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A prospective study on the dynamics of the clinical and immunological evolution of human *Leishmania (L.) infantum chagasi* infection in the Brazilian Amazon region

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ABSTRACT

This prospective study was carried out from October 2003 to December 2005 and involved a cohort of 946 individuals of both genders, aged 1–89 years, from an endemic area for American visceral leishmaniasis (AVL), in Pará State, Brazil. The aim of the study was to analyze the dynamics of the clinical and immunological evolution of human *Leishmania (L.) infantum chagasi* infection represented by the following clinical-immunological profiles: asymptomatic infection (AI); symptomatic infection (SI = AVL); subclinical oligosymptomatic infection (SOI); subclinical resistant infection (SRI); and indeterminate initial infection (III). Infection diagnosis was determined by the indirect fluorescent antibody test and leishmanin skin test. In total, 231 cases of infection were diagnosed: the AI profile was the most frequent (73.2%), followed by SRI (12.1%), III (9.9%), SI (2.6%) and SOI (2.2%). The major conclusion regarding evolution dynamics was that the III profile plays a pivotal role from which the cases evolve to either the resistant, SRI and AI, or susceptible, SOI and SI, profiles; only one of the 23 III cases evolved to SI, while most evolved to either SRI (nine cases) or SOI (five cases) and eight cases remained as III.

1. Introduction

One of most interesting features regarding the interaction between *Leishmania (L.) infantum chagasi*, the etiological agent of American visceral leishmaniasis (AVL), and the human immune response is the clinical and immunological spectrum that may result from this interaction. Indeed, a well-defined understanding of this spectrum may provide a more complete view regarding the repertoire of alternatives used by the human immune response against *L. (L.) i. chagasi* infection. Thus, it was considered that the clinical spectrum might range from an asymptomatic stage in resistant individuals with a vigorous T-cell response (hypersensitivity) to a symptomatic stage in susceptible individuals, in which a specific suppression of T-cell response (hyposensitivity) may lead to classic AVL.[1,2] Between these two polar stages, however, certain individuals may show an intermediate condition known as subclinical oligosymptomatic infection, in which the clinical and immunological features are not clearly defined.[3,4]

In Brazil, although the aim of some studies has been to try to characterize the clinical and immunological spectrum of human *L. (L.) i. chagasi* infection, this has been principally realized on children under 15 years old and

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based only on the antibody response of infected individuals, which has raised some difficulties concerning a complete view of human immune response against the infection.[5–7] In other words, these studies have generally used humoral immunodiagnostic methods, such as the enzyme-linked immunosorbent assay (ELISA), to determine the diagnosis of an active *L. (L.) i. chagasi* infection, leading to underestimation of the possibility that certain clinical and/or immunological features could also be associated with the T-cell response of infected individuals living in endemic areas.

In view of these considerations and the lack of information regarding the interaction between human *L. (L.) i. chagasi* infection and human immune response in the Brazilian Amazon region, we considered the results of a prospective study combining the clinical status of infected individuals with their humoral and T-cell immune responses. This study was realized for a period of more than 2 years with a cohort of 946 individuals of both genders, aged ≥1 year, in an endemic area for AVL in the municipality of Barcarena, PA, Brazil. The humoral response was measured by indirect fluorescent antibody test (IFAT) and the T-cell response by leishmanin skin test (LST). Using this diagnostic approach, we studied the dynamics of the clinical and immunological evolution of human *L. (L.) i. chagasi* infection, aiming to improve current understanding concerning the repertoire of immune response against this infection.

2. Materials and methods

2.1. Study area and population

This follow-up study was carried out in Santana do Cafezal village, which is situated on the banks of the river Cafezal, 7 km from the administrative centre of Barcarena municipality (01°30′S; 48°37′W) and is considered to be within the metropolitan region of Belém, PA, northern Brazil.[8] The population enrolled in this study consisted of a cohort of 946 individuals (568 males and 378 females) aged 1–89 years.[8]

2.2. Study design

As the present study was performed to analyze the dynamics of the clinical and immunological evolution of human *L. (L.) i. chagasi* infection, it was necessary to follow the design and planning of a prospective analysis on the prevalence and incidence of infection during a follow-up period of more than 2 years (October 2003 to December 2005). Thus, the IFAT and the LST were chronologically used at the same timepoint as the prevalence and incidence surveys; i.e. for all individuals previously selected for the prevalence survey and at 6, 12 and 24 months. These tests were performed only on those individuals that were negative both in the prevalence and in the previous incidence survey. However, in cases of reactivity by LST alone, which represents a genetic characteristic of immunological resistance to infection,[9] the individuals were removed from subsequent LST surveys, similar to that proposed in a longitudinal study in Sudan.[10] In cases revealing reactivity for both tests, the individuals were tested only by IFAT. Finally, in cases of reactivity by IFAT alone, which, contrary to LST, represents an immunological status of susceptibility to infection, the individuals remained under investigation by both tests, with the aim of analyzing the evolution of both immune responses. For a number of different reasons, such as holidays or travel, a loss of almost 10% (94 individuals) from the original sample occurred over the 2 year follow-up period. In addition, the total population was also divided into three age groups (1–10, 11–20 and ≥21 years, consisting of 260, 218 and 468 individuals, respectively), aimed at analyzing the age distribution of infection.

2.3. Clinical evaluation of infected individuals

All individuals presenting any type of immune reaction, by LST and/or IFAT, were clinically examined (a complete physical examination) in order to identify any signs and/or symptoms that could be recognized as classical features of AVL, as well as symptoms (fever, asthenia, pallor and slight hepatomegaly) of subclinical oligosymptomatic infection. Only those cases presenting typical features of AVL received conventional antimony therapy, as recommended by the Brazilian AVL control program.[11] In principal, the subclinical oligosymptomatic cases were only followed-up to confirm their spontaneous clinical resolution in the following month or two, as observed in a prospective study realized in Maranhão State, in the northeastern region of Brazil.[6]

2.4. Criteria for identification of human infection

The definition of a human case of *L. (L.) i. chagasi* infection was based on the presence of reactivity to IFAT or LST or both tests. Considering that human HIV co-infection could interfere with this diagnostic approach, it is important to state that up to the onset of the study, no case of human HIV infection in the study area was recorded by the Health Care Secretary of Barcarena municipality. Serological reactions of ≥80 (IgG) titer and intradermal reactions forming papules or indurations of ≥5 mm in diameter were regarded as the positive cut-off for the IFAT and LST, respectively.[12,13] A scale of semi-quantitative results was used to express the specificity of the IFAT and LST with scores varying from + to ++++, as follows: for the IFAT, serological titers of 80–160 (IgG) were regarded as +, 320–640 as ++, 1280–2560 as +++ and 5120–10 240 as ++++; for the LST, exacerbated intradermal reactions (≥16 mm) were regarded as ++++, strongly positive (13–15 mm) as +++, moderately positive (9–12 mm) as ++ and weakly positive (5–8 mm) as +. By combining the clinical status of infected individuals with the semiquantitative scale of scores for LST and IFAT specificity, it was possible to identify the clinical-immunological profiles of infection,[8] which were followed up in the present investigation: asymptomatic infection (AI) (LST+/++++ and IFAT−); symptomatic infection (SI = AVL) and subclinical oligosymptomatic infection (SOI) with the same immune profile (LST− and IFAT+++/++++); subclinical resistant infection (SRI) (LST+/++ and IFAT+/++); and indeterminate initial infection (III) (LST− and IFAT+/++).