Rev. Inst. Med. trop. S. Paulo
39(4): 223-226, July/August, 1997

AMASTIGOTES FORMS OF *Trypanosoma cruzi* DETECTED IN A RENAL ALLOGRAFT

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SUMMARY

Trypanosoma cruzi, the causative agent of Chagas'disease assumes two distinct forms in vertebrate hosts: circulating trypomastigote and tissular amastigote. This latter form infects predominantly the myocardium, smooth and skeletal muscle, and central nervous system. The present work describes for the first time the detection of amastigote forms of *T. cruzi* in the renal parenchyma of a kidney graft recipient one month after transplantation. The patient was serologically negative for Chagas'disease and received no blood transfusion prior to transplant. The cadaver donor was from an endemic area for Chagas'disease. The recipient developed the acute form of the disease with detection of amastigote forms of *T. cruzi* in the renal allograft biopsy and circulating trypomastigote forms. The present report demonstrates that *T. cruzi* can infect the renal parenchyma. This mode of transmission warrants in endemic areas of Chagas'disease.

KEYWORDS: Chagas'disease; *Trypanosoma cruzi*; Kidney transplantation.

INTRODUCTION

Chagas'disease, or American trypanosomiasis, is a zoonosis caused by *Trypanosoma cruzi*, which is endemic in most countries of south and central America, mainly in Argentina[1,2,8,17], Chile[15] and Brazil[11,12,14].

After kidney transplantation, patients in endemic areas may develop the acute phase of the disease either as a consequence of reactivation[5,17], transmission by the graft[4,6,7,9] or by the bite of triatomid bugs after the transplant[16].

The first case reported of acute Chagas'disease after kidney transplantation did not discuss the etiopathogenesis of the infection[10]. Transmission of the disease through the graft was first proposed by CHOCAIR et al. (1981), and later observed by others[3,5,6,7,9,17].

The causative agent of Chagas' disease assumes two distinct forms in vertebrate hosts, i.e., circulating trypomastigote and tissular amastigote. The transmission of Chagas' disease by transplanted organs probably occurs only if there is tissular parasitism, since the graft is extensively perfused prior to transplantation.

In all reported cases on post-transplant Chagas' disease, the diagnosis was established by the detection of trypomastigote forms in the peripheral blood, xenodiagnosis and/or by serology. Diagnosis based on the presence of amastigote in the renal parenchyma has not been reported.

The purpose of this work is to describe a case of acute Chagas'disease following kidney transplantation with the detection of *T. cruzi* in the renal tissue, which corroborates the hypothesis that this zoonosis can be transmitted by an infected graft.

CASE REPORT

A 37-year-old white male university teacher, born and living in Bauru, state of São Paulo, Brazil, with terminal chronic renal failure secondary to crescentic glomerulonephritis, on continuous ambulatorial peritoneal dialysis for 5 months, was submitted for his first renal transplantation from a cadaveric donor in October, 1993. The 46-year-old white male donor, with 2 matches on Dr loci, was seronegative for Chagas'disease.

Prior to surgery the recipient was negative for Chagas'disease by passive hemagglutination and IgG indirect

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immunofluorescence and had never received any blood transfusion or lived in endemic areas for Chagas'disease.

There was immediate function of the graft after transplantation. The initial immunosuppressive medications were azathioprine 2 mg/kg/d; prednisone 1 mg/kg/d and cyclosporine 6 mg/kg/d. On the 12th post-transplantation day the creatinine level was 1.8 mg/dl and increased to 2.5 mg/dl on day 18. The patient was treated with methylprednisolone 1 g/day for 3 days without any response. A renal biopsy revealed acute tubular necrosis (ATN). Creatinine levels reached 5.1 mg/dl on day 25. A renal biopsy at that day showed persistent ATN.

As no clinical amelioration occurred and the patient became febrile (38.4-39.0°C), an third renal graft biopsy was performed on day 31. It revealed persistent ATN, cytomegalovirus inclusions in tubular cells and lymphomononuclear interstitial nephritis, which showed macrophages with pseudocysts of amastigotes in the cytoplasm. This finding was confirmed by a positive immunohistochemical stained with a rabbit polyclonal anti-*T. cruzi* (Figure 1 A-D). Peripheral blood Giemsa-stained smears revealed a large number of trypomastigotes.

The persistence of ATN was associated to the presence of vascular component of an acute rejection.

The vasculitis was observed in the 2nd and 3rd biopsy and was defined by the presence of leukocytes adherent to an in between the endothelial cell, and by fibrous thickening of the intima.

On this occasion the patient's hemogram showed 27.3% hematocrit, 9.3 g/dl hemoglobin, 6100/mm^3 white cells (4% band cells, 89% segmented cells, 6% lymphocytes and 1% monocytes), 75,000/mm^3 platelets and 1% atypical lymphocytes. Serology for Chagas'disease was inconclusive in passive hemagglutination, positive in IgG indirect immunofluorescence and ELISA; serology for cytomegalovirus was positive in IgM-ELISA. Cerebrospinal fluid was clear, colorless and cell-free, and presented normal levels of total proteins, glucose and chlorides. Treatment with 6 mg/kg/day benzonidazole and 3 mg/kg/day gancyclovir was started. The patient became afebrile but renal function remained unchanged.

On day 50 another biopsy was performed which revealed ATN in regeneration, and absence of tissular amastigote cysts or cytomegalovirus inclusions. Serological tests, Giemsa-stained smears examination and xenodiagnosis for Chagas'disease were repeated and were negative. The patient died due to conditions unrelated to Chagas'disease and the family did not authorize necropsy.

COMMENTS

The amastigote forms of *T. cruzi* are most frequently found in the myocardium, smooth and skeletal muscle and central nervous system[11,12]. The presence of amastigotes in the renal parenchyma has never been reported in chagasic patients, or infected animals, either in presence or absence of immunosuppression.

Chagas'disease may occur in renal transplantation either by endogenous reactivation, even in serologic negative patients, since there occur 2% of false negative results by passive hemagglutination[13], by transfusion with infected blood or by exogenous infection after the patient's return to the endemic area[17].

In addition, although previous investigations on chagasic infections in patients submitted to renal transplantation strongly indicated that Chagas'disease may be transmitted by the transplanted kidney, this form of transmission has not been completely accepted because the presence of amastigotes in the renal parenchyma had not been previously reported.

In this study, the source of infection was not fully identified because the recipient and the donor were both serologically negative for Chagas'disease prior to renal transplantation. The recipient received no blood transfusion before or after renal transplantation, had always lived in the state of São Paulo, that it is not considered to be an endemic area and where the incidence of Chagas'disease is around 2.5%[13] and, was hospitalized throughout the course of the disease.

The kidney was obtained from a patient who lived in an endemic area for Chagas'disease where the prevalence of infection in blood donors may be as high as 7.5%[13], which suggested that the infection was transmitted to the recipient by the graft.

The fact that no amastigotes were observed in the two first biopsies may be explained by the low level of parasitism or a sample error. After a long period of immunosuppressive therapy, associated with large doses of methylprednisolone and an infection by cytomegalovirus, which induces also immunosuppression, the level of parasitism increased and amastigotes were detected in the third renal biopsy.

The present report demonstrates that *T. cruzi* may infect the renal parenchyma. Therefore, kidneys from donors in the subclinical stage of Chagas'disease may be the source of infection in renal transplantation.

RESUMO

Detecção de formas amastigotas do *Trypanosoma cruzi* em enxerto renal

A doença de Chagas é zoonose transmitida pelo *Trypanosoma cruzi*, o qual apresenta duas formas distintas no hospedeiro vertebrado, a tripomastigota circulante e a amastigota tecidual. Esta última parasita freqüentemente os tecidos musculares cardíaco, liso e estriado, e o tecido nervoso. Até o presente momento nunca foram detectados formas amastigotas em parênquima renal. O presente relato descreve, pela primeira vez, a detecção de formas amastigotas do *T. cruzi* em parênquima

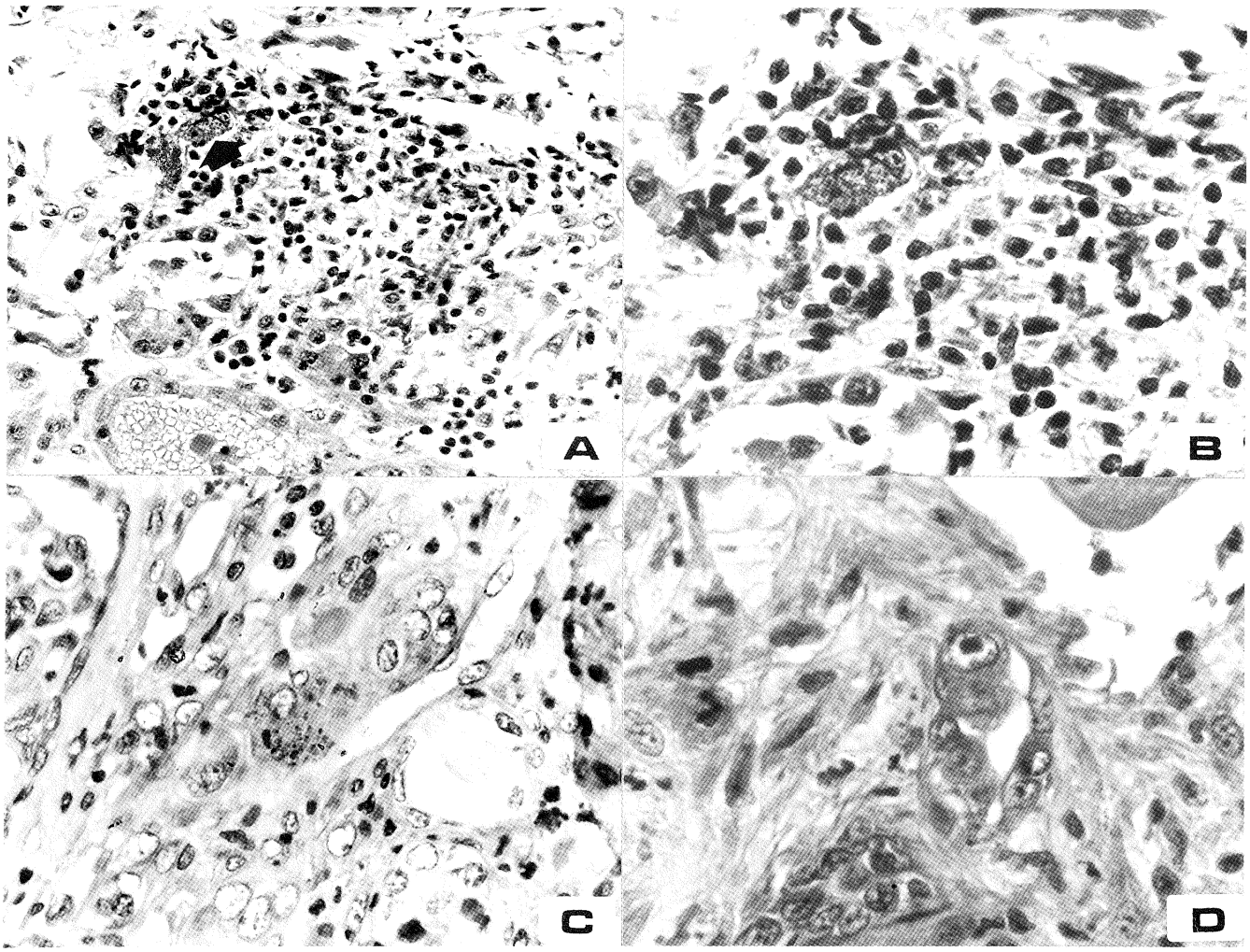

Fig. 1 – A) Lymphomononuclear interstitial nephritis with an amastigote pseudocyst (arrow). (HE; 100X); B) Higher magnification showing the pseudocyst (HE; 400X); C) Immunoperoxidase stained with a rabbit polyclonal anti-*T. cruzi* serum (400X); D) Cytomegalovirus inclusions in tubular cells (HE; 400X).

renal em receptor de enxerto de rim, com testes sorológicos negativos para a doença de Chagas e ausência de transfusões prévias, observado 1 mês após o transplante renal com doador cadáver proveniente de região endêmica. O paciente desenvolveu doença de Chagas aguda com detecção de formas tripomastigotas circulantes. Como a única forma de transmissão desta zoonose pelo enxerto é através de órgão parasitado com formas amastigotas, sugere-se fortemente que o rim transplantado foi o responsável pela transmissão da doença de Chagas, no presente caso. Esta é a via de infecção que deve ser levada em consideração em transplantes nas áreas endêmicas.

ACKNOWLEDGEMENT

We thank to Dr. Maria Irma Seixas Duarte for the immunohistochemical stainings.

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Recebido para publicação em 21/05/1997
Aceito para publicação em 15/09/1997