patients infected with *T. cruzi* allows speculation regarding differential host immunoregulatory mechanisms that may contribute to these differences in morbidity. The remainder of this article will summarize findings concerning responses against *T. cruzi* antigens, and their immunoregulation, and responses against idiotypes (Ids) associated with these anti-*T. cruzi* responses. It will present, analyze and hypothesize about immunologic profiles which correlate with the presentation of different clinical forms of the infection.

In Chagas' disease, peripheral blood mononuclear cells (PBMC) proliferate to soluble (Morato *et al.*, 1986) or nitrocellulose-fixed (Gazzinelli *et al.*, 1990) parasite antigen. If the *in vitro* responses to soluble epimastigote antigens (EPI) are subdivided into levels of responsiveness and analyzed, almost all of the low responders are clinically classified as "cardiac" (C) patients (Gazzinelli *et al.*, 1988a; Colley *et al.*, 1990; Morato, M.J.F. *et al.*, submitted for publication). The responses of these patients are also augmented more, relative to those of "indeterminate" (I) patients, by the removal of adherent cells (Morato, M.J.F. *et al.*, submitted for publication). PBMC from chronic I patients usually respond well to EPI and these responses and are not apparently regulated by adherent cells. These data, coupled with the observations that C patient PBMC responses are often partly augmented by the addition of indomethacin (Morato, M.J.F. *et al.*, submitted for publication), suggests a role for prostaglandin-mediated adherent macrophage suppression in the development or expression of clinical disease. It is possible that strong cellular responses against some of the parasite components in EPI are essential to hold the parasite in check, thus decreasing the chance of parasite-induced related morbidity.

There have been a number of studies examining humoral responses of patients infected with *T. cruzi* (Kuhn, 1981; Snary, 1985; Nogueira, 1986). Recent research has compared patients' antibody responses by Western immunoblotting against separated EPI in parallel with their cell-mediated responses by T cell-Western blotting (Gazzinelli *et al.*, 1988a, 1990). These experiments have shown some differences between C and I patients. PBMC from I patients responded more often to high molecular weight EPI components (100-150 kD) while both C and I patients cells responded to molecules in the 28-32 kD and 48-57 kD ranges. But while there was an apparent difference in the recognition by T cells of separated antigens, none of the specific antigen bands were recognized preferentially by sera of patients with one clinical form *vs.* another. These studies further describe a glycoprotein component of EPI which migrates diffusely between 57 and 51 kD (GP57/51) (Scharfstein *et al.*, 1986; Murta *et al.*, 1988). GP57/51 antigen is recognized by all patients' sera and cells, and a given patient's PBMC responses to this purified antigen correlate with their responsiveness to crude EPI. It appears that a major portion of the cellular reactivity against crude EPI is due to responses

specific for GP57/51. The biological significance of GP57/51 and its potential use as a diagnostic antigen are the focus of ongoing studies.

The role of Id/anti-Id interactions in the regulation and maintenance of the immune response was first proposed by Jerne in 1974 (Jerne, 1974). It has been demonstrated since that time that repeated, long-term exposure to antigens can lead to the development of dominant cross-reactive Ids (CRI), and that these CRI and anti-Ids against them may play multiple roles in the responses to immunizations or during infections (Bona, 1987; Kearney, 1989; Cerny and Hiernaux, 1990). Id/anti-Id interactions have been demonstrated to be integral to the ability of the host to regulate some responses to immunologic challenges and have also been implicated in the pathogenesis of certain autoimmune diseases. We have begun to explore the possibility that Id/anti-Id systems, which occur in Chagas' disease, are related to the clinical differences that occur in this infection.

In studies which parallel findings in human schistosomiasis mansoni (Colley *et al.*, 1989), chagasic patients' PBMCs proliferate upon exposure to anti-EPI immunoaffinity-purified antibodies (anti-EPI) from patients' serum (Gazzinelli *et al.*, 1988b, c; Colley *et al.*, 1990). As in schistosomiasis, PBMC from patients who have never been exposed to *T. cruzi* are not stimulated by these antigen specific antibodies. Controls for these experiments demonstrate that neither normal Ig or immunoaffinity-purified antibodies to antigens of other non-related parasites stimulate PBMC of chagasic patients to proliferate.

Gazzinelli and co-workers (Gazzinelli *et al.*, 1988b) observed that anti-EPI purified from sera of chronic chagasic patients with cardiac disease (Id-C) were generally more stimulatory for PBMC (or T lymphocytes) than anti-EPI Id isolated from sera of indeterminate patients (Id-I). This was seen regardless of the clinical form of the donor of the responding cells. This was the first demonstration of anti-Id T lymphocytes in Chagas' disease, and the correlation with clinical disease suggested a possible role for Id/anti-Id responses in the generation of chronic chagasic cardiopathy.

This Id stimulation of auto-anti-Id T cells from Chagas' patients was observed to require the presence of an adherent cell population. Recently, a difference in the requirements for chronic chagasic T cell-recognition and response to Id-C and Id-I became evident while examining this role of adherent cells in antigen processing (Colley *et al.*, 1990; Gazzinelli, R. T. *et al.*, submitted for publication). As expected, antigen (EPI) stimulated responses of all patients are completely ablated in the presence of 25 μM chloroquine. This level of chloroquine is known to block lysosomal enzymatic degradation by raising the pH of lysosomes and thus blocking processing and presentation of antigen to T cells (Unanue, 1984). Chloroquine also inhibited Id-I stimulation of PBMC from either C or I patients, but strikingly, when Id-C preparations were used as the stimulus, two different response patterns were observed in the presence of chloroquine (Colley *et al.*, 1990; Gazzinelli, R.T. *et al.*, submitted for publication). The PBMC responses of one group of patients (Group 1) resembled the responses to either Id-I or EPI. Their anti-Id-C responses were markedly inhibited by chloroquine (responses were decreased by greater than 40%). In contrast, a second group of patients (Group 2) had anti-Id-C responses which were not significantly inhibited or were augmented in the presence of chloroquine. Most Group 1 patients were I, and surprisingly, all 14 Group 2 patients had severe Chagas' disease-related morbidity. This is a striking clinical correlation. It is possible that the direct (non-processed; non-MHC-presented) stimulation of anti-Id T cells seen with cells and Ids from C-patients could be related to the immunopatho-

genesis of the disease.

Recent experiments in progress (Reis *et al.*, 1989) have shown that anti-Id specific rabbit sera can detect Id differences in the anti-EPI Abs from pooled or individual sera from C *vs.* I patients (i.e. Id-C *vs.* Id-I). Preliminary competitive ELISA assays and Western immunoblot analyses of these Ids confirm that the Ids on Id-I are associated with the primary structure of Ig heavy and light chains, while the Ids on Id-C are defined by conformation, requiring the presence of intact, non-denatured immunoglobulin molecules. These data are completely compatible with those results from the *in vitro* anti-Id T cell proliferation studies where processing and presentation was required for Id-I stimulation, but not Id-C stimulation, and delineated the different clinical forms.

Presently, it is conjectural how differential processing requirements for anti-Id T cell responses among patients with different clinical forms of Chagas' disease is related to pathogenesis. It is at least possible that the lesions could result from repeated, unregulated direct stimulation of anti-Id T cells, and thus be a unique form of autoimmunity based on an Id etiology. Further dissection of the response at both the cellular and Id levels may clarify why *T. cruzi* infection in one patient stabilizes and remains asymptomatic, while in another it leads to severe morbidity.

Maternal fetal effects of idiotypes in Chagas' disease

In areas endemic for Chagas' disease individuals become infected early in life and maintain life-long, chronic infections. In areas of high prevalence, this pattern predicts that many women of child bearing age are actively infected at the time of their pregnancy. This situation could create an opportunity for the mothers' infection, or their immunological responses to that infection, to greatly influence the developing repertoire and future reactivity of her child. An active infection at the time of pregnancy would lead to placental or milk transfer of the mothers' high titer IgG antibodies (bearing their Ids) and circulating *T. cruzi* antigens to the developing child. The impact of these Ids or antigens on the future development of pathology when that child becomes infected with *T. cruzi* is currently only speculative. This may, however, explain the observation in many parasitic infections that people from non-endemic areas (*i.e.*, who would be unlikely to be born of infected mothers) are much more likely to suffer severe disease than are residents of the endemic area. People born of infected mothers may be primed *in utero* to elicit regulatory or non-pathogenic Id/anti-Id responses by specific maternal Ids or anti-Ids on antibodies developed during their chronic infections (Bona, 1987; Kearney, 1989; Cerny and Hiernaux, 1990).

Specific anti-Id priming *in utero* (possibly caused by antigen, Id or anti-Id) has been demonstrated in humans by Eloi-Santos and her colleagues in studies of the responsiveness of cord blood mononuclear cells (CBMC) from neonates born of mothers with Chagas' disease (Eloi-Santos *et al.*, 1989). CBMC from neonates born of mothers infected with *T. cruzi* responded to soluble antigen from epimastigotes but not from unrelated antigens (Eloi-Santos *et al.*, 1988). Their studies also showed that anti-EPI antibodies, but not anti-schistosome egg antigen antibodies (anti-SEA) or normal human Ig, stimulated these CBMC. Furthermore, anti-EPI Ids did not stimulate CBMC from neonates of uninfected or *Schistosoma mansoni*-infected mothers. These data strongly support the hypothesis that *in utero* sensitization of anti-Id T-cells occurs. We further hypothesize that such powerful influences on the developing repertoire of the neonate play an important role in determining the

responses and regulatory mechanisms expressed by most children in endemic areas when they are subsequently exposed to the same chronic infection.

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NIGERIAN ONCHOCERCIASIS: EPIDEMIOLOGICAL PERSPECTIVE

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Abstract: Onchocerciasis is a widespread filarial disease in Nigeria that produces grave socio-economic effects. The great majority of the communities are mesoendemic for onchocerciasis while only few are hyperendemic especially in the savanna zone. Sex-related infection depends on the degree of endemicity while age-infection increases gradually with advancing age. Visible and palpable nodules are more abundant around the pelvic region. The nodules are countered more in the rainforest zone even when microfilarial density (MFD) is moderate, while the nodules are less numerous in the savanna form with high MFD. The microfilariae in concert with host's immune response precipitate various skin lesions. The resulting pruritus, scratching and itching are generalized in the rainforest and localized in the savanna. In the eye, various ocular lesions are associated with the death of microfilariae. There is high incidence of eye lesion in the savanna zone than in the rainforest zone, especially the anterior lesions. The epidemiological picture presented by onchocerciasis in Nigeria is the summation of a complex array of contributing factors, both intrinsic to the microfilariae and resulting from the host-immune response, bioclimatic factors and vector species complex.

INTRODUCTION

Human onchocerciasis is a chronic parasitic disease caused by *Onchocerca volvulus*. In Nigeria the main vectors are the *Simulium damnosum* Theobald, 1903 complex of which there are at least 26 species (Dunbar, 1976). The distribution of these cytospecies is to a larger extent related to the two main climatic regions in West Africa: rainforest and savanna

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(Townson and Meredith, 1979). It is not important to note the absolute demarcation because there are cases of savanna-type microconditions within the rainforest zone and pockets of rainforest environment within the savanna zone especially along river banks. The six main vector cytospecies in West Africa have been grouped according to phytogeographical conditions as follows: savanna species (*S. sirbaum* and *S. damnosum* s.s.), rainforest species in big rivers (*S. santipauli* and *S. soubrense*), rainforest species in small rivers (*S. yahense* and *S. squamosum*) and in rainforest pockets within the savanna zone (*S. squamosum*).

Onchocerciasis was first reported in Nigeria by Parsons (1908). It seems that onchocerciasis produces grave socio-economic effects and much human suffering to about 750,000 Nigerians (Dipeolu and Gemade, 1983). Yet, the geographical distribution as well as its complications are inadequately coordinated in the country. We have therefore used available literature to make for a better understanding of epidemiology of Nigerian onchocerciasis.

Some believe that the South American disease has been imported from Africa through the slave trade, because only negro offsprings whose ancestors have been forced to work around the coastal region in the 16th to 18th centuries are infected and the clinical features is likely to be of African type. On the other hand, the disease in Guatemala and South Mexico are apparently different from the South American regions: clinical manifestations are characteristic and the parasite is transmitted by a different species of blackfly unlike to those of South America.

Although there are marked differences in the clinical manifestations and the vector-parasite complexes between the Guatemalan and West African disease (Duke, 1974; De Leon and Duke, 1966), no distinct morphological differences in the parasites from the two regions are ever recognized by Franz (1980). Results on the chromosomes of Nigerian and Guatemalan *O. volvulus* (Hirai *et al.*, 1987) showed that both parasites had basically the same chromosomal construct (2n=8, xy type) which corresponded to the findings of Bazanez *et al.* (1983) in Venezuela. Also the nuclei number of 88.0 distributed in the CS-NR portion of the microfilariae of Nigerian *O. volvulus* (Mimori *et al.*, 1986) suggests that there is no significant taxonomical difference in *O. volvulus* from Nigeria and Guatemala; 87.8 (Tada *et al.*, 1981) and 81.7 nuclei number from Amazonas State, Venezuela (Yarzabal *et al.*, 1983). Even though results so far indicate that the Nigerian *O. volvulus* is biologically quite close to the Guatemalan species, polymorphism of *O. volvulus* by iso-enzyme technique and gene analysis are needed to strongly back up the argument.

Prevalence and Distribution

One of the satisfactory methods of studying the epidemiological aspects of onchocerciasis is by the direct demonstrating of *O. volvulus* microfilariae in skin biopsies. About 750,000 Nigerians are infected by human onchocerciasis (Dipeolu and Gemade, 1983). This disease is wide spread in Nigeria (Crosskey, 1954; Bradley, 1976; Edungbola *et al.*, 1983; Nwoke, 1986; Nwoke *et al.*, 1989). These studies have shown that according to the recommendation of the WHO (1966), few areas in Nigeria must be regarded as hypoendemic, many mesoendemic while few others as the middle Hawal area, Jarawa District on the Jos Plateau are hyperendemic areas (Fig. 1).

The prevalence of Nigerian onchocerciasis shows significant variations of villages with those villages near the breeding sites of vectors having the highest infection rates (Onwuliri

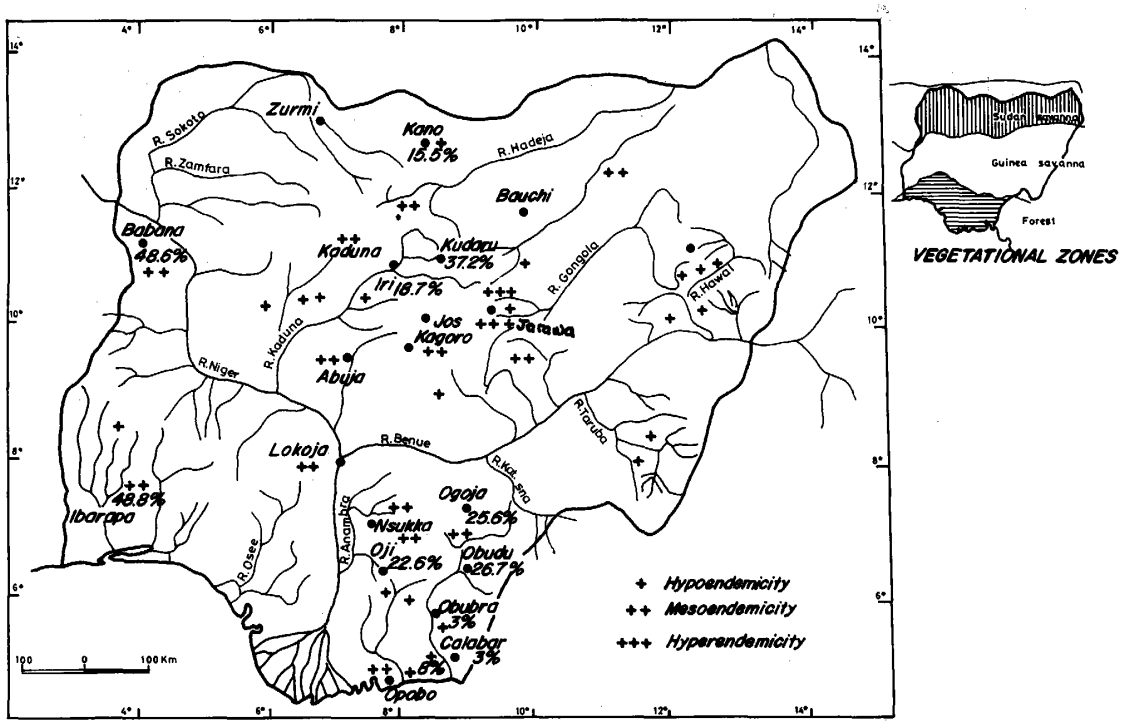

Figure 1 Endemicity of onchocerciasis (river blindness) in Nigeria (After several authors).

et al., 1978). The many workers (Edungbola *et al.*, 1983; Somorin, 1983; Nwoke, 1986) have shown that the onchocerciasis infection rate increases gradually with advancing age in Nigeria. This infection pattern bears witness to the chronic and cyptic nature of human onchocerciasis infections (Buck, 1974). In Nigeria, children make frequent visits to the foci of infection to swim, collect water for domestic use, hunt and fish. These activities together with farming are maintained throughout life in these rural communities. As a result the contact with infected *Simulium* are permanently maintained and therefore the age-related prevalence and intensity shows a gradual increase.

Sex-related onchocercal infection in Nigeria varies depending on the degree of endemicity. In hyperendemic villages male subjects have slightly higher but insignificant infection rate than females. This is because inhabitants in such hyperendemic villages live in homes very close to the foci of infection. This results that even when there are sex differential in occupation, both sexes are more or less equally exposed to the same number of infective vectors (Bradley, 1976; Edungbola *et al.*, 1983). On the other hand, in hypo- and meso-endemic villages and living far away from foci of infection male subjects show significantly higher intensity and prevalence rate than females. This is because the surroundings of such villages are not easily accessible to vector flies and therefore occupational exposure tend to play more prominent role (Crosskey, 1954). In occupational groups, field workers, the nomadic or migrant fulani tribesmen are more affected by onchocerciasis (Onwuliri *et al.*, 1987).

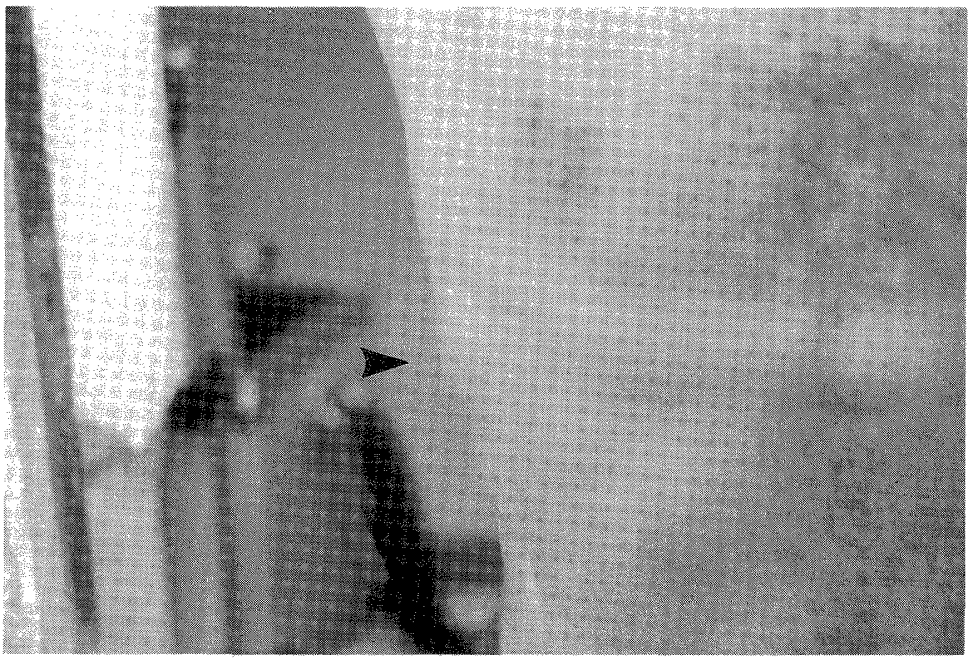

Photo. 1 Onchocercal nodule in the right thorax of a patient at Maijemu, Jos Plateau.

The Adult Parasites and Nodules

The adult parasites of *O. volvulus* are characteristically contained in fibrous subcutaneous nodules (Photo. 1). However, the great frequency of individuals with microfilariae of *O. volvulus* demonstrable by skin biopsies, but without visible or palpable nodules indicates that adult worms may be lying free, unencapsulated in the subcutis (Nnochiri, 1964; Nwoke *et al.*, 1988); or may be small and deeply located (Leichsenring *et al.*, 1990).

In Nigeria (Dipeolu and Gemade, 1983; Nwoke *et al.*, 1987) and most other African countries (Browne, 1961; Leichsenring *et al.*, 1990) the great majority of detectable nodules occur in the pelvic region. This is in contrast to which obtains in Guatemala where as many as 70% of the nodules are found in the head region (Lagraulet *et al.*, 1964). Yemen onchocerciasis rarely present with onchocercomata (Somorin, 1983).

There exist two form of nodules in West Africa. In the rainforest form, the disease is characterized by abundant nodules and moderate microfilariae density (MFD) while in the savanna form the nodules are less numerous with characteristic high MFD in the skin (Onwuliri *et al.*, 1987; Nwoke, 1986).

The Microfilariae

The microfilariae are distributed widely in the dermis and the eyes. Most investigators believe that the distribution of microfilariae in the skin is correlated with the onchocercal nodules. In Nigeria and West Africa visible and palpable nodules are mainly in the lower parts of the body and the highest density of microfilariae is recorded in pelvic region

(Kershaw *et al.*, 1954; Edungbola, 1982; Nwoke *et al.*, 1989). In East Africa, Nelson (1958) noted that the microfilariae are mostly concentrated around the buttocks and upper thigh, whereas the skin below the knee is less affected. On the other hand, Guatemalan onchocerciasis is characterized by high MFD in the upper part of the body which corresponds to the anatomical distribution of nodules (De Leon and Duke, 1966).

In most onchocerciasis-endemic countries including Nigeria, the MFD has shown varying concentrations in the skin depending on the time of the day or month of the year, the strain of parasite or environmental conditions. In the Haute-Volta, the microfilariae were much higher in the skin at 16 hr than at 10 hr (Lartique, 1967) while in Cameroon Duke *et al.* (1967) reported an elevation in the MFD during the afternoon in both the rainforest and Sudan-savanna forms which they noted correlated well with the curves for temperature/saturation deficiency and for the biting density of *S. damnosum* vectors. However, they noted that the fluctuation in MFD were too small to affect the results of diagnosis. Also Picq and Jardel (1973) in Haute-Volta reported insignificant changes in the skin MFD. In Guatemala, Tada and Figuerroa (1974) observed that the daily variations of MFD were not consistent. Hashiguchi *et al.* (1983) in Guatemala observed that the pattern of microfilarial intake by *S. onchraceum* did not correlate well with the diurnal pattern of MFD. Recently Nwoke (1988) in the savanna part of Nigeria observed insignificant variations in the seasonal, daily and diurnal MFD. However, direct sun-shine on the skin of patients working in the field and low temperatures (8-15°C) had a significant reducing effect on MFD in Nigeria (Nwoke, 1986).

Onchodermatitis (Skin Lesions)

The subcutaneous microfilariae, in concert with the host's immune response precipitate various dermatological lesions such as pruritus with papular rash, depigmentation and atrophy. The most common dermatological manifestations of onchocerciasis in Nigeria are pruritus, scratching and itching (Edungbola *et al.*, 1983) which may be generalizes in the rainforest type, localized in the savanna or American type and segmental in the Yemen type (Somorin, 1983). Pruritus may be accompanied by a nonspecific rash which may be macular, papular or pustular. In Nigeria, pruritus occurs in all ages but is significantly higher among older age groups. After some period of scratching lesions develop dry, scaly and in elastic skin with folds resulting in lichenification. It is often impossible distinguish the skin changes from those seen vitamin A deficiency patients. Rodger (1962) suggested that the parasite competes for vitamin A in the skin or that vitamin A deficiency predisposes the skin to heavier infections. Sowda, the Yemen type of onchocerciasis characterized by dermatic hyperpigmented (black) segmented limb associated with enlarged inguinal lymphatic gland has been observed in the rainforest of Nigeria (Somorin, 1983).

The lichenified pruritus areas also may be associated with pigmentary changes (hyper- or hypopigmentation) resulting in a striking dermatologic feature commonly called leopard skin (Photo. 2). In Nigeria, this clinical manifestation of onchocerciasis is associated with long-standing onchodermatitis. Leopard skin affects the skin and anatomical sites such as the penis, buttock, thigh, scrotum, inguinal fold and axillae (Connor and Palmieri, 1985; Nwoke *et al.*, 1989) which are not readily exposed to biting blackflies. Connor and Palmieri (1985) revealed progression of leopard skin: increasing number of microfilariae accumulates and degenerates in the upper dermis which consequently provokes acute inflammation at first,

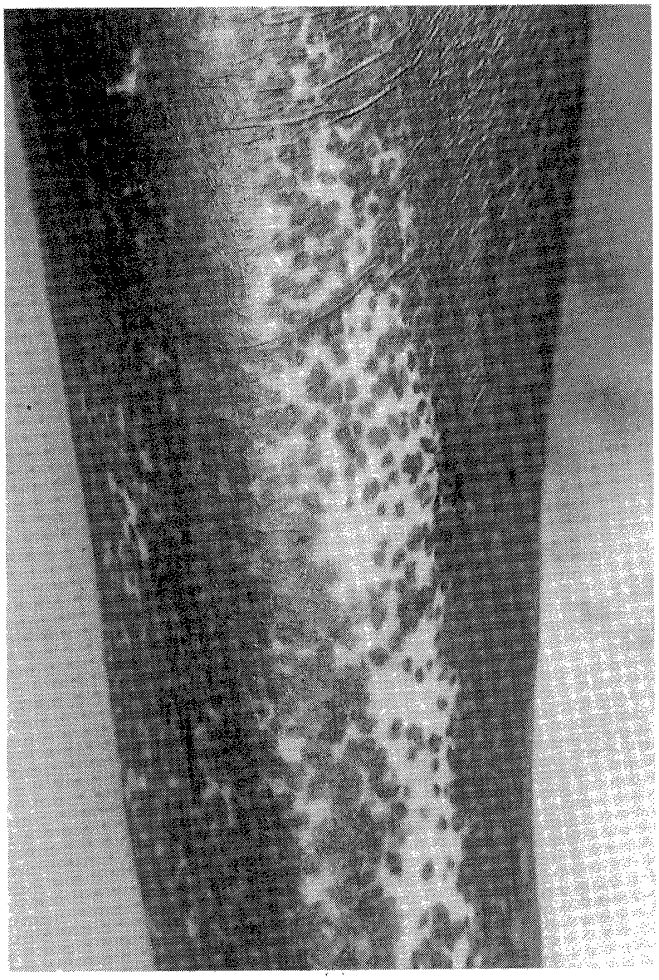

Photo. 2 Leopard skin on the shin in a patient at Maijemu, Jos Plateau, characterized by focal depigmentation.

around degenerating microfilariae, then chronic. There is melanin drop into the upper dermis where it is phagocytosed and eventually carried to regional lymph nodes. Nigerian onchocerciasis is characterized by relatively high prevalence rate of leopard skin in the savanna zone than in the rainforest (Edungbola *et al.*, 1983). This has been attributed to differences in the pathogenicity and antigenic peculiarities of the savanna and rainforest strains of the parasites or to differences in the host immunological responses (Anderson and Fuglsang, 1977; Anderson *et al.*, 1974).

Onchocercal Lymphadenitis

Antigens released from microfilariae lead to the deposition of immune complex in tissues, which in turn causes inflammation and fibrosis and eventually obstructive lymphadenitis. Lymphadenitis may sometimes lead to hanging groin and elephantiasis (Buck, 1974; Gibson and Connor, 1978). Adenolymphocele is a feature of onchocerciasis in Zaire, Central Africa Republic, Chad, Cameroon and Nigeria and perhaps other African countries. These pendu-

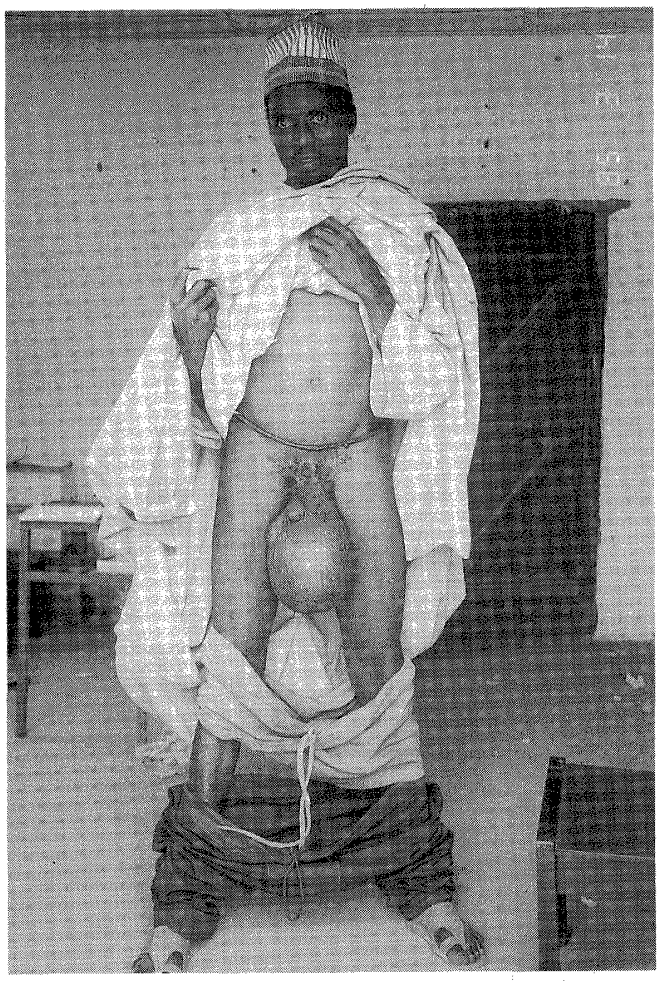

Photo. 3 Enlarged scrotum in a patient at Maijemu, Jos Plateau.

lous sacs are observed in male adults and often contain both femoral and inguinal lymph glands. Somorin (1983) in Nigeria and Connor and Palmieri (1985) in Africa described female patients of onchocerciasis with fullness in the femoral and inguinal regions revealing underlying uni- or bilateral lymphadenopathy. No difference has been recorded between the prevalence of onchocercal lymphadenitis in the savanna and rainforest zones of Nigeria. In Nigeria, Nwoke (1986, 1989) observed that these dreadful malformations due to onchocercal lymphadenitis cause a lot of socio-cultural discomfort to patients (Photo. 3). In affected patients with the pendulous genital sacs, sexual life is greatly affected.

Ocular Lesions

Ocular complications are the most serious clinical manifestations of onchocerciasis. Loss of vision can manifest either as anterior or posterior lesions. In Nigeria, Dipeolu and Gemade (1983) estimated that 30,000-40,000 people have ocular complications of onchocerciasis. The pathologic picture in ocular onchocerciasis is the summation of a complex array of contribut-

ing factors, both intrinsic of microfilariae and resulting from the immune response and bioclimatic factors. The release of large amount of microfilariae antigens have more serious consequences in the eye and skin than in the organs with a more efficient antigen-cleaning system, such as the spleen and liver (Mackenzie *et al.*, 1985).

Ocular onchocerciasis was found to be more severe in the savanna region than in the rainforest of Nigeria (Budden, 1963; Anderson and Fuglsang, 1974). Duke (1976, 1981) noted that the microfilariae of the savanna strain were more invasive and more pathogenic on the cornea than those of the rainforest strain. In addition, the influence of climatic factors cannot be ruled out completely. Sclerozing keratitis having a much higher prevalence in the savanna than in the rainforest (Duke and Anderson, 1972) caused the invasion of microfilariae on the cornea. Microfilariae trend to involve that parts of the cornea which is exposed to light (WHO, 1973). However, it is possible to observe a comparatively low blindness rate in onchocerciasis hyperendemic communities in the savanna zone of Nigeria. According to Nwoke (1986) and Nwoke *et al.* (1987) this low blindness rate may be attributed to the habitual migration of disabled people in the savanna zone to urban centers to beg for alms, consequently reducing the actual number of blind people in the rural communities.

Ocular lesions of onchocerciasis are much more prevalent among productive age group in Nigeria (Budden, 1963; Dipeolu and Gemade, 1983; Onwuliri *et al.*, 1987). The pattern of socio-economic liability that has emerged due to onchocerciasis blindness in the settlements has been damaging (Bradley, 1976; Nwoke *et al.*, 1988; Nwoke, 1989). Onchocerciasis in Nigeria has been instrumental to abandonment of some river valleys especially in the savanna that are agriculturally fertile (Bradley, 1976).

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ナイジェリアにおけるオンコセルカ症の疫学

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オンコセルカ症はナイジェリアに広く流行しており，社会経済的に多大の悪影響を及ぼしている。ナイジェリアにおけるオンコセルカ症の分布，および合併症についての知見は不十分であるため，これまでに報告された研究を整理し，ナイジェリアにおけるオンコセルカ症の記述疫学的な記載を行った。ナイジェリアの大部分の地区は，WHOの基準にしたがうとオンコセルカ症の中等度の流行地であるが，サバンナ地帯に高度流行地が点在する。地区でのオンコセルカ症の感染率における性差は，流行の程度に依存し，低流行地で性差が著しく，男性で高率である。また，感染率は加齢とともに増加する。視認および触知可能な皮下腫瘤は，腰部に多く存在する。熱帯雨林地帯ではミクロフィラリア密度が少ないにも関わらず，皮下腫瘤を多く認めるが，サバンナ地帯ではミクロフィラリア密度が多いにも関わらず，皮下腫瘤は少ない。ミクロフィラリアは宿主の免疫反応と相まって様々な皮膚病変を引き起こす。皮膚掻痒症の発現は熱帯雨林では一般的であるが，サバンナ地帯では一部に留まる。様々な眼科病変は，死亡したミクロフィラリアによって引き起こされることが明らかになってきた。眼，特に前房の病変は，熱帯雨林地帯よりもサバンナ地帯に多い。ナイジェリアにおけるオンコセルカ症は，ミクロフィラリア自身の性質および宿主の免疫反応，生物気候学的因子，伝播するブユの亜種の多要因によって複雑な疫学像を呈している。

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THE ETIOLOGICAL FACTOR FOR EOSINOPHILIA AND HYPERGLOBULINEMIA E IN BRAZILIAN SCHOOL CHILDREN

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Abstract: The etiology of peripheral blood eosinophilia and hyperglobulinemia E, which are commonly found among residents in tropical area, were studied with two groups of Brazilian children from different primary schools. These two groups of children had different infection rates with soil-transmitted helminthiases, but similar positive rates of antibody against a common allergic antigen. The prevalence rate of children with eosinophilia or hyperglobulinemia E was significantly higher in a group with higher infection rate with soil-transmitted helminthiases than in another group with lower infection rate. It was suggested that eosinophilia and hyperglobulinemia E in this area were mainly caused by soil-transmitted helminthiases.

INTRODUCTION

Peripheral blood eosinophilia and hyperglobulinemia E (hyperIgE) are common laboratory findings among residents in tropical areas (Johansson *et al.*, 1968; Mahmoud, 1984). It has been empirically supposed that these abnormal findings are caused by helminth infections, which are major health problem in tropical areas. However, the comparisons in the number of eosinophils and serum IgE level between helminth infected and uninfected subjects in one community usually failed to show prominent differences (Bruce-Tagoe *et al.*, 1977; Kaplan *et al.*, 1980).

Allergic diseases are another etiological factor for eosinophilia and hyperIgE especially in residents in temperate areas. It has been demonstrated that the morbidity of allergic diseases was not so low even in tropical residents (Lynch *et al.*, 1984). Therefore the participation of allergic diseases on eosinophilia and hyperIgE in tropical residents also

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should be considered.

To know the etiology of eosinophilia and hyperIgE in tropical residents, we examined the number of eosinophils and serum IgE level in two groups of Brazilian children living in a city, in relation to soil-transmitted helminthiases and allergic diseases.

METHODS

Participants

Recife, located in the northern part of Brazil, is a port city with a population of one million. Two primary school in Recife were chosen for testing. One in Varzea (VAR) located in the suburbs, where sanitary condition is poor. The other in Madalena (MAD) is in the center of the city with good sanitation. The stool and peripheral blood were collected from 92 school children in VAR and 18 school children in MAD (age range from 6 to 12 years old).

Stool

Fecal samples were collected and examined for helminth eggs by modified Kato-Katz method.

Blood

The number of eosinophils was microscopically counted with Hinkelman's staining. The presence of eosinophils more than 500/mm^3 was considered as eosinophilia. Serum IgE level were measured by using Phadezym IgE PRIST kits (Pharmacia fine chemicals) and were expressed as international units per ml (IU/ml). HyperIgE was defined as serum IgE level more than 500 IU/ml. The level of anti-house dust IgE antibody was determined by Radio Allergo Sorbent Test (Pharmacia fine chemicals). Scores higher than 2 were considered as positive.

Statistical analysis

Data were analysed by the application of χ^2 test or student's t test.

RESULTS

Stool examination

Results of the stool examination are shown in Table 1. Fifty eight (63%) of 92 children

Table 1 Results of stool examinations among school children in Recife, Brazil

	No.(%) children positive for			
	helminth eggs	A.l.	T.t.	A.l. & T.t.
School in VAR (N=92)	58 (63%)*	19	20	19
School in MAD (N=18)	1 (6%)	0	1	0

A.l.: *Ascaris lumbricoides*, T.t.: *Trichuris trichiura*

* This percentage was significantly higher than that of school in MAD ($p<0.001$).

Table 2 Number of eosinophils and serum IgE level among primary school children in Recife, Brazil

	School in VAR (N=92)	School in MAD (N=18)	P§
No. eosinophils* (/mm³)	1,053±80	317±51	
No.(%) children with eosinophila†	66 (72%)	3 (17%)	<0.001
Serum IgE level* (IU/m*l*)	1,202±114	525±175	
No.(%) children with hyperIgE‡	67 (73%)	5 (28%)	<0.001

* Number indicate mean±SEM.
† Number of eosinphils more than 500/mm³.
‡ Serum IgE level more than 500 IU/m*l*.
§ P value calculated from the percentages.

in VAR were found to be positive for helminth eggs (*Ascaris lumbricoides* alone, 19; *Trichuris trichiura* alone, 20; Both, 19). Of the 18 children in MAD, only one (6%) was positive for helminth eggs. The prevalence rate of soil-transmitted helminthiases were significantly higher in VAR children than in MAD children (P<0.001).

Anti-house dust IgE

As a marker of allergic diseases, anti-house dust IgE antibody was measured in 32 children in VAR and 18 children in MAD. Only 3 (9%) and 2 (11%) students were positive in VAR and MAD children, respectively. Therefore, with respect to the prevalence of anti-house dust IgE antibody, no significant difference was observed between these two groups.

Number of eosinophils and IgE level

Prominent increase of the mean number of eosinophils and serum IgE level were observed in the children of VAR (eosinophils: 1,053±80/mm³, IgE: 1,202±114 IU/m*l*) (Table 2). The percentage of children with eosinophilia (eosinophils>500/mm³) was 72%, and children with hyperIgE (IgE>500 IU/m*l*) was 73%. Significant correlation was

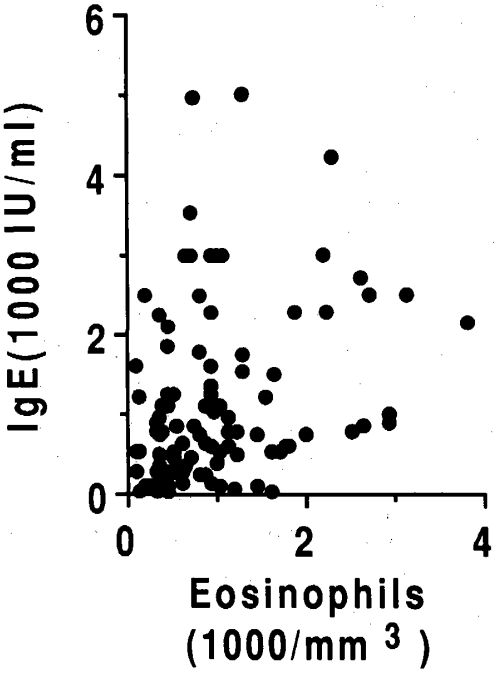

Figure 1 Correlation of the number of eosinophils and serum IgE level in primary school children in VAR. The number of eosinophils showed a significant correlation with serum IgE level (r=0.24314, p<0.05).

demonstrated between the number of eosinophis and IgE level ($p<0.05$, Figure 1). Therefore, it is supposed that frequently observed eosinophilia and hyperIgE in VAR children were caused by the same etiology, such as soil-transmitted helminthiases.

On the other hands, in MAD children, the mean number of eosinophils and serum IgE level were nearly normal values (eosinophils: 317 ± 51 mm^3, IgE: 525 ± 175 IU/ml) (Table 2). The percentage of children with eosinophilia was 17% and, children with hyperIgE was 28%. When percentages of children with eosinophilia were compared between VAR and MAD children, significantly higher percentage was observed in VAR than in MAD ($P<0.001$). Similarly, the percentage of children with hyperIgE was significantly higher in VAR than in MAD ($P<0.001$).

Discussion

Eosinophilia and hyperIgE has been known to occur in association with helminth infections or allergic diseases (Bennich and Johansson, 1971; Gleich and Adolphson, 1986). Many previous studies demonstrated that eosinophilia and hyperIgE were common in communities with high prevalence of helminth infections (Johansson *et al.*, 1968; Mahmoud, 1984). However, it has been also reported that the number of eosinophils and serum IgE level were not significantly different between helminth infections positive and negative subjects within a community (Bruce-Tagoe *et al.*, 1975; Kaplan *et al.*, 1980).

In the present study, eosinophilia and hyperIgE were frequently found in VAR children compared with MAD children. The difference between these two communities was also apparent in regard to the rate of helminthic infections. These results strongly suggest that eosinophilia and hyperIgE in VAR children were caused by soil-transmitted helminthiases.

These two schools were located in the same city, and race and life style of the children was almost the same. The recent epidemics of schistosomiasis and filariasis were not found in this city (Dr. Ivete Barbosa, personal communication), suggesting that helminth infections other than soil-transmitted may be excluded from the etiology for eosinophilia and hyperIgE.

It has been reported that the incidence of allergic diseases in tropical area is identical to those in temperate area, especially in urban district (Lynch *et al.*, 1984). Anti-house dust IgE antibody was chosen as a marker of allergic diseases in this study, since house dust antigen is the most popular allergen in tropical residents (Lynch *et al.*, 1984). The prevalence rates of anti-house dust IgE antibodies were almost the same in children of both VAR and MAD, suggesting that allergic diseases were not involved in the major etiologies for eosinophilia and hyperIgE in VAR children.

The number of eosinophils and serum IgE level in VAR children were significantly correlated. Therefore, it is likely that infected helminths concurrently caused eosinophilia and hyperIgE. Helminthic infections preferentially induce one of the subsets of helper T lymphocytes (TH2), which produce both interleukin-4 (IL-4) and interleukin-5 (IL-5) (Mosmann and Coffman, 1989). These lymphokines have the function to elicit coincidently hyperIgE and eosinophilia, namely IL-4 accelerates B lymphocytes to secrete IgE (Stavnezer *et al.*, 1988), and IL-5 promote proliferation of eosinophils in bone marrow (Coffman *et al.*, 1989). It is also possible that IgE antibody, produced by stimulations with helminths, subsequently gives signals to mast cells to release eosinophil chemotactic factor which cause eosinophilia.

Additional investigation into the precise mechanisms for eosinophilia and hyperIgE in these patients with soil-transmitted helminthiases is needed.

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ブラジル，レシフェ市内小学生の好酸球増多症，高 IgE 血症の病因

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熱帯地住民に高率にみられる好酸球増多症および高 IgE 血症の病因を究明するため，ブラジル，レシフェ市内の 2 つの小学校生徒を対象に，末梢血好酸球数，血清総 IgE 値を測定した。また，蠕虫感染の指標として糞便内蠕虫卵検査，アトピー性疾患の指標として血清抗ハウスダスト IgE 抗体価の測定を施行した。2 校の生徒間で，抗ハウスダスト IgE 抗体陽性率には差がなかったが，糞便内蠕虫卵陽性率には著明な差を認めた。検出された虫卵は，全て土壌伝播蠕虫卵であった。好酸球増多症（好酸球数500/mm³以上），高 IgE 血症（IgE 値500 IU/m*l* 以上）を呈する生徒は，糞便内蠕虫卵陽性率が高い小学校において，有意に高率に認められた。

以上から，この地方における小学生の好酸球増多症および高 IgE 血症の病因として，土壌伝播蠕虫感染が考えられた。

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EVALUATION AND CHARACTERIZATION OF PARTIALLY PURIFIED SKIN TEST ANTIGENS PREPARED FROM *LEISHMANIA PANAMENSIS* PROMASTIGOTES

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Abstract: The present study was designed to evaluate skin test preparations prepared from *Leishmania panamensis* promastigotes in 30 active cutaneous leishmaniasis patients. The crude antigen preparation (CA) used was 10,000×*g* supernatant of the parasites-homogenate. The soluble extract was further resolved into 4 preparations (FA-1 to -4) with the aid of a Sephacryl S-200 gel filtration. There was no significant difference in the positive ratio and the average induration size between CA (10 μg protein/test) and Montenegro's antigen (MA; 5×10^6 parasites/test). The reactivity of the delayed-type hypersensitivity to 10 μg dose of CA was shown with much the same intensity in the 25 μg dose of CA. In FAs (10 μg protein dose, except for 7.5 μg in FA-4), the positive ratio was as follows: 90.0% in FA-1, 77.8% in FA-2, 75.0% in FA-3 and 37.5% in FA-4. The positive ratio and the intensity of skin test response in FA-4 were remarkably low in comparison with those in CA or MA. Significant difference was found in the intensity of response between FA-3 and CA or MA. Based on these results, therefore, we concluded that 10 μg protein dose of CA of *L. panamensis* and same dose of the fractionated preparations, FA-1 and -2, were very suitable for the diagnosis of cutaneous leishmaniasis in endemic areas of the New World. Furthermore, it was estimated that at least some or all of the 5 proteins, approximately 66, 55, 45, 28, and 26 kD, were related to a specific delayed-type hypersensitivity in cutaneous leishmaniasis of the New World.

INTRODUCTION

The intradermal skin test is widely used for a presumptive diagnosis of visceral and cutaneous leishmaniasis in endemic areas of the Central and South America. Although the antigen commonly used is a suspension of whole parasites in phenolized saline (Buss, 1929),

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a little information on other preparations prepared from promastigote-extracts have been also reported (Furtado and Pellegrino, 1956; La Placa *et al.*, 1975; Shaw and Lainson, 1975; Reed *et al.*, 1986). Among them, Reed *et al.* (1986) have reported that a crude soluble extract prepared from ruptured *Leishmania chagasi* promastigotes was highly sensitive and specific for the diagnosis of American visceral leishmaniasis. We have also demonstrated that a similarly prepared soluble extract obtained from *L. braziliensis* complex was very useful for the screening of cutaneous leishmaniasis in endemic areas of Ecuador (Furuya *et al.*, 1989). In order to gain a better information on a standardization of skin test preparation and the antigen dose, we designed preliminary examinations to evaluate skin test preparations prepared from *L. panamensis* promastigotes. The present paper reveals that the soluble promastigotes extract and fractionated preparations of the soluble extract are highly sensitive for the diagnosis of active cutaneous leishmaniasis, and a characterization of protein components of the preparations will be discussed in this paper.

Materials and Methods

Skin test preparations

Crude antigen preparation (CA): *L. panamensis* (MHOM/PA/71/LS94) obtained from Dr. P. Desjeux, PDP, WHO (formerly Instituto Boliviano de Biologia de Altura, Bolivia) was cultured with the medium described by Pan (1984). After washing of parasites with a balanced salt solution, the harvested promastigotes were ruptured by a freeze-thawing procedure and centrifuged at $10,000 \times g$ for 30 min at 4°C (Furuya *et al.*, 1989). The supernatant was filtered through 0.45 μ sterile filter and lyophilized as CA.

Fractionated antigen preparation (FA): The above mentioned soluble extract was further resolved into 4 preparations, designated FA-1, -2, -3, and -4, with the aid of a Sephacryl S-200 (Pharmasia, Uppsala, Sweden) column being in equilibrium with 0.02 M phosphate buffered saline (pH 7.2). The solution of each peaks was condenced by ultrafilter (MW cut-out 10,000; Advantex Co., Japan), dialyzed against PBS, and then centrifuged by $10,000 \times g$ for 30 min at 4°C. After filtration, these FAs were adjusted 100 μg protein concentration per m*l*, except for 75 μg in FA-4.

Montenegro's antigen preparation (MA): 5×10^7 whole promastigotes per m*l* in sterile saline containing 0.5% phenol was used (Bray, 1985).

Skin test

Skin test was made on 30 patients with active cutaneous leishmanial lesions. The preparations were injected intradermally in 0.1 m*l* in flexor surface of the forearm. The skin test area was observed for erythema and induration at 48 hrs. Induration size of more than 5 mm (mean value of length and breadth) at the injection site was considered a positive reaction.

Analysis of components of CA and FAs

CA and FAs were solubilized with 60 mM Tris-HCl buffer (pH 6.8) containing 2% SDS, 10% glycerol, and 5% 2-mercaptoethanol. Samples were analyzed by SDS-PAGE as described by Laemmli (1970). After electrophoresis gel was stained by 0.25% Coomassie brilliant blue R250.

RESULTS

Skin test

Intradermal skin test using MA, CA, and FAs was carried out against 30 patients. The results were shown in Tables 1 and 2. Most of the patients had one or two active cutaneous lesions infected at 3 to 8 months ago. Parasites were confirmed from the lesions of 29 patients by smear specimens or culture method. Ten strains of *Leishmania* isolated from these patients were characterized by performing zymodeme, serodeme and schizodeme analysis (McMahon-Pratt *et al.*, 1982; Lopes *et al.*, 1984): 9 strains were identified as *L. panamensis,* and one strain was *L. braziliensis* (Table 1). The detailed characterization of these strains will be reported elsewhere.

Intensity of induration size of skin test using CA and MA was shown in Table 2. The CA at 25 μg protein dose gave positive response in all 14 individuals, but, a superabundant response was also recognized in 2 subjects (Table 2). The 10 μg protein dose of CA and MA gave 87.5% (14/16) and 93.8% (15/16) positivity, respectively. No response against CA and MA was observed in one patient. This patient had numerous lesions on the upper side of his right arm and had been treated with a corticosteroid for 3 months. There was no significant difference in the positive ratio and the average induration size between 25 and 10 μg dose of CA. Moreover, the reactivity of the delayed-type hypersensitivity (DTH) to 10 μg dose of CA was shown with much the same intensity in the MA.

As shown in Fig. 1, the soluble promastigotes extract was separated seven to eight peaks by Sephacryl S-200 gel filtration. An evaluation of skin test using FAs was made on 10

Table 1 Intradermal skin test responses to Montenegro's antigen (MA), crude antigen (CA), and fractionated antigens (FA) prepared from *Leishmania panamensis* in 10 individuals with active cutaneous leishmanial lesions

No.	Sex*	Age	Months after infection	Induration size (mm)						Parasite	
				MA	CA	FA-1	FA-2	FA-3	FA-4	Smear†	Species‡
1	M	28	3	9	8	6	7	6	4	+	L.p.
2	M	41	4	16	20	25	19	8	5	+	L.p.
3	M	23	3	12	10	16	18	16	9	+	L.b.
4	F	25	3	10	11	20	2	2	2	+	L.p.
5	M	19	3	6	4	5	5	5	4	+	L.p.
6	M	22	4	10	11	4	2	3	2	+	L.p.
7	M	21	4	16	17	10	16	7	3	+	L.p.
8	M	21	4	20	12	7	7	7	6	+	L.p.
9	M	38	12	12	15	22	N.D.	N.D.	N.D.	+	
10	M	40	?	13	18	10	10	N.D.	N.D.	−	
Positive ratio				100.0	90.0	90.0	77.8	75.0	37.5		
Average induration size (±SD)				12.4 (4.1)	12.6 (4.9)	12.5 (7.7)	9.6 (6.6)	6.8 (4.3)	4.4 (2.3)		

*M, male; F, female † +, positive; −, negative

‡ L.p., *Leishmania panamensis*: L.b., *Leishmania braziliensis*

Table 2 Frequency distribution of induration size of leishmanial skin test using crude antigen (CA) and Montenegro's antigen (MA) in 30 patients with active cutaneous leishmanial lesions

Induration size (mm)	CA		MA
	25 (μg protein)	10 (μg protein)	5×10^6 (parasites)
< 5		2	1
5 ~10		3	4
11~15	6	7	6
16~20	6	3	3
21~25		1	2
>26	2*		
Average induration size	20.1±12.7	13.3±7.2	13.1±5.8

* Induration size of 2 examinees was 38×27 mm and 80×40 mm, respectively.

patients. The positive ratio against each FAs was as follows: 90.0% (9/10) in FA-1, 77.8% (7/9) in FA-2, 75.0% (6/8) in FA-3, and 37.5% (3/8) in FA-4. Among 8 subjects received all of the FAs, 3 patients reacted to all of the FAs, 2 patients reacted to FA-1, -2 and -3, and 1 patient only reacted to FA-1. In the last patient (No. 4), however, 6, 13, and 6 mm size of erythema was observed in the injection site of FA-2, -3, and -4, respectively. On the other hand, one patient (No. 6), showing positive reaction against MA and CA, did not react against all of the FAs. There was significant difference in positive ratio of skin test response between FA-4 and CA or MA ($p<0.001$). Furthermore, significant difference was found in the average induration size between FA-3 and CA or MA ($p<0.025$).

SDS-PAGE profiles of CA and FAs

Lane 1 in Fig. 2 shows the Coomassie brilliant blue R250-stained profile of the CA, and lanes 2 to 5 show the pattern of FAs. About 35 bands were recognized in lane 1 (CA). Seven bands were weakly stained in lane 2 (FA-1), 25 bands were recognized in lane 3 (FA-2), 18 bands were observed in lane 4 (FA-3), and 9 bands in lane 5 (FA-4). Among these bands, 4 bands migrating in the region approximately 66, 55, 45, and 26 kilodalton (kD) were common to all of the FAs. SDS-PAGE proved that most of protein components of the soluble promastigotes extract of *L. panamensis* was recovered in FA-2 area by Sephacryl S-200 gel filtration.

Discussion

Until recently, no exact information has been available on species or subspecies level characterization of the genus *Leishmania* of cutaneous leishmaniasis in this country. Three strains of *Leishmania* isolated from active cutaneous leishmaniasis patients have been first characterized as *L. panamensis* by isoenzyme electrophoresis and monoclonal antibodies

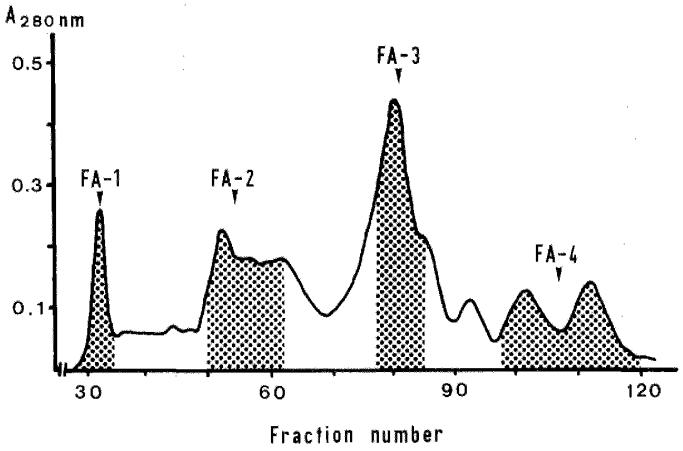

Figure 1 Effluent pattern of soluble protein extract of *L. panamensis* by Sephacryl S-200 gel filtration. Each of the shadded area of figure was collected and concentrated by ultrafilter as a FA preparation of skin test. column, 2.5×60 cm; buffer, 0.02 M phosphate buffered saline (pH 7.2); sample, 2.5 m*l* of soluble protein extract of *L. panamensis*; flow rate, 18 m*l*/hr; fraction, 3 m*l*/tube.

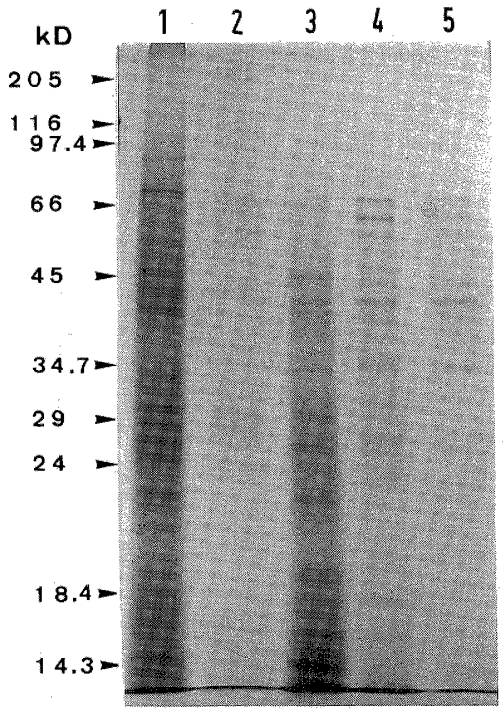

Figure 2 SDS-PAGE profile of soluble extract preparations prepared from *L. panamensis*-promastigotes. Lanes: 1, crude antigen preparation (CA; 45 μg protein); 2, fractionated antigen preparation FA-1 (14 μg protein); 3, FA-2 (48 μg protein); 4, FA-3 (28 μg protein); 5, FA-4 (17 μg protein). The electrophoresis was done at 6.5 mA for 12 hrs. The positions of molecular size are indicated. kD, kilodaltons. 12% gel.

(Mimori *et al.*, 1989). Of the 26 strains newly isolated in this country, 23 were identified as *L. braziliensis* complex (12 *L. panamensis*, 7 *L. guyanensis* and 4 *L. braziliensis*) by enzyme electrophoresis (Armijos *et al.*, 1990). In the present studies, therefore, we used *L. panamensis* promastigotes for preparing of the present skin test preparations.

Although a standerdization of antigen concentration in intradermal skin test for visceral and cutaneous leishmaniasis had not yet been done, 50 to 25 μg protein dose is used normally (Kerdel-Vegas, 1982; Bray, 1985; Reed *et al.*, 1986). In the present study, it was definitely shown that 10 μg dose of CA was able to stand comparison with a relatively large number of promastigotes of MA in detecting DTH in patients with active cutaneous lesions. There was no significant difference in the positive ratio and the average induration size between 25 μg and 10 μg antigen dose of CA. In the test with 25 μg protein dose of CA, superabundant intradermal response was observed in a few active and some cured cutaneous leishmaniasis individuals (data not shown). From these results, 10 μg protein dose of soluble promastigotes extracts (CA) of *L. panamensis* will be suitable for the diagnosis using intradermal skin test against cutaneous leishmaniasis in the New World.

For a characterization of skin test antigens, it has been recently reported that two defined glycoconjugates purified from *L. amazonensis* was able to induce a specific DTH response to infected susceptible and resistant mice strains, and that one of the glycoconjugates was a degradation product of a 17 kD antigen present in promastigotes and amastigotes (Rodrigues *et al.*, 1986a, b). Partially purified antigens containing 94 to 64 kD proteins, derived from *L. infantum* or *L. major* promastigotes and isolated under reducing conditions with SDS-PAGE, were also able to induce specific DTH reactions in mice (Frommel *et al.*, 1988). In the present studies using partially purified preparations, no significant difference was observed in the positive ratio and the average induration size between FA-1 and -2 and CA or MA. By SDS-PAGE analysis of CA and FAs, 5 bands migrating the region approximately 66, 55, 45, 28 and 26 kD were common to the both FAs. From these results, it is assumed that at least some or all of these 5 antigens of *L. panamensis* may be related to a specific DTH response in active cutaneous leishmaniasis patients infected with *L. braziliensis* complex.

Cross-reactivity at the skin test level between different leishmanial species has been demonstrated in humans and experimental animals (Manson-Bahr, 1961; Adler and Gunders, 1964; Bryceson *et al.*, 1970), although the reactions to heterologous organisms appeared to be of lesser magnitude (Weissberger *et al.*, 1973; Neal and Miles, 1976). Recently, Reed *et al.* (1986) also reported that a crude soluble promastigotes extract prepared from a heterologous parasite, *L. amazonensis*, was clearly less effective than a crude extract prepared from a homologous parasite, *L. chagasi*, in detecting DTH in cured American visceral leishmaniasis patients. In the present examinations, it was found that the present preparation was highly sensitive in the intradermal skin test against active cutaneous leishmaniasis patients suffering from heterologous organisms, *L. braziliensis*. There was no appreciable difference in the intensity of responses in patients caused by homologous and heterologous organismus. The results suggest that the present CA and FAs contain some common antigens, may be highly antigenic components, to *L. braziliensis*. It was concluded that the soluble extracts of *L. panamensis* would be very useful for diagnosis of active or cured cutaneous leishmaniasis caused by *L. braziliensis* complex in the endemic areas of the New World.

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Leishmania panamensis promastigote 型原虫から精製した皮内反応用抗原の効果判定とその特性

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Leishmania panamensis promastigote 型原虫から作成した種々皮内反応用抗原の効果を30人の皮膚型リーシュマニア症患者で判定した。原虫ホモジネートの10,000×*g* 遠心上清を粗抗原（CA）とし，更に Sephacryl S-200 ゲルを用いて 4 画分（FA-1 から FA-4）を得た。CA（10 μg タンパク量/テスト）による皮内反応の陽性率，および硬結径は Montenegro 抗原（MA; 5×10^6 原虫/テスト）でのそれらと比較して優位差がなかった。更に，10 μg タンパク量の CA によって誘発される遅延型皮内反応の反応の強さは，同抗原液を25 μg タンパク量で用いた場合と同等であった。分画抗原（FA-4 は7.5 μg タンパク量，他は10 μg タンパク量）での皮内反応陽性率は FA-1 が90.0%，FA-2 が77.8%，FA-3 が75.0%，FA-4 が37.5%であった。これら 4 抗原のうち，FA-4 は陽性率および反応の強さの両面で，又 FA-3 は反応の強さの点で CA や MA でのそれらと比較して著しく劣っていることが判明した。以上の結果から，*L. panamensis* 原虫から作成した皮内反応用抗原のうち，10 μg タンパク量の CA および同タンパク量の FA-1，FA-2 分画抗原が新大陸での皮膚型リーシュマニア症の診断に適していることが結論づけられた。更に，これらの抗原液を構成しているタンパク質のうち，少なくとも66, 55, 45, 28, 26 kD タンパクの全て，又は一部が新大陸での皮膚リーシュマニア症の遅延型皮内反応惹起に関与している可能性が示唆された。

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