Genetic Polymorphism and Molecular Epidemiology of *Leishmania (Viannia) braziliensis* from Different Hosts and Geographic Areas in Brazil

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Received 18 November 2002/Returned for modification 31 January 2003/Accepted 31 March 2003

Numerical zymotaxonomy and variability of the internal transcribed spacers (ITS) between the small and large subunits of the rRNA genes were used to examine strain variation and relationships in natural populations of *Leishmania (Viannia) braziliensis*. A total of 101 strains from distinct hosts and Brazilian geographic regions were assigned to 15 zymodemes clustered in two major genetic groups. The great number of isolates (48.5%) placed in zymodeme IOC/Z-27 were collected on the Atlantic coast. The high molecular diversity found in populations in the Amazon Basin was related to the great number of sandfly vector(s) in that region. The results of the restriction fragment length polymorphism analysis of the ITS depicted considerable intraspecific variation. Genotypic groups A, B, and C contained 39, 40, and 22 isolates, which were divided into 16, 10, and 15 genotypes, respectively. The genetic polymorphism observed demonstrates the degree of diversity of *L. (V.) braziliensis* strains from different regions where they are endemic. The results reinforce the clonal theory for *Leishmania* parasites showing the genetic diversity of this pathogen and an association of *L. (V.) braziliensis* genotypes with specific transmission cycles, probably reflecting an adaptation of different clones to the vector species involved.

Infections with the parasitic protozoan *Leishmania (Viannia) braziliensis* Vianna 1911 (Kinetoplastida: Trypanosomatidae) or strain variants are recognized as causing human illness in many areas of (sub)tropical America (at least 15 countries), where it constitutes a significant public health problem. Many of these parasites seem to have a unique life cycle, with different phlebotomine sandfly (Diptera: Psychodidae) vectors and/or animal reservoirs and a different geographic distribution (13). This pathogen is capable of producing a variety of clinical manifestations, such as (i) cutaneous leishmaniasis (CL), which may heal spontaneously; (ii) mucosal leishmaniasis (ML), a hyperergic invasive ulcerative form that progresses in the absence of any apparent cellular defect (15); and (iii) disseminated CL (4).

Most of the environmental factors affecting the epidemiology of the various leishmaniasis are still poorly understood. Wild mammals serve as reservoirs for most of the New World *Leishmania* spp. (21), but there is increasing evidence that some of the human pathogenic *Leishmania* strains can be maintained in both sylvan and urban cycles. In the case of *L. (V.) braziliensis*, the principal vertebrate hosts in the sylvan cycle have not been identified, but there is evidence that dogs, horses, and donkeys may serve as reservoir hosts of this parasite (15). The existence of an urban cycle involving peridomestic sandfly species for *L. (V.) braziliensis* reflects the ability of these parasites and their vectors to adapt to changes in their original forested habitats with important public health implications. Studies using molecular techniques to characterize *L. (V.) braziliensis* populations from different regions have shown a relationship between level of similarity among the parasite populations (12, 24) and their geographic range, but recent data have also indicated that the considerable variability detected among these parasites is more probably related to the sandfly vector(s) and/or animal reservoir(s) involved in the transmission cycles (18).

Pathogens that produce many different genetic variants are more prone to infect multiple hosts (37). Although several studies have discussed the polymorphism observed in natural populations of different *Leishmania* species (8, 25, 34), until now there has been little information available about the genetic variability of the parasites and the correlation with eco-epidemiological features of the disease (16). The risk factors for clinical leishmaniasis are still poorly understood and probably are influenced by host and parasite features.

Considering the public health importance of leishmaniasis caused by *L. (V.) braziliensis* in Brazil and the role of the genetic polymorphism of the parasites in the epidemiology of the disease, we applied multilocus enzyme electrophoresis (MLEE) and the analysis of the restriction fragment length polymorphism (RFLP) of the internal transcribed spacers (ITS) of the rRNA genes as typing methods to determine the level of genetic variation in natural population of *L. (V.) bra-
Leishmanial strains. The parasites used in the present study and the sources of original stocks are listed in Table 1. We analyzed Leishmania (V.) braziliensis isolates from four geographic localities in Brazil that are regions where cutaneous leishmaniasis is endemic: Rio de Janeiro, Espírito Santo, and Pernambuco, as well as in the Amazonian region. Leishmania (V.) braziliensis reference strains and isolates used in the present study are maintained at DIFIOCRUZ (registration no. 731 [WFCC World Data Center on Microorganisms Directory]).

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**TABLE 1. Results of molecular typing and provenance details of 101 L. (V.) braziliensis strains collected over a 23-year period that were studied for their genetic relatedness**

<table>
<thead>
<tr>
<th>Zymodeme&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Genotypic type&lt;sup&gt;b&lt;/sup&gt; (no. of stocks)</th>
<th>Geographic origin&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Clinical form&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B6 (1)</td>
<td>RJ, Angra dos Reis</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B6 (1)</td>
<td>RJ, Ituca</td>
<td>ML</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B6 (1)</td>
<td>RJ, Maricá</td>
<td>ML</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B5 (1), B6 (8), B9 (1)</td>
<td>RJ, Mesquita</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B6 (3)</td>
<td>RJ, Paraty</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B9 (1)</td>
<td>RJ, Quinclam</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B5 (1), B6 (5), B7 (1), B8 (1)</td>
<td>RJ, Rio de Janeiro</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B6 (1)</td>
<td>RJ, São Gonçalo</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B6 (1)</td>
<td>RJ, Teresópolis</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>A12 (1), A16 (1)</td>
<td>ES, Afonso Cláudio</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>A1 (1)</td>
<td>ES, Água Doce do Norte</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B4 (1)</td>
<td>ES, Aracruz</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>A16 (1)</td>
<td>ES, Castelo</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B3 (1)</td>
<td>ES, Fundão</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>A9 (1)</td>
<td>ES, Itaguaçu</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>A7 (2)</td>
<td>ES, Itarana</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>A13 (1), A16 (1)</td>
<td>ES, Muniz Freire</td>
<td>CL</td>
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<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B2 (4)</td>
<td>ES, Santa Leopoldina</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B10 (2)</td>
<td>ES, Serra</td>
<td>CL</td>
</tr>
<tr>
<td>Z-27 (MHOM/BR/66/M2903)</td>
<td>B1 (5)</td>
<td>ES, Viana</td>
<td>CL, ML</td>
</tr>
<tr>
<td>Z-31 (IPAR/BR/80/IM231)</td>
<td>C13 (1)</td>
<td>RO, BR-319Rd, Km866</td>
<td></td>
</tr>
<tr>
<td>Z-32 (ICAR/BR/86/IM2978)</td>
<td>C1 (1)</td>
<td>RO, Cachoeira Samuel</td>
<td></td>
</tr>
<tr>
<td>Z-34 (MHOM/BR/88/IM3476)</td>
<td>C2 (1)</td>
<td>AM, Tapauá River</td>
<td></td>
</tr>
<tr>
<td>Z-35 (MHOM/BR/88/IM3483)</td>
<td>C3 (1)</td>
<td>AM, Tapauá River</td>
<td></td>
</tr>
<tr>
<td>Z-35 (MHOM/BR/88/IM3483)</td>
<td>C5 (1), C6 (2), C7 (4), C8 (3), C9 (2)</td>
<td>AM, Coari–Urucu River</td>
<td></td>
</tr>
<tr>
<td>Z-35 (MHOM/BR/88/IM3483)</td>
<td>C4 (1)</td>
<td>AM, Manaus</td>
<td></td>
</tr>
<tr>
<td>Z-45 (MHOM/BR/91/JRS)</td>
<td>B1 (5)</td>
<td>PE, Porto Velho</td>
<td></td>
</tr>
<tr>
<td>Z-33 (MHOM/BR/91/IM3708)</td>
<td>B10 (2)</td>
<td>AM, Coari–Urucu River</td>
<td></td>
</tr>
<tr>
<td>Z-33 (MHOM/BR/91/IM3708)</td>
<td>B6 (1)</td>
<td>AM, Coari–Urucu River</td>
<td></td>
</tr>
<tr>
<td>Z-33 (MHOM/BR/91/IM3708)</td>
<td>C11 (1)</td>
<td>AM, Coari–Urucu River</td>
<td></td>
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<tr>
<td>Z-33 (MHOM/BR/91/IM3708)</td>
<td>C12 (1)</td>
<td>AM, Coari–Urucu River</td>
<td></td>
</tr>
<tr>
<td>Z-33 (MHOM/BR/91/IM3708)</td>
<td>C14 (1)</td>
<td>AM, Barcelos</td>
<td></td>
</tr>
<tr>
<td>Z-33 (MHOM/BR/91/IM3708)</td>
<td>A4 (3), A13 (2)</td>
<td>PE, Amaraji</td>
<td></td>
</tr>
<tr>
<td>Z-33 (MHOM/BR/91/IM3708)</td>
<td>A3 (1), A5 (1)</td>
<td>PE, Amaraji</td>
<td></td>
</tr>
<tr>
<td>Z-33 (MHOM/BR/91/IM3708)</td>
<td>A2 (1)</td>
<td>PE, Amaraji</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The zymodeme number of the Instituto Oswaldo Cruz (classification was established by numerical zymotaxonomic analysis) is given. The reference strain designation code is given in parentheses as follow host (I for Insecta [CAR, Psychodopygus carrerai; PAR, Psychodopygus paraensis] M for Mammalia [AGO, Agouti paca; DID, Didelphis marsupialis; HOM, Homo sapiens])country of origin/year of isolation/original code.

<sup>b</sup> Denotes the identity between isolates by using all polymorphic rDNA results.

<sup>c</sup> Codes for states in Brazil (AM, Amazonas; ES, Espírito Santo; PE, Pernambuco; RJ, Rio de Janeiro; RO, Rondônia) are given.

<sup>d</sup> Leishmaniasis lesions in humans (CL, cutaneous; ML, mucosal).

The observed genetic variability and the relationships among the isolates were the basis for investigating whether the genetic differences in *L. (V.) braziliensis* reflect distinct ecoepidemiological features of the infection since these strains are endemic in the distinct areas studied, thus bringing to light fundamental information for future control programs of the disease.
drugase (EC 4.2.1.3), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glucose
phosphate isomerase (EC 5.3.1.9), isocitrate dehydrogenase NAD and NADP
(EC 1.1.1.42), malate dehydrogenase (EC 1.1.1.37), malic enzyme (EC 1.1.1.40),
mannose phosphate isomerase (EC 5.3.1.8), nucleosidase (EC 3.2.2.1), 6-phos-
phogluconate dehydrogenase (EC 1.1.1.43), phosphoglucomutase (EC 1.4.1.9),
Leu-Pro dipeptidase (EC 3.4.13.9), and Leu-Gly dipeptidase (EC 3.4.11.1). The
bands produced on the gels were numbered according to enzymatic mobility, and
the enzyme profiles were subjected to numerical analysis and used to group the
samples in zymodesmes. Leishmanial strains with the same electrophors were
classified into the same zymodeme (5). One strain from each zymodeme was
chosen as the reference for that zymodeme (Table 1).

Intergeneric region typing (IRT). Genomic RFLP analysis of the ITS between
the small and large subunits of the ribosomal DNA (rDNA) locus (PCR-RFLP
ITSrDNA) of leishmanial parasites used in the present study (Table 1) was done,
as described elsewhere (6). The ITS were PCR amplified and digested with
restriction enzymes (dial, EcoRI, FspI, HaeIII, HinfI, RsaI, and TaqI). Digestion
products were separated by high-resolution electrophoresis in a 12%
polyacrylamide gel by using the Genephor apparatus (Amersham Pharmacia
Biotech). The fragment sizes were estimated relative to molecular weight mark-
ers and the banding patterns were used to group the isolates in genotypes (each
genotype grouped isolates with the same banding pattern for all of the restriction
enzyme used) and were also subjected to numerical analysis.

Numerical analysis of MLEE or PCR-RFLP ITSrDNA data. For this numer-
cal analysis, a matrix with the presence or absence of the bands was built and
analyzed by using the NTSYS software program (version 2.1; Exeter Software).
The similarity matrices constructed by using the simple matching coefficient ($S_{sm}$
= $m/n$, where $m$ is the number of matches and $n$ is the total number of charac-
ters) were transformed into phenograms by the UPGMA (unweighted pair-
method with arithmetic averages) algorithm (35). The confidence of the
groups was tested applying a bootstrap analysis with 1,000 replicates using Free-
Tree software (version 0.9.1.50). The average and standard deviation of the
similarities observed among individuals from each area (considering the clusters
observed by the genetic analysis) where the infection is endemic were calcu-
lated as a parameter of genetic diversity.

RESULTS

MLEE. Fourteen enzyme loci of 101 stocks of $L$. ($V$.) braziliensis from different hosts and Brazilian geographic areas
were analyzed, and the electromorphic profiles were compared to reference strains revealing 15 zymodesmes (Table 1). The
most common zymodeme (Z-27) grouped 49 isolates from Espírito Santo and Rio de Janeiro States. In contrast, 31
isolates collected in a rural area in Pernambuco State were classified into five zymodesmes. Furthermore, most of the 22
samples from the Amazon and Rondônia States were individu-
ally assigned to separate zymodesmes. However, the analysis of
allelic variation occurring in stocks compared to the reference
strain MHOM/BR/1966/M2903 of $L$. ($V$.) braziliensis revealed that some zymodesmes differ from each other in only one en-
zymatic locus position (data not shown).

Affinities between zymodesmes were calculated by using the
simple matching similarity coefficient and were transformed
into a phenogram by using mean distances between groups
(Fig. 1A). The 15 $L$. ($V$.) braziliensis zymodesmes could be
clustered into two major groups, with high level of statistic
support as demonstrated by the bootstrap value obtained for
each group (Fig. 1A). Each group clustered strains collected on
either the Atlantic coast or the Amazon region. One group
(comprising isolates from CL or ML patients) was less diverse
compared to the other group (comprising isolates from hu-
mans and various wild animals and sylvan sandflies). Further-
more, the later group contained two parasites (Z-53 and Z-69)
that clustered independently of other zymodesmes. These two
zymodesmes were assigned to the second group based on their
geographic origin and because they presented a higher level of
similarity with the zymodesmes of this group than to the ones of
the other group.

Genotyping. The IRT allowed the examination of polymor-
phisms among $L$. ($V$.) braziliensis zymodesmes (Fig. 2). All leish-
manial isolates indicated in Table 1 were analyzed. It was possible to characterize 41 genotypes, each representing paras-
ites with unique ITS fragment profiles. By using numerical
analysis, the strains were studied for their genetic relatedness
(Fig. 1B). Three statistically supported groups were observed.
Genotypic groups A, B, and C comprised 39, 40, and 22 iso-
lates, which were divided into 16, 10, and 15 related genotypes,
respectively. It was observed that zymodesmes into which more
than one isolate was assigned are represented by more than one
genotype: Z-27 included 49 samples and 16 genotypes, Z-35 included 14 samples and 7 genotypes, Z-45 included 16
samples and 7 genotypes, and Z-72 included 6 samples and 4
zymodesmes (Table 1). Except for Z-27, all of the other zymo-
demes representing large clusters included isolates from the
same geographic locality.

Isolates characterized as the same zymodeme (Z-27) were
clustered into two independent clusters (A and B). Cluster A
comprised isolates from rural localities in Pernambuco and
Espírito Santo. Cluster B accounted for strains related with
domestic animals and peridomestic sandflies that were as-
signed to the same zymodeme (Z-27). Group C included only
isolates from the Amazonian region. This group was more
diverse, presenting some genotypes (C12, C13, C14, and C15)
that clustered independently from the others, but with more
affinity for the genotypes of group C than for the ones of group
A or B. A clonal population (genotype B6) classified in this
group contained 21 isolates from different geographic locations
in the Espirito Santo and Rio de Janeiro states. In contrast,
eight isolates characterized as genotype A4 were assigned to
separate zymodesmes. Furthermore, parasites showing a high
degree of genetic similarity were found to cause either CL or
ML in humans (Table 1).

Geographic distribution and genetic diversity of the geno-
types. A geographic structuring was observed for some geno-
types but not for others that was related to genotypes from
more distant areas where the organisms were endemic (Fig. 1
and 3). The genetic similarity average based on the MLEE and
IRT data was calculated for each locality, and it was assumed
as a parameter to evaluate the level of genetic diversity. We
observed that the isolates from the Amazonian region pre-
sented the highest level of genetic diversity and that the iso-
lates from Rio de Janeiro were more homogeneous (Fig. 3).
The isolates from Espírito Santo were considered as being
involved in two distinct transmission cycles. The Espirito Santo
isolates from urban areas grouped together with the isolates
from Rio de Janeiro (group B) and those from rural areas
clustered with isolates from Pernambuco (group A) (Fig. 3).

DISCUSSION

Advances in molecular technology are facilitating the study of
the ecology of Leishmania clonal populations by providing
information on (i) sources of infection, (ii) transmission pat-
terns, (iii) response to treatment, and (iv) the importance of
immunity in preventing reinfection (27). In the New World, the
Leishmania (Viannia) parasites (7, 8), the main causal agents
of American CL, represent a biologically diverse group of microorganisms showing considerable intraspecies variability (5, 6, 12) that complicates taxonomic classification and epidemiological studies.

The present systematic molecular study revealed *L. (V.) braziliensis* strains to be an extremely diverse population. The multilocus data on a diverse collection of strains from different hosts and ecologic regions in Brazil indicate a clonal popula-

FIG. 1. (A) MLEE phenogram showing the similarity between groups of *L. (V.) braziliensis* zymodemes. Unless indicated otherwise by the number in parentheses, IOC/Z contains a unique isolate. (B) Molecular tree showing the clustering of *L. (V.) braziliensis* genotypes classified in this work (PCR-RFLP ITS rDNA). The bootstrap values (italic numbers) are shown for the principal clusters. Details about strains are given in Table 1. The three main clusters represent parasites collected from different hosts and geographic areas in Brazil (i.e., the Atlantic coast [A and B] or the Amazon region [C]). Isolates characterized as IOC/Z-27 (outlined with continuous and dashed lines) presented genotypes in either group A and B. A, Strains collected in rural localities (Espírito Santo and Pernambuco) where different sandfly and animal species are involved in the transmission cycle; B, strains from urban areas (Espírito Santo and Rio de Janeiro) where transmission is related with a cycle involving domestic animals and preidomestic sandfly species; and C, strains from the Amazon region (in this case, the parasites are maintained in an enzootic cycle involving various wild animals and sylvan sand flies).
tion structure, with some zymodemes (e.g., IOC/Z-27) widely distributed and others seemingly unique and localized to a particular endemic focus (e.g., IOC/Z-35 and IOC/Z-45). The IRT analysis resulted in a very high degree of discrimination among isolates from diverse regions, confirming the ability of this technique to discriminate between closely related parasites (6). The three distinct genotypic groups described herein may represent important genetic variation within *L. (V.) braziliensis*, which could explain the plasticity of these parasites and of their ability to adapt to changing ecological conditions. Changes in the ITSrDNA locus, which is a noncoding region, should not reflect the ability to adapt to ecological changes. However, because of the high level of polymorphism in the composition of the ITSrDNA locus, several molecular epidemiological studies have been conducted by using this tool. Analysis of the ITS regions of the nuclear rRNA operon for different parasites has demonstrated the utility of this marker in detect genetic diversity and the association of this with several epidemiological aspects (9, 26).

The present study also indicates that most of the genotypes are specific to some geographic areas (e.g., genotype B1 contains isolates from one geographic location, in Espírito Santo, that were collected over a distinct period of time [between 1982 and 1995]), showing the importance of clonal dissemination versus sexual reproduction for this organism in nature, at least occurring in some regions. The stability of this genotype adapting to some environmental modifications through years in the same area is a clear proof of the aforementioned statement.

*L. (V.) braziliensis* can infect vertebrate hosts of different species and orders (21) and has been associated with a number of different sandfly species (15). Our analyses indicated that the molecular diversity found in parasites from the Amazon Basin is apparently related to the great number of sandfly vector(s) and/or animal reservoir(s) involved in the transmission cycles (13, 14). In contrast, the *L. (V.) braziliensis* populations circulating in the Brazilian Atlantic coast (in the present study, representing 81.2% of the isolates collected from human beings and dogs in old established communities in nonforested areas) showed a lower level of heterogeneity than did the Amazonian strains and have peridomestic sandfly species such as *Lutzomyia intermedia* and *Lutzomyia whitmani* as the principal suspected vectors (1, 3, 10). The presence of the same genotypes in humans and dogs in the same area suggests that specific transmission cycles could be defined by using the proposed methodologies.

A total of 49% of the parasites collected in areas (Rio de Janeiro or Espírito Santo) where sylvan animals are probably not involved in the transmission cycle were clustered together into the same zymodeme (IOC/Z-27) of the *L. (V.) braziliensis* reference strain (MHOM/BR/1966/M2903), which was isolated from a patient with American CL in the Serra dos Carajás, Pará State. In this case, a sylvan sandfly, *Psychodopygus wellcomei*, has been determined to be the vector (20, 31). In contrast, isolates from Pernambuco, where grass mice (*Bolomys lasiurus*) and black rats (*Rattus rattus*) have been found to be parasite reservoirs (S. P. Brandão-Filho, M. E. F. Brito, F. G. Carvalho, E. A. Ishikawa, E. Cupolillo, and J. J. Shaw, Abstr. Wordleish 2000, abstr. 100, 2000), were grouped into five distinct zymodemes. Factors that predispose pathogens to

![Acrylamide (12%) gel electrophoresis comparison of ITS fragment patterns generated with the restriction enzymes BstUI (A) and HhaI (B), among selected strains of *L. (V.) braziliensis* from Brazil.](http://jcm.asm.org/)

**FIG. 2.** Acrylamide (12%) gel electrophoresis comparison of ITS fragment patterns generated with the restriction enzymes BstUI (A) and HhaI (B), among selected strains of *L. (V.) braziliensis* from Brazil. The codes in each lane represent the genotypes and the respective zymodemes (IOC/Z). The genotypic groups correspond to those described in Fig. 1. (A) Differences among groups A, B, and C; (B) differences among isolates from the same zymodeme.
infect multiple hosts include high levels of genetic diversity and opportunities for cross-species transmission (37). The extent of host adaptation is therefore linked to and limited by the genetic variability. Pathogens that contain more accumulating mutations should produce more genetic variants and are more likely to be generalists. An alternative explanation to the extent of host adaptation of some organisms is to consider that the infection of a given host is only related to opportunity; therefore, parasites that infect several different hosts are more likely to be isolated in different transmission cycles.

The RFLP-ITSrDNA data show that parasites from urban areas (Espírito Santo and Rio de Janeiro), where transmission is associated with Lutzomyia intermedia, cluster together. Another cluster comprises parasites from rural localities in Espírito Santo and Pernambuco, where Lutzomyia whitmani is the principal vector species. Other studies using different molecular markers have demonstrated a geographic structuring of Leishmania parasites (2, 16, 23, 33, 34), and populations of L. (V.) braziliensis isolates collected from geographically closed areas seem to be more genetically similar (12, 24). However, the present study indicates that the geographic structuring of L. (V.) braziliensis reflects the plasticity of these parasites to adapt to different vector species (or populations of this vector) involved in the transmission cycle. Furthermore, our results indicate an association between the diversity of vectors involved in the transmission of L. (V.) braziliensis and the genetic diversity of the parasites. Evaluation of the phosphoglycan composition of these leishmanial populations is fundamental to an understanding of the vector-parasite interaction (32). Another important approach is to study the population structure of the sandfly vectors in areas where the genetic diversity of L. (V.) braziliensis has been determined.

It was recently suggested that the dispersion of Leishmania clones is associated with the vector and reservoir movements (18). However, what is important to note is the plasticity of leishmanial parasites, their ability to adapt to changes in ecological conditions, and a possible evolutionary relationship between them and their vectors as observed in the Old World (28).

The clinical expression of leishmanial infection is dependent on a number of different factors (15). The identification of virulence factors that contribute to the pathogenesis of L. (V.) braziliensis infections are poorly understood, mainly because of the lack of good in vivo models. A better understanding of the genetic basis of pathogenesis in ML is needed, as are studies on the role of host factors in immune susceptibility to this form of the disease. Our genotyping studies did not identify a pathogenic clone specifically associated with the development of ML. However, in other analyses we were able to detect distinct genotypic types of a single L. (V.) braziliensis isolate from a patient with ML (unpublished data). Competitive interactions among coinfecting clones could affect host immune responses and play a role in determining the immunopathology of the progressive and destructive form of ML. Some underlying
causes of its hyperergic response might include the following factors: (i) the resistance of some parasite clones to elimination (27) and the persistence of “allergic” antigen that evokes hyperergic hypersensitivity inflammatory responses and (ii) autoimmune phenomena related to antigens cross-reactive between leishmanial parasites and host tissues (11, 29).

In conclusion, the combination of MLEE and IRT-SrDNA is useful for studies of the interface of population genetic and epidemiology. The application of these molecular techniques could allow us to better determine the genetic diversity of various *Leishmania* parasites circulating in nature. This information is expected to yield new approaches to mitigating parasite transmission and virulence in humans and eventually to improve parasite control.

ACKNOWLEDGMENTS

We thank Luiz Eduardo de Carvalho Paes for technical assistance and Hooman Momen and Octavio Fernandes for the critical revision of the manuscript.

This work was supported by Fundação Oswaldo Cruz, Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro FAPERJ, Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq, and PRONEX III/CNPq/MCT (Brazil).

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