lactic acidosis, it is an extremely useful measurement. As we scale up HIV care worldwide, we must look to use existing technologies in novel settings if we are to combat the epidemic effectively.

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Received: 28 November 2005; revised: 9 December 2005; accepted: 12 January 2006.

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Tracing the origin of Brazilian HTLV-1 as determined by analysis of host and viral genes

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We compared the genetic diversity of the Brazilian human T-cell lymphotropic virus type 1 isolates with those found in KwaZulu–Natal (KZN), South Africa, and with the genetic background of the hosts. The seroprevalence rate in KZN was 1.7%. All sequences belonged to the A subgroup. The presence of South African sequences in two different clusters from Brazil, and the finding of the β^A-globin haplotype in infected hosts are consistent with the transmission of this virus from southern Africa to Brazil.

The origins of human T-cell lymphotropic virus type 1 (HTLV-1) in Salvador, a Brazilian city in the Bahia State, are not fully understood and are difficult to trace. One hypothesis suggests that the virus was introduced into south America from Africa, during the post-Columbian slave trade [1]. The majority of Africans who came to Salvador during this period of time were from west Africa, where only the HTLV-1 C subgroup of the Cosmopolitan (a) subtype has been found. Previous studies reported that HTLV-1 strains from Salvador belong to the A subgroup [2], as it was also previously demonstrated in KwaZulu–Natal (KZN), South Africa [3]. We attempted to resolve this discrepancy by studying the β^A-globin haplotype of HTLV-1-infected individuals living in Salvador [2]. The detection of HTLV-1a subgroup A among the Bantu people from Salvador could suggest that Brazil strains may have originated from southern Africa. To examine this possibility, we conducted detailed sequence and evolutionary analyses, comparing the genetic diversity and molecular phylogenies between Brazil and KZN HTLV-1 isolates, and with the genetic background of the infected hosts.

A total of 1435 samples were collected from HIV-1-uninfected and infected treatment-naive individuals obtained in Durban, KZN, South Africa, and surrounding areas, after approval from the University of KZN Ethical Board. Plasma were screened for HTLV-1/2 antibodies by enzyme immunoassay. DNA was extracted from peripheral blood mononuclear cells (enzyme immunoassay-positive samples) using QIAamp (Qiagen, USA). *Pol* gene-nested polymerase chain reaction was performed to differentiate between HTLV-1 and 2 [4]. Long-term repeat (LTR) fragments were amplified from 29 South Africa DNA samples and from 10 samples from Salvador collected in a previous study [2]. The products were purified and sequenced directly on a 3100 genetic analyser (Applied Biosystems, California, USA). Phylogenetic trees of 724 basepair LTR sequences were generated using the neighbour-joining and maximum-likelihood (ML) methods of PAUP* software, version 4.0b10 [5]. Two sets of LTR sequence alignments from mother–infant pairs in KZN were available for an estimation of the HTLV-1 evolutionary rate (nucleotide/site/year). The evolutionary rate of each set was calculated using a homogeneous Poisson model, as previously described [6]. Genotyping of human β^A-globin was performed on 10 HTLV-1-infected individuals (five South Africa and five Brazil) as previously described [4]. The haplotype patterns for South African and Brazil isolates were compared with those typical haplotypes from the Central African Republic (CAR; Bantu), Benin, Senegal and Cameroon.

The HTLV-1 seroprevalence in KZN was 1.7% (24). The average intersequence diversity among Brazil LTR sequences was significantly higher than among KZN sequences (1.42 × 0.7%), even when epidemiologically linked samples were excluded from the KZN analysis (0.78%). As expected, sequences from transmission pairs were highly conserved (divergence of 0.1%) with only one polymorphism being detected in the LTR region of one family. Phylogenetic analysis showed that all South Africa and Brazil sequences belonged to subgroup A of the HTLV-1a (Fig. 1). Two distinct clusters of Latin American sequences (A and B) were identified within the A subgroup, both supported by high bootstrap and by ML. At the main cluster (A), two new isolates from KZN (HTLV04 and HTLV06) formed a monophyletic outgroup, a finding also supported by both bootstrap and ML. The second cluster (B) contained a new KZN isolate

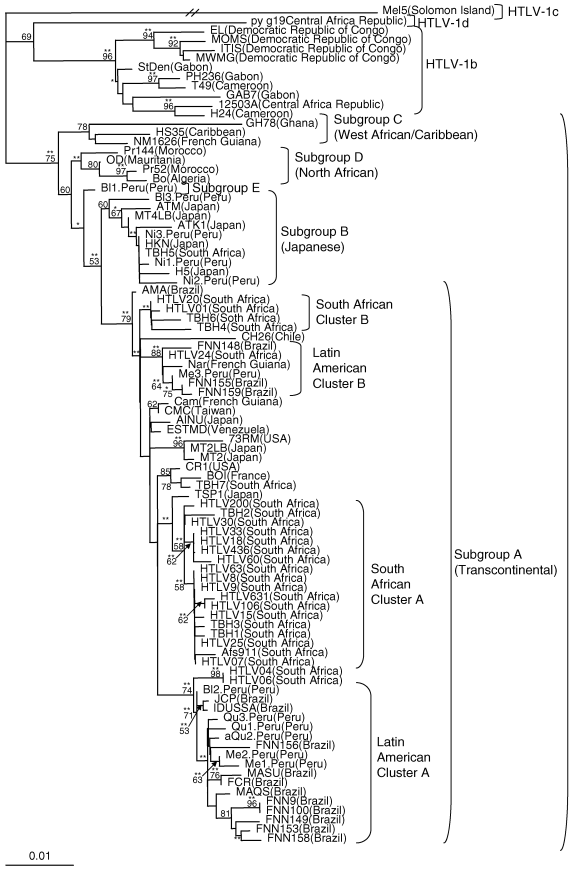

Fig. 1. Rooted neighbour-joining tree of human T-cell lymphotropic virus type 1 strains based upon a 724 bp of the total long-term repeat region. The Tamura-Nei evolutionary model with (-distribution was selected and the tree was drawn using TreeView, version 1.4. The bootstrap values (above 50% and using 1000 bootstraps) on the branches represent the percentage of trees for which the sequences at the right end of the branch form a monophyletic group. Mel5 is used as the outgroup. Geographical origin and ethnic origin are given between parentheses. Newly sequenced long-term repeats included in this analysis (in bold) are the following Salvador and South African isolates. The ** means that the maximum-likelihood method was highly significant with a *P* value of less than 0.001 or significant with a *P* value of less than 0.005. The GenBank accession numbers of the new strains are DQ005546–DQ005574.

(HTLV24). Analysing all 20 β^A-globin chromosomes, we identified two Senegal, six Benin and 12 CAR haplotypes. Among South Africa, four were homozygous for either the Benin/Benin ($n = 2$) or CAR/CAR ($n = 2$) haplotypes; and one was Benin/CAR heterozygote. Two Brazil were homozygous (CAR/CAR) and three (60%) were heterozygous: two for the Senegal/CAR, and one for the Benin/CAR haplotype. Based on a single mutation in the LTR region of one mother–child pair, the minimum and maximum age of transmission was calculated to be 20 and 102 years. The average HTLV-1 evolutionary rate (756 sites) from both transmission pairs was estimated to be 2.16×10^{-5}, with the upper and lower 95% intervals estimated to be 1.30×10^{-4} and 1.13×10^{-6}, respectively. To increase the statistical power of our analysis, we increased our sample size, including published evolutionary data from an additional 16 transmission chains [6]. The combined datasets contained three LTR mutations, and resulted in an average evolutionary rate of 4.49×10^{-6}, with lower and higher intervals in the range of 1.08×10^{-6} and 1.34×10^{-5}. The total transmission time (t) for the collective dataset (18 transmission pairs) was calculated to be between 539 and 1203 years.

The high degree of relatedness is consistent with the transmission of HTLV-1a subgroup A from South Africa to Brazil, presumably during the slave trade process. Moreover, three LTR sequences from South Africa actually segregated within the Brazil clusters, suggesting that there were probably multiple introductions of HTLV-1 from South Africa to Brazil. Transmission between South Africa and Brazil is also consistent with the similar prevalence rates in KZN (1.7%) and Salvador (1.76%), a city where more than 80% of the population is of African origin [2,7]. The finding that all HTLV-1-infected individuals in our study had the same β^A-globin haplotype is also consistent with the transmission from southern Africa. The source of the CAR haplotype in South Africa is not known, but may reflect the migration of the Bantu population from north to South Africa during the last 3000 years, an event that gave origin to the Zulu tribes of South Africa. Alternatively, the Benin haplotype may have been introduced recently (during the past 300 years) [8]. Finally, the low level of diversity observed among South African isolates was supported by evolutionary analysis of two mother-to-child transmission chains, when the attempts to improve the accuracy of this value, by including data from 16 previously published transmission chains [6], resulted in an estimated evolutionary rate of 4.49×10^{-6}. Unlike the HIV-1, HTLV-1 shows little evidence of adaptation or natural selection, exhibiting a low evolutionary rate. As a result, spatial and demographic processes, such as multiple introductions of the virus during the slave trade, are likely to be among the main processes shaping the structure of phylogenetic trees. Understanding the differences between HTLV-1 and HIV-1 may lead to new insights for controlling the spread and genetic evolution of these important human pathogens.

Acknowledgements

The authors are grateful to Taryn Page and Natalie Graham for the technical assistance and Estrelita van Rensburg for providing the samples.

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Sponsorship: This work was partly supported by grants from the Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB) and from the Wellcome Trust (UK) grant 061238/2/00/2 (S.C.).

Received: 1 June 2005; revised: 21 July 2005; accepted: 10 August 2005.

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