LEISHMANIA DONOVANI: AN OPPORTUNISTIC MICROBE ASSOCIATED WITH PROGRESSIVE DISEASE IN THREE IMMUNOCOMPROMISED PATIENTS

ROBERTO BADARÓ EDGAR M. CARVALHO
HEONIR ROCHA ARISTIDES C. QUEIROZ
THOMAS C. JONES[1]

Division of Immunology, Federal University of Bahia, Salvador, Bahia, Brazil; and Division of International Medicine, Cornell University Medical College,[1] New York, USA

Summary Three cases are described showing that *Leishmania donovani* can cause progressive disease in immunocompromised hosts. The first patient was receiving corticosteroid therapy for ulcerative colitis and the second corticosteroids and cyclophosphamide for proliferative glomerulonephritis; in the third patient, leishmaniasis occurred after a long episode of hepatosplenic schistosomiasis and salmonella bacteraemia which was treated with chloramphenicol. In two cases, the patients had moved away from areas of *L donovani* transmission many years before the progressive disease occurred, consistent with long-term survival of the organism in normal hosts. *L donovani* should be added to the growing list of opportunistic microbial infections.

Introduction

VISCERAL leishmaniasis has long been known to affect children and adults with poor nutrition. Even in the poorest areas, it is a rare disease, with a prevalence of 1–5 per 1000 inhabitants.[1–4] There is evidence that the causative organism, *Leishmania donovani*, can infect and initiate a long-term immune response in 25–50% of individuals living in endemic areas.[2,4–6] The strongly positive delayed hypersensitive skin test reactions obtained many years after exposure[6] and the few cases of disease that have developed in individuals long after leaving endemic areas[7] suggest that the organism persists and remains viable even in healthy people for long periods. The reported variation in incubation periods, from days to years, also supports this view.

The concept of opportunistic microbial infections has been developed during the past 30–40 years for organisms which persist for long periods in mammalian tissues but usually do not cause disease in the normal host. Such organisms include cryptococcus, pneumocystis, *Toxoplasma gondii*, cryptosporidium, *Mycobacterium avium*, and cytomegalovirus.[8–11] We suggest that *L donovani* should be added to this list of organisms.

Case-reports

Patient 1

This 27-year-old woman born in Tucano, in the interior of the State of Bahia, Brazil, was entirely well until the seventh month of her first pregnancy. She had a spontaneous abortion and then an illness of 2 months' duration characterised by fever, diarrhoea with blood and mucus, abdominal pain, and tenesmus, which was diagnosed as ulcerative colitis. She had associated weight loss, hypoalbuminaemia (1·7 g/dl), anaemia (haemoglobin 4·9 g/dl), and leucopenia (white-blood-cell count 2×10^9/l). Physical examination revealed only abdominal tenderness and an enlarged liver 2–3 cm below the costal margin. Liver function tests were within normal limits. Stool examination revealed *Giardia lambia* infection, which was treated appropriately. The patient was treated with high-dose corticosteroids for 5 weeks. She also received antibiotics and blood transfusion on three occasions. The fever persisted and was attributed to pyogenic infection documented during week 2 by a positive blood culture for group D streptococcus, as well as the appearance of pulmonary infiltrates and ascites. During week 2, the spleen tip was noted for the first time and progressive enlargement of the liver and spleen occurred during the next 3 weeks until the liver could be felt 8 cm below the costal margin and the spleen 10–12 cm below the left costal margin. She died 5 weeks after diagnosis and start of corticosteroid therapy.

It is important to note that this patient moved from Tucano to Salvador, a city without endemic visceral leishmaniasis, at the age of 7 years and never returned to the endemic area. She is not aware of any significant febrile illnesses during her childhood in Tucano.

At necropsy, changes in the colon were found consistent with the diagnosis of ulcerative colitis. In addition, the spleen, liver, lymph-nodes, and bone marrow all showed histological changes consistent with leishmaniasis. The spleen weighed 1250 mg. Histology showed atrophy of lymphocyte follicles, and macrophages contained *L donovani* organisms. The liver showed Kupffer cell hyperplasia and leishmania organisms. Mononuclear cell hyperplasia and numerous organisms were found in both lymph-nodes and bone marrow.

Patient 2

This 11-year-old boy was well until 8 months before his admission to hospital in 1977, when generalised oedema, ascites, proteinuria (4·6 g/dl), hypercholesterolaemia (263 mg/dl), and hypoalbuminaemia (2·0 g/dl) developed; nephrotic syndrome was diagnosed. His blood urea nitrogen was 15 mg/dl and serum globulin 2·6 g/dl. He was treated with corticosteroids for 6 months. A renal biopsy sample showed generalised proliferative glomerulonephritis, and cyclophosphamide was added to the prednisolone therapy. After 7 months, a remission was achieved and the steroid treatment gradually reduced. Signs and symptoms recurred in 1980, 1982, and 1983, requiring treatment with prednisone and indomethacin.

The boy was born in an area with endemic leishmaniasis but had moved away 7 years before the disease was diagnosed. He visited the area occasionally and in September, 1983, spent a couple of days in the village where he was born. 2 months later high fever started and continued for 2 months, associated with hepatosplenomegaly. This boy had an excellent nutritional status and did not present the classic picture of visceral leishmaniasis—weight loss, epistaxis, cough, diarrhoea, anaemia, neutropenia, and hypoalbuminaemia. Because he came from an area of Brazil endemic for visceral leishmaniasis, we looked for antileishmania antibodies, which were present in high titre (1/2048 immunofluorescent test).[12] Bone-marrow examination showed increased monocytes and plasmocytes, but leishmania organisms were not identified on a smear. A culture for *L donovani* was contaminated. Because of the specificity of the serological test at this titre, and because of the clinical pattern of the illness, antimony therapy was started. The symptoms decreased and hepatosplenomegaly resolved. After 2 months, the antileishmania antibody titre had fallen to 1/512 and a skin test with leishmania antigen became positive (>5 mm).[6] Corticosteroid treatment to control the glomerulonephritis was restarted. The boy has remained stable, without recurrence of hepatosplenomegaly, for 2 years.

Patient 3

This 20-year-old man has lived in an area of the State of Bahia where cases of visceral leishmaniasis have been carefully recorded for 30 years.[4,13,14] It had an epidemic pattern of disease during 1970–76 but has had only two cases of the disease since 1979. In 1976, when the patient was 12 years old, active visceral leishmaniasis was diagnosed on the basis of the clinical pattern of fever and hepatosplenomegaly and identification of *L donovani* in

bone-marrow smear. He was treated with antimony, recovered, and remained well during the next 7 years. After the effective therapy, he moved to a part of his home town where no case of visceral leishmaniasis has been reported for the past 10 years. In 1981, as part of a study to evaluate immunological response in treated visceral leishmaniasis, the blastogenesis response of his lymphocytes was measured. The tritiated thymidine uptake of cultures stimulated with leishmania antigen was 2513±174 cpm compared with 569±39 cpm in unstimulated cultures. This response is typical of successfully treated visceral leishmaniasis.[15]

In 1983, fever and weight loss occurred. The patient was admitted to hospital and the well-recognised combination of hepatosplenic schistosomiasis and prolonged *Salmonella typhi* bacteraemia was documented.[16,17] He was treated with chloramphenicol, with only partial recovery, but disappearance of the bacteria was documented. Shortly after discharge and over the next 4 months, gradually increasing hepatosplenomegaly and recurrence of fever were observed. Bone-marrow aspirate revealed *L donovani* organisms and the patient was seropositive for *L donovani* at a titre of 1/8000. He was treated with antimony with resolution of his symptoms.

Discussion

The criteria for an organism to be an important cause of opportunistic infection are that it is present in the tissues of normal hosts for long periods and that it is maintained in that state by normal immunological mechanisms. On the basis of the three cases of visceral leishmaniasis reported here, *L donovani* satisfies these criteria. Patient 1 was not exposed to *L donovani* for over 20 years, but during immunosuppression rapidly progressive, fatal visceral leishmaniasis developed. Although patient 2 could have been an unusual presentation of naturally acquired leishmaniasis, we are convinced that the disease developed only because of his immunosuppression. Patient 3 showed recurrence of visceral leishmaniasis during an illness and therapy known to be associated with suppression of the immune response.[18] His case clearly differs from the well-known relapse that may occur within a few months of antimony therapy for visceral leishmaniasis.[1,19]

Three other protozoa are already considered important causes of opportunistic infections—pneumocystis, *Toxoplasma gondii*, and cryptosporidium.[20–22] *T gondii* may cause mild illness at the time of initial infection, then it develops a life-long cyst stage which has been shown to reactivate and lead to serious, fatal disease in immunosuppressed patients, particularly those with acquired immunodeficiency syndrome (AIDS).[22] The main differences between toxoplasma and leishmania are the organ site and the microbial stage allowing persistent infection. In toxoplasma, the site is brain or muscle; in leishmania, organisms may persist in granulomas in the liver where they have been identified in symptom-free infected individuals, but this is not certain.[23] Cryptosporidium is an intestinal pathogen, which causes a short illness in healthy individuals but a severe, progressive disseminated disease in AIDS patients. This organism is a common cause of symptomless infection in tropical and subtropical countries.[24]

Visceral leishmaniasis is characterised by pronounced abnormalities in host immune response, including hypergammaglobulinaemia,[25] reduced delayed-type hypersensitivity response to various antigens,[26] depressed lymphocyte blastogenesis to leishmania antigen,[15] presence of serum suppressor factors of lymphocyte blastogenesis,[27] and high levels of circulating immune complexes.[28] Although some of these abnormalities are consequences of the disease, we have found that infected healthy subjects who later acquire the disease have depressed lymphocyte response to leishmania antigen before clinical manifestations appear.[29] In contrast, subjects infected and able to control the disease can mount a good cellular immune response to leishmania antigen. These findings indicate that leishmania infection can be controlled by the host immune response and that acquisition of disease is associated with reduced immune responsiveness to leishmania antigen.

Antibody titres to *L donovani* are very high during active disease and they can be used for the diagnosis of visceral leishmaniasis.[5,12,25] Antileishmania antibody titres fall after treatment, but they may remain positive for long periods.[30] Persistence of high serum antibody titres is also common in other protozoal infections in which the microbe remains viable for decades in host tissue, such as Chagas' disease and toxoplasmosis. In addition, many such infections, including tuberculosis, toxoplasmosis, and leishmaniasis, are associated with life-long skin test reactivity to the relevant antigen.[6] Based on these observations, we recommend a search for *L donovani* amastigotes and measurement of antibody titres to *L donovani* in all immunocompromised hosts who have ever lived in an area endemic for leishmaniasis if they present with fever of unknown origin associated enlarged liver and/or spleen.

This work was supported by National Institutes of Health grant AI 16282.

Correspondence should be addressed to R. B., Hospital Prof Edgard Santos, Rua João das Botas, s/n-Canela, 40000 Salvador, Bahia, Brazil.

REFERENCES

1. Thakur CP. Epidemiological, clinical and therapeutic features of Bilhar kala-azar (including post kala-azar dermal leishmaniasis). *Trans R Soc Trop Med Hyg* 1984; **78:** 391–98.
2. Ho M, Siongok TK, Lyerly WH, Smith DH. Prevalence and disease spectrum in a new focus of visceral leishmaniasis in Kenya. *Trans R Soc Trop Med Hyg* 1982; **76:** 741–46.
3. Greenwood BM, Ajdukiewick AB, Conteh S, Hagan P, Mabey DCW, Panton LJ. Leishmaniasis in the Gambia. Is its incidence increasing? *Trans R Soc Trop Med Hyg* 1984; **78:** 407–09.
4. Badaró R, Lorenço R, Lisboa A, Teixeira R, Johnson WD Jr, Jones TC. An epidemiological and clinical study of American visceral leishmaniasis. Anais do XIX Congresso da Sociedade Brasileira de Medicina Tropical 1983; 59–60.
5. Badaró R, Reed S, Barral A, Orge G, Jones TC. Evaluation of the micro enzyme-linked immunosorbent assay (ELISA) for antibodies to American visceral leishmaniasis: standardisation of parasite antigen to detect infection-specific responses. *Am J Trop Med Hyg* 1986; **35:** 72–78.
6. Reed SG, Badaró R, Masur H, et al. Selection of a specific skin test antigen for American visceral leishmaniasis. *Am J Trop Med Hyg* 1986; **35:** 74–85.
7. Ma DDF, Concannon AJ, Hayes J. Fatal leishmaniasis in renal transplant patients *Lancet* 1979; ii: 311–12.
8. Peterson PK, Simon RL. Infection in organ transplant recipients. In: Mandell G, Bennett J, Douglas RG, eds. Principles and practice of infectious disease New York: John Wiley & Sons, 1985: 1676–80.
9. Gottlieb MS, Schroff R, Schauber HM, et al. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy honosexual men. *N Engl J Med* 1981; **305:** 1425–31.
10. Pape JW, Litaud B, Thomas F, et al. Characteristics of the acquired immunodeficiency syndrome (AIDS) in Haiti. *N Engl J Med* 1983; **309:** 945–50.
11. Masur H, Michaelis MA, Wormser GT, et al. Opportunistic infection in previously healthy women. *Ann Intern Med* 1982; **97:** 533–39.
12. Badaró R, Reed SG, Carvalho EM. Immunofluorescent antibody test in American visceral leishmaniasis: sensitivity and specificity of different morphological forms of two leishmania species. *Am J Trop Med Hyg* 1983; **32:** 480–84.
13. Pessoa SB, Silva LHP, Figueredo J. Calazar endêmico em Jacobina (Estado da Bahia) *Bol Fund Gonçalo Muniz* 1956; **7:** 1–13.
14. Carvalho JS, Prata A. Mielograma no calazar. *Arq Bras Med Naval* 1958; **68:** 65–84
15. Carvalho EM, Teixeira R, Johnson WD. Cell mediated immunity in American visceral leishmaniasis. Reversible immunosuppression during acute infection. *Infect Immun* 1981; **33:** 498–02.
16. Teixeira R. Typhoid fever of protracted course. *Rev Int Med Trop São Paulo* 1959, **2:** 65–72.
17. Rocha H, Kirk JW, Hearey CD Jr. Prolonged Salmonella bacteremia in patients with *Schistosoma mansoni* infection. *Arch Intern Med* 1971; **128:** 254–57.
18. Lipsty JJ, Anderson NP, Lietman PS. Suppression of graft-versus-host reactions by D and L chloramphenicol. *Cell Immunol* 1976; **23:** 278–82

References continued at foot of next column

CELL MEMBRANE SODIUM TRANSPORT: A CORRELATION BETWEEN HUMAN RESISTANCE VESSELS AND LEUCOCYTES

C. AALKJAER
S. D. PARVIN
R. F. BING
A. M. HEAGERTY
P. R. F. BELL
J. D. SWALES

Department of Biophysics, University of Aarhus, Denmark; and Departments of Medicine and Surgery, University of Leicester, Clinical Sciences Building, Leicester Royal Infirmary, Leicester, UK

Summary Sodium efflux was studied in leucocytes and resistance vessels from omental fat from 18 subjects undergoing laparotomy. The efflux of sodium was considerably faster from resistance vessels than from leucocytes, but there was a significant correlation between total efflux rate constants in the two tissues. This was attributable to a highly significant correlation between active sodium pumping as assessed by the ouabain-sensitive sodium efflux rate constants observed in both tissues. These results indicate that intrinsic characteristics of cell membrane electrolyte transport are shared by the leucocyte and vascular smooth muscle in human beings. Therefore, leucocytes can be used to provide information about altered vascular ion handling.

Introduction

INTEREST in the mechanisms underlying human essential hypertension has been aroused by numerous reports of disturbances in sodium handling by plasma membranes.[1] Most of this work has been on easily accessible tissues, such as erythrocytes and leucocytes. In white blood cells, a consistent finding has been a decreased ouabain-sensitive efflux rate constant and a slightly raised intracellular sodium content.[2,3] The direct relevance of this to hypertension is that leucocyte sodium fluxes are thought to be representative of sodium handling by smooth-muscle cells of resistance blood vessels.[4] Cellular sodium metabolism has been studied recently in resistance vessels.[5] Therefore, we decided to investigate whether membrane sodium movements could be correlated in leucocytes and resistance vessels from the same subjects.

Patients and Methods

Patients

18 patients were studied, of whom 15 were male. The mean age was 63±2·9 years, and mean blood pressure was 149±5·2/80±2·7 mm Hg supine and 140±5·5/82±3·5 mm Hg standing. All subjects had been admitted for elective surgical procedures which required laparotomy. Leucocytes were obtained by means of peripheral venesection, and resistance vessels were obtained from a sample of omentum taken at the time of operation. All subjects gave informed consent and the study was approved by the local ethical committee.

Measurement of Leucocyte Sodium Efflux Rate Constant

This was done with the method of Milner et al.[6] Venous blood (60 ml) was drawn into heparinised tubes and mixed with 'Plasmagel' (Uniscience, Cambridge) for 30 min at 37°C to allow most of the erythrocytes to sediment and leave the leucocytes in suspension. The suspension was centrifuged at 37°C at 300 *g* for 7 min and the remaining erythrocytes removed by means of hypotonic lysis.

After resuspension of the cells in 'Medium 199' (Gibco, Paisley, Scotland) 5 μCi of ^{22}Na (Radiochemicals, Amersham) was added and the cells were allowed to incubate at 37°C for 30 min to reach a steady state. The leucocytes were then washed twice in unlabelled medium 199, and samples were taken at timed intervals in the presence and absence of ouabain (1 mmol/l) and cell pellets counted for ^{22}Na content. The sodium efflux rate constant was calculated with the use of linear regression analysis to give the slope of the rate of efflux.

Measurement of Sodium Transport in Resistance Vessels

This was done with the method of Aalkjaer and Mulvany.[5] Omental biopsy samples were placed in a physiological salt solution (PSS) and brought to the laboratory, where 8 to 12 3 mm segments of artery with a diameter of approximately 200 μm were dissected out for flux determinations. For the flux measurements the vessels were kept in PSS at 37°C for 1 h and then loaded in PSS with ^{22}Na (1 Ci/mol) at 37°C for 30 min. After the loading the vessels were washed for 13 min by taking them through a series of vials each containing PSS at 37°C. The last vial (11 min to 13 min) contained 1 mmol/l ouabain (Sigma). The rate constants for the total efflux and ouabain-resistant, and ouabain-sensitive flux were then calculated for each vessel from the washout at specific times. The geometric means of the 8–12 determinations of the total, the ouabain-resistant, and the ouabain-sensitive rate constants were then taken as representative for an individual.

Results are expressed as mean±SEM and correlations were obtained by plotting values for leucocytes and blood vessels from the same subject against each other.

Results

Efflux Rate Constant

The mean sodium efflux rate constants (ERC) for resistance vessels in 18 patients were: total, 6·93±0·6 h^{-1}; ouabain resistant, 3·3±0·3 h^{-1}; ouabain sensitive, 3·5±0·4 h^{-1}.

The mean sodium ERC for leucocytes in the same 18 patients were: total, 2·06±0·17 h^{-1}; ouabain resistant, 0·66±0·09 h^{-1}; ouabain sensitive, 1·4±0·15 h^{-1}.

There was a significant correlation between total sodium ERC in the resistance vessels and leucocytes ($r=0·48$, $p<0·05$, fig 1). There was no correlation between ouabain-resistant ERC ($r=-0·18$, $p>0·1$, fig 2), but there was a highly significant correlation between ouabain-sensitive ERC in the two tissues ($r=0·64$, $p>0·01$, fig 3).

R. BADARÓ AND OTHERS: REFERENCES—*continued*

19. Chulay JD, Bhatt SM, Muigai R, et al. A comparison of three dosage regimens of sodium stibogluconate in the treatment of visceral leishmaniasis. *J Infect Dis* 1983; **148:** 148–55.
20. Masur H, Michaelis MA, Greene JB, et al An outbreak of community acquired *Pneumocystis carinii* pneumonia: initial manifestation of cellular immune dysfunction. *N Engl J Med* 1981; **305:** 1431–38.
21. Current WR, Reese MV, Ernst JC, et al. Human cryptosporidiosis in immunocompetent and immunodeficient persons: studies of an outbreak and experimental transmission. *N Engl J Med* 1983; **308:** 1252–57.
22. Wong B, Gold JWM, Brown AE, et al. Central nervous system toxoplasmosis in homosexual men and parenteral drug abusers. *Ann Intern Med* 1984; **100:** 36–42.
23. Pampiglione FG, Manson-Bahr PEC, Girinzi F, Girint G, Parenti A, Trotti GC. Studies on Mediterranean leishmaniasis. Two asymptomatic cases of visceral leishmaniasis. *Trans R Soc Trop Med Hyg* 1974; **68:** 447–53.
24. Soave R, Ma P. Cryptosporidiosis: traveler's diarrhea in two families. *Arch Intern Med* 1985; **145:** 70–72.
25. Rezai HR, Ardehali SM, Amirhakimi G, Kharazmi A. Immunological features of kala-azar. *Am J Trop Med Hyg* 1978; **27:** 1079–83.
26 Andrade TM, Teixeira R, Andrade JAF, Pereira C, Carvalho EM. Hipersensibilidade de tipo retardado na leishmaniose visceral *Rev Inst Med Trop S Paulo* 1982; **24:** 298–302.
27 Carvalho EM, Bacelar O. Lymphocyte reactivity to mitogens in American visceral leishmaniasis. *Bras J Med Biol Res* 1983; **16:** 35–41.
28. Carvalho EM, Andrews BS, Martinelli R, Dutra M, Rocha H. Circulating immune complexes and rheumatoid factor in schisttosomiasis and visceral leishmaniasis. *Am J Trop Med Hyg* 1983; **32:** 61–68.
29. Carvalho EM, Badaró R, Barral A, Pedral-Sampaio D, Bacelar O. Leishmaniose visceral. Associação entre depressão da resposta celular a antígeno de leishmania e aparecimento de doença clinicamente manifesta. XI Reuniao de Pesquisa Básica em Doença de Chagas 1984; 111–12.
30. Badaró R, Carvalho EM, Orge MGO, Teixeira RS, Rocha H. Immunidade humoral e celular em individuos curados de leishmaniose visceral. *Rev Soc Bras Med Trop* 1985; **18:** 77–83.