HAL
archives-ouvertes.fr

HTLV-2B Strains, Similar to Those Found in Several Amerindian Tribes, Are Endemic in Central African Bakola Pygmies.

Philippe Mauclère, Philippe Vicente Afonso, Laurent Meertens, Sabine Plancoulaine, Sara Calattini, Alain Froment, Monique van Beveren, Guy de Thé, Lluis Quintana-Murci, Renaud Mahieux, et al.

► To cite this version:

Philippe Mauclère, Philippe Vicente Afonso, Laurent Meertens, Sabine Plancoulaine, Sara Calattini, et al.. HTLV-2B Strains, Similar to Those Found in Several Amerindian Tribes, Are Endemic in Central African Bakola Pygmies.. Journal of Infectious Diseases, Oxford University Press (OUP), 2011, 203 (9), pp.1316-23. 10.1093/infdis/jir031 . pasteur-00590844

HAL Id: pasteur-00590844
https://hal-pasteur.archives-ouvertes.fr/pasteur-00590844

Submitted on 6 May 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

MAJOR ARTICLE

HTLV-2B Strains, Similar to Those Found in Several Amerindian Tribes, Are Endemic in Central African Bakola Pygmies

Philippe Mauclère,[1,a] Philippe Vicente Afonso,[1,a,b] Laurent Meertens,[1,a] Sabine Plancoulaine,[1,3] Sara Calattini,[1] Alain Froment,[4] Monique Van Beveren,[1] Guy de Thé,[1] Lluis Quintana-Murci,[2] Renaud Mahieux,[1,c] and Antoine Gessain[1]

[1]Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, [2]Human Evolutionary Genetics, Institut Pasteur, [3]Laboratoire de Génétique Humaine des Maladies Infectieuses, Institut National de la Santé et de la Recherche Médicale, and [4]Institut de Recherche pour le Développement, Musée de l'Homme, Paris, France; and [5]Centre Pasteur du Cameroun, Yaoundé, Cameroun

Background. The presence and origin of endemic foci of human T-lymphotropic virus type 2 (HTLV2) infection in Africa remain a matter of debate.

Methods. To better appreciate such determinants, we performed a survey of 1918 inhabitants from Cameroon forest areas, including 1051 Bakola Pygmies and 867 Bantus.

Results. The overall HTLV-1/2 seroprevalence was 4% (49 cases of HTLV-1 and 27 cases of HTLV-2 infection). Both infections were mainly restricted to the Bakola Pygmies, with surprisingly no HTLV-2 infections in the Bantu population. Both HTLV-1 and HTLV-2 seroprevalences increased with age. There was evidence of ongoing HTLV-2 transmission in this population. Lymphoid T cell lines producing HTLV-2 were established. HTLV-2 long terminal repeat sequences (672 base pairs) obtained from 7 infected Bakola were highly similar to each other (<1% nucleotide divergence), as well as to Amerindian HTLV-2B strains. Analyses on a complete sequence (8954 base pairs) confirmed that it was a typical HTLV-2 subtype B strain. Along with molecular clock analysis, these data strongly suggest that HTLV-2 has been endemic in the Bakola Pygmy population for a long time.

Conclusions. This study demonstrates clearly an HTLV-2 endemicity with ongoing transmission in an African population. Furthermore, it give insights into central questions regarding the origins and evolution rate of HTLV-2 and the migrations of infected populations.

Human T-lymphotropic virus type 1 (HTLV-1), simian T-lymphotropic virus type 1 (STLV-1), HTLV-2 [1], STLV-2, STLV-3, and the recently discovered HTLV-3 [2, 3] and HTLV-4 [3] constitute a group of related human and simian retroviruses, the primate T-lymphotropic viruses (PTLVs), that share common biological and molecular features. HTLV-1 is the causative agent of adult T cell leukemia/lymphoma and of tropical spastic paraparesis/HTLV-1–associated myelopathy (TSP/HAM). By contrast, HTLV-2, which also has some transforming capacities in vitro, has been in vivo mainly associated with TSP/HAM–like diseases [4] and with lymphocytosis [5].

A striking characteristic of HTLV-1 and HTLV-2 epidemiology is the presence of clusters of high prevalence, often located near regions of low endemicity [6]. High HTLV-1–endemic clusters have thus been described in areas of southern Japan and Gabon, as well as in populations of African origin in French Guyana and Colombia [7]. Similarly, HTLV-2 is endemic among several, often remote, Amerindian tribes [4, 8–11]. There is also evidence for a 30-year-old epidemic of HTLV-2 infection among intravenous drug users, mainly in the United States but also in Europe [4, 12–14]. Despite these differences, both retroviruses share similar epidemiological determinants: higher prevalence in women, increase with age, and similar modes of transmission,

Received 28 May 2010; accepted 30 November 2010.

Potential conflicts of interest: none reported.

Correspondence: Antoine Gessain, MD, PhD, Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, CNRS URA 3015, Département de Virologie, Institut Pasteur, 75724 Paris Cedex 15, France (agessain@pasteur.fr).

[a]P.M., P.V.A., and L.M. contributed equally to the study.

[b]Present affiliation: Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD.

[c]Present affiliation: Oncogenèse Rétrovirale, Institut National de la Santé et de la Recherche Médicale, Ecole Normale Supériere de Lyon, Lyon, France.

The Journal of Infectious Diseases 2011;203:1316–23

© The Author 2011. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com

0022-1899 (print)/1537-6613 (online)/2011/2039-0001$14.00
DOI: 10.1093/infdis/jir031

Downloaded from jid.oxfordjournals.org at Institut Pasteur, M?diath?que scientifique on May 6, 2011

including mother-to-child transmission by prolonged breast-feeding [4, 6].

The origin of the puzzling geographical (or often ethnic) distribution of HTLV-1 and -2 is unclear. It is probably linked to a founder effect followed by persistent viral transmission in specific populations. The latter may be favored by genetic background, as well as cultural and environmental cofactors.

Most strains of PTLV-1 cannot be separated into distinct phylogenetic lineages according to their origin species. In Central Africa, STLV-1 strains from chimpanzees or mandrills cannot be distinguished from HTLV-1 strains of molecular subtype B and D, respectively [7, 15, 16]. This phylogenetic intermixing has been interpreted as evidence for past and recent interspecies transmission episodes [15, 17]. The situation for PTLV-2 is different: HTLV-2 and STLV-2 form distinct monophyletic clades, with no evidence for recent interspecies transmissions [17, 18].

Molecularly, the HTLV-2 sequences are subdivided in 2 major subtypes (A and B); both have been found in Amerindians and intravenous drug–using populations. Subtype B is referred to as the paleo-Indian subtype [6, 9, 11, 19, 20]. A variant of subtype A (called subtype C) is nearly exclusive in Brazilian populations [4, 21].

As a consequence, HTLV-2 was originally thought to be a New World–restricted virus, brought to the Americas through the migration of the original Mongoloid settlers through the Bering Strait some 25,000 ± 15,000 years ago [17, 18]. However, sporadic cases of HTLV-2 infection, with limited molecular characterization, were described in different African areas [22–25], which complicated the overall picture. Furthermore, in 1998, a unique highly variant strain (called subtype D) was characterized from an Efe Pygmy living in the Democratic Republic of Congo (DRC) [26]. The nucleotide divergence between HTLV-2D, -2A, and -2B viruses (corresponding to a estimated divergence earlier than 40,000 years ago) could suggest an African origin for HTLV-2 [18]. We then isolated a HTLV-2 subtype B strain (Pygcam-1) from a Bakola Pygmy living in Cameroon [27]. This virus was mostly identical to HTLV-2 sequences found in Amerindian populations, pointing at such issues as HTLV-2 origins and evolution rate [17, 27, 28].

The molecular data concerning these "African" HTLV-2 strains remained limited, as they were based mostly on analyses of sporadic cases. In fact, one could always argue about a recent introduction of HTLV-2 in Africa. Its endemic presence remained to be demonstrated.

To get new insights into epidemiological determinants and origins of HTLV-2 in Africa, we developed a cross-sectional study among Bantus and Bakola populations living in the Southern Cameroon rain forest area. We therefore assessed the overall HTLV-1 and -2 seroprevalence in these populations and compared the main epidemiological determinants of HTLV-1 and HTLV-2–infected Bakola and Bantu populations. We also isolated and characterized several new HTLV-2 strains that we integrated into phylogenetic and molecular clock studies.

METHODS

Population Studied

Bakola Pygmies are an ethnic group of around 5000 individuals, living mainly in the Ocean Region in South Cameroon (Supplemental Figure 1). This group is spread throughout the rain forest territories. They were traditionally hunter-gatherers but now practice rudimentary agriculture and trade with neighboring Bantu farmers [29].

Between 1994 and 1998, a series of missions were conducted in several settlements of Bakola, as well as in surrounding Bantu villages. A 5-mL blood sample was taken on EDTA for each participant. The overall study was completed after authorization by the national (Ministry of Health) and local (village/settlement chiefs) authorities and personal consent of every participant (or parental consent for minors).

HTLV-1 and -2 Serological Tests

Plasma samples were screened for antibodies against HTLV-1 and -2 with enzyme-linked immunosorbent assay (ELISA) (Platelia HTLV NEW, Sanofi Diagnostic Pasteur), as well as an in-house indirect fluorescent antibody (IFA) test [27]. A confirmatory Western blot (HTLV 2.3, Diagnostic Biotechnology) was performed on samples found positive and/or borderline in ELISA and IFA testing with stringent criteria to define HTLV-1 or -2 seropositivity.

Viral Detection and Isolation

Peripheral blood mononuclear cells (PBMCs) were cultured [27]. Indirect IFA and cytofluorographic analyses were performed on these cells [30]. Production of p24 antigen was detected by means of ELISA (Coulter).

Polymerase Chain Reaction

DNA was extracted, either from buffy-coats or from PBMCs (cultured or not). Polymerase chain reaction (PCR) tests for the HTLV *pol* fragment were carried out with the SK110-SK111 primers [31]. A Southern blot was then performed on the amplified DNA using either SK112 (for HTLV-1) or SK188 (for HTLV-2) as labeled probes.

Seven 672 base-pair–long terminal repeat (LTR) fragments were amplified using the primers BSDR4-BSQF2 and sequenced [32]. The complete sequence (8955 base pairs) of 1 HTLV-2 strain was obtained after PCR amplification, cloning, and sequencing of 9 distinct proviral fragments.

Phylogenetic and Molecular Clock Analyses

Multiple alignments were obtained with the DAMBE (University of Ottawa) program. They were then submitted to the Modeltest program (University of Vigo) (version 3.6) to select the best model to apply to phylogenetic analyses. These

Downloaded from jid.oxfordjournals.org at Institut Pasteur, M?diath?que scientifique on May 6, 2011

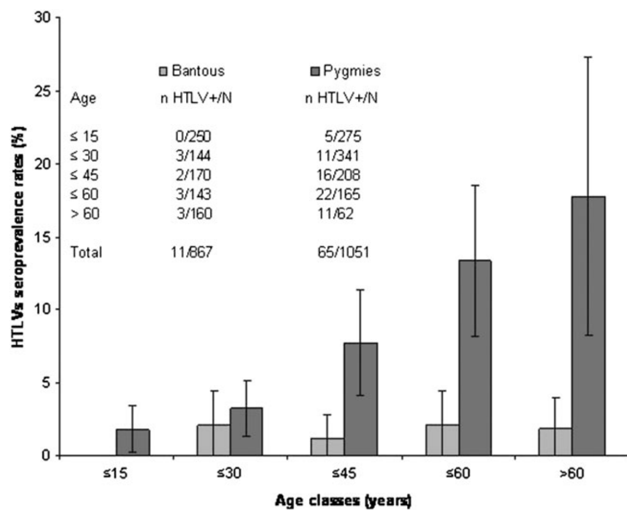

Figure 1. Human T-lymphotropic virus (HTLV) seroprevalence is higher among Bakola Pygmies than among Bantus. The seroprevalence rates were given with a 95% confidence interval according to age among 1051 Bakola Pygmies (*dark gray*) and 867 Bantus (*light gray*).

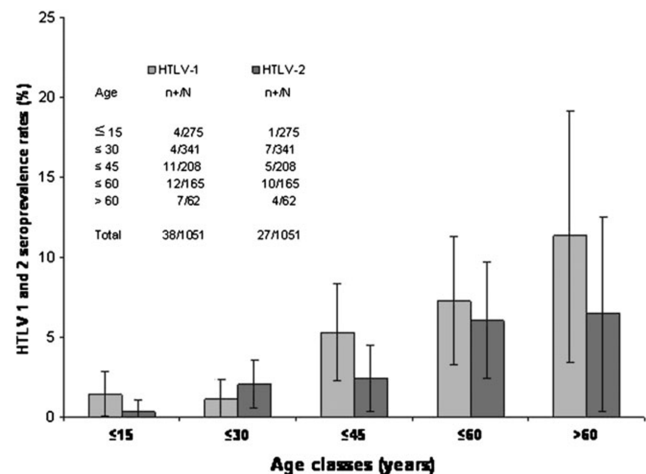

Figure 2. Both human T-lymphotropic virus type 1 (HTLV-1) and HTLV-2 are endemic among Bakola Pygmies. HTLV-1 (*light gray*) and HTLV-2 (*dark gray*) seroprevalence rates are given with a 95% confidence interval among 1051 Bakola Pygmies from Cameroon, Central Africa. HTLV-1 and HTLV-2 antibody titers were obtained using indirect fluorescent antibody testing by 2-fold serial dilution. HTLV-1–positive plasma samples were more reactive on MT2 cells than on C19 cells (geometric mean of the titers, 1/55 vs 1/23; $P < .001$), whereas HTLV-2–positive plasma samples were more reactive on C19 than on MT2 cells (1/57 vs 1/11; $P < .001$).

parameters were implemented in the PAUP program (version 4.0b10) to infer trees according to the neighbor-joining or maximum likelihood methods. To test the robustness of the tree topologies, 1000 bootstrap replicates were performed.

Molecular clock analyses were performed on the HTLV-2 LTR alignment. The evolutionary rate was estimated by using a Bayesian Markov chain Monte Carlo (MCMC) molecular clock method using the BEAST software package [33]. Both strict and relaxed molecular clock hypotheses gave similar evolutionary rates. The general time-reversible substitution model (gamma plus invariant sites) of 6 gamma categories was used. The population size was considered constant, ranging from 0 to 100,000,000 [28]. The chain length from MCMC analysis was 20 million, with sampling every 10,000 steps. The divergence dates were systematically determined for the monophyletic groups of interest, a priori introduced in the BEAST program. Convergence of the MCMC was assessed by calculating the effective sampling size (ESS) with the program Tracer. All parameter estimates showed significant ESSs (>150).

Statistical Analysis

Proportions were compared using the 2-sided χ^2 test or Fisher exact test when needed. To best fit the data, age was log-transformed. Geometric means of the titers were compared using a 2-sided paired Student t test. All analyses were performed using SAS software, version 9.1 (SAS Institute).

RESULTS

Bakola Pygmy Population, But Not Bantu, Infected With HTLV-2

The survey was performed among 1918 persons (1011 women and 907 men; mean age, 32.8 years [range, 2–83 years]) (Figure 1). The studied populations included 1051 Bakola Pygmies (561 women, 490 men) and 867 Bantus (450 women, 417 men).

Detection of HTLV Infection by Means of Serological Analyses. Among the 1918 samples tested, 680 samples had positive results at ELISA, and 320 had either clearly positive or uncertain (reactive but with a high background) results at IFA testing. All 759 samples with positive or uncertain seroreactivities by means of ELISA and/or IFA testing were tested by Western blot. Typical HTLV-2 pattern was detected for 27 plasma samples, whereas 49 exhibited a complete HTLV-1 reactivity pattern. However, most (591) of the plasma samples exhibited a partial reactivity pattern, with indeterminate profiles; these were considered HTLV-seronegative in the statistical analysis. Many (187) of these had a typical HTLV *gag*-indeterminate pattern, probably a marker of *Plasmodium falciparum* infection [31]. The 92 remaining plasma samples showed no Western blot reactivity.

Confirmation of the Reliability of Serological Studies by Means of PCR Studies. DNA samples from 336 different individuals, for whom we had available buffy-coats or PBMCs, were extracted and then tested using HTLV-1 and -2 *pol* primers. Twenty-one originated from the HTLV-2–seropositive persons and 17 from the HTLV-1–seropositive individuals. The 298 other samples were from persons with an indeterminate ($n = 158$) or a negative ($n = 140$) Western blot result. There was a perfect concordance between serological and PCR results, except for 4 samples. On one hand, proviral DNA could not be

Downloaded from jid.oxfordjournals.org at Institut Pasteur, M?diath?que scientifique on May 6, 2011

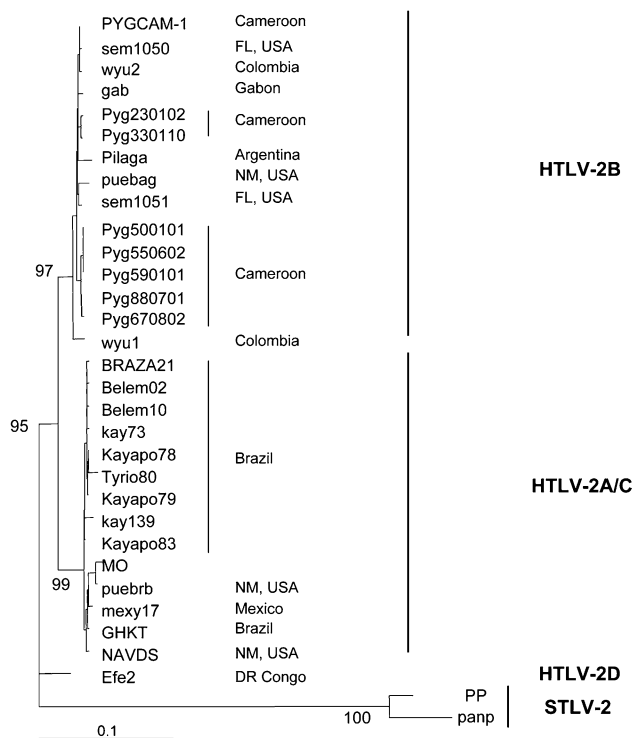

Figure 3. Strains from Bakola Pygmies belong to the human T-lymphotropic virus type 2B (HTLV-2B) subgroup. Phylogenetic tree generated with the neighbor-joining method, performed in the PAUP program (version 4.0b10), on a 629 base pair fragment of the LTR using the endemic sequences (from human immunodeficiency virus–negative individuals) available in GenBank and the 7 new LTR sequences from the Bakola Pygmy strains obtained in this study (noted as Pygxxxxxx in the figure and referenced as sequences GU212848-GU212853 in GenBank). Bootstrap support (1000 replicates) is noted on the branches of the tree. The tree was rooted with simian T-lymphotropic virus type 2 (STLV-2) sequences. The branch lengths are drawn to scale, with the bar indicating 0.1 nucleotide replacement per site.

amplified for 1 person with a typical HTLV-2 Western blot result; on the other hand, 3 individuals with an indeterminate Western blot result were positive by means of PCR for HTLV-1 (2 cases) or HTLV-2 (1 case). This confirms that the strategy that we followed is appropriate for large epidemiological studies; it also shows that we might be slightly underestimating the retroviral prevalence.

Comparison of the Epidemiological Features of HTLV-1 and HTLV-2 Infection. Among the 1918 plasma samples tested, the overall HTLV-1/2 seroprevalence was 4% ($n = 76$). HTLV-1/2 infection was significantly more frequent among the Bakola than among the Bantus (6.1% vs 1.3%; $P < .001$) (Figure 1). Surprisingly, HTLV-2 infection was restricted to the Bakola population (2.6%, ie, 27 HTLV-2–positive individuals; $P < .001$) (Figure 2). Among this group, seroprevalence increased with age for both viruses, reaching 11.3% for HTLV-1 and 6.5% for HTLV-2 in the elderly (>60 years old) (Figure 2). Although HTLV-2 infection was more prevalent in women than in men ($P = .03$), this was not the case for HTLV-1 ($P = .22$). Geographical analysis indicated a wide distribution of HTLV-1 and HTLV-2–infected individuals in the different territories of the Bakola (Supplemental Figure 1).

HTLV-2 Is Still Being Transmitted in the Bakola Population. The presence of HTLV-2 in few children, together with the analysis of some genealogical trees showing familial clusters (husband-wife and mother-child) (Supplemental Figure 2), indicates an ongoing transmission of HTLV-2 within this population.

To isolate the virus, PBMCs from 7 HTLV-2–seropositive Pygmies were cultured. In 6 cases, cells proliferated rapidly, and after 2–3 weeks some very large cells were detected. These were positive by IFA test for HTLV, and HTLV-1/2 p24 antigen was detected in the supernatant of these cultures. After 2 months of culture, 10%–60% of cells were HTLV positive by IFA. These cells had an activated T cell phenotype ($CD2^+$, $CD3^+$, $CD\ 19^-$, HLA DR^+, $CD25^+$) and were either $CD4^+$ or $CD8^+$ or had both markers. Three cultures were further expanded and cocultivated with BJAB cells. After 3–5 days, expanding HTLV-2–positive syncitia were observed.

Similarity of the HTLV-2B Strains Endemic in Bakola Pygmies to Those Present in Some Amerindian Populations Phylogenetic Analysis of LTR Sequences. LTR fragments (625 base pairs) were obtained from 7 Bakola who were living in 4 independent settlements. The sequences were very similar to each other and to the Pygcam-1 strain [27]: nucleotide genetic divergence was lower than 1.2%. With the exception of the Pygcam-1 and Gab strains [34], the HTLV-2B clade used to be composed exclusively of Amerindian sequences (Figure 3). Surprisingly, the new Pygmy strains showed a high similarity to these Amerindian strains (0%–1.2% divergence). Moreover, neither the Bakola nor the Amerindian strains constituted a separate monophyletic clade within the B-subtype (Figure 3).

Phylogenetic Analysis of Complete Sequences. A complete proviral sequence (8955 base pairs) was obtained from Bakola Pygmy PYG550602. Phylogenetic analysis performed on the concatenated *gag-pol-env-tax* genes (7263 base pairs) confirmed that the new sequence is unique and belongs to the HTLV-2B subgroup (Figure 4).

Molecular Clock Analysis. The evolution rate of retroviruses can be widely variable. It can depend on the virus itself: PTLV-1 and PTLV-2 have different evolution rates [18], supposedly because of different replication strategies. The evolution rate can also depend on the host species [35]. Finally, the evolution rate of HTLV-2 is different between individuals who do and do not use intravenous drugs [28, 36]. For these reasons, we chose to perform the molecular clock analysis on HTLV-2 LTR sequences isolated only from individuals who do not use intravenous drugs.

We tested 2 different hypotheses for the calibrating date of the molecular clock using only HTLV-2 sequences:

Downloaded from jid.oxfordjournals.org at Institut Pasteur, M?diath?que scientifique on May 6, 2011

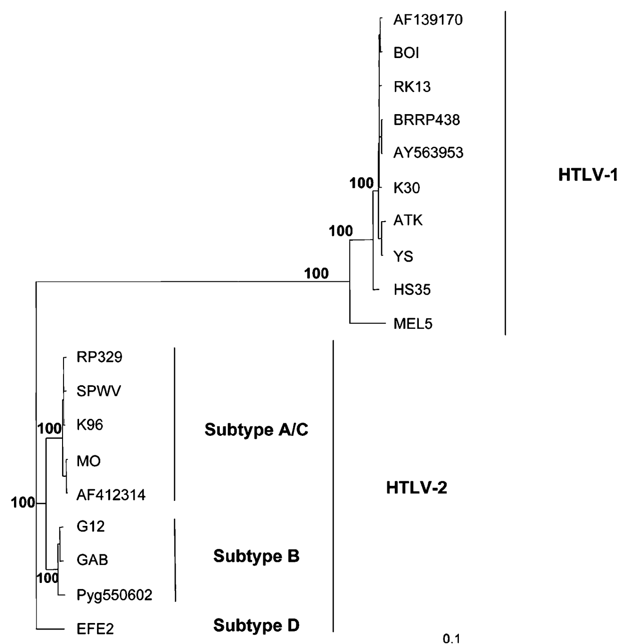

Figure 4. The strain from the Bakola Pygmy Pyg550602 belongs to the human T-lymphotropic virus type 2 (HTLV-2) genotype. The phylogenetic tree was generated with the neighbor-joining method, using 7263 base pairs of the concatenated *gag-pol-env-tax* genes of the new Pyg550602 strain obtained in this study (Pyg550602; GenBank accession no. GU212854) and all the complete sequences of HTLV-1 and HTLV-2 available in GenBank. Bootstrap values (1000 replicates) are noted on the branches of the tree. Branch lengths are drawn to scale, and the bar indicates a 0.1-nucleotide substitution per site.

1. The HTLV-2 groups contain the HTLV-2D sequence, which is believed to be of African origin, and the HTLV-2A/C sequences, which are believed to be of Amerindian origin. Therefore, the divergence date of the HTLV-2 group might be 100,000 ± 25,000 years ago, corresponding to the date of human spread out of Africa;
2. Because HTLV-2A and HTLV-2C contain only Amerindian sequences, the common ancestor might have emerged at 25,000 ± 5000 years ago, which is the date of human migrations over the Bering Strait.

Both hypotheses gave very similar dates for the emergence of the HTLV-2 B subtype: 24,000 ± 500 years ago (ESS = 2904) and 26,000 ± 300 years ago (ESS = 2832), respectively (Figure 5).

DISCUSSION

The Bakola Pygmies are one of the very few populations in which both HTLV-1 and HTLV-2 are naturally endemic. Previously, only intravenous drug–using groups had been shown to be infected by both retroviruses [12, 37]. Our study is the first, to our knowledge, to show that HTLV-2 endemicity, with ongoing viral transmission, is clearly established in a large African population. Surprisingly, HTLV-2 strains isolated in Bakola are nearly identical to strains isolated from several populations of Native Americans living in different areas of the Americas [6, 9–11, 17–19]. Such unexpected findings raise several questions.

Why Is the Level of HTLV-1 and HTLV-2 Infection Much Higher in the Bakola Pygmy Than in the Bantu Populations Living Nearby?

The causes of a retroviral endemic focus restricted to the Bakola are not clear. The focus might result from a founder effect followed by persistent viral transmission facilitated by environmental or cultural situations. Such a hypothesis has been proposed to explain several HTLV-1 endemic foci [6], as well as the presence of HTLV-2 found in several Native American populations [4, 8].

The risk of interspecies transmission seems higher for Bakolas than for Bantus: the first are hunter-gatherers, whereas the latter are mostly farmers. One could argue that Bantus also hunt. However, among Bakola, the majority of men and many women actively hunt and butcher nonhuman primates [29]. In contrast, in Bantu populations, the hunting activity is restricted to few men. Therefore, the cumulative risk of encountering STLV-infected nonhuman primates and acquiring PTLV is higher in Bakola than in Bantu populations.

Retroviruses might also have a higher probability of being maintained among Bakolas than among Bantus. In fact, anthropological studies have revealed that duration of breast-feeding, a major factor for HTLV vertical transmission, is much longer for Pygmies (often reaching 2–3 years) than in Bantu populations. Moreover, intimate contacts between Pygmies and Bantus are very limited, mainly restricted to few episodes of sexual intercourse between Bantu men and Pygmy women. In fact, recent genetic studies based on mitochondrial DNA indicated that only Pygmy-to-Bantu maternal gene flow occurred [38]. As HTLV-1 and -2 seem preferentially transmitted from men to women [4, 39], such rare contacts are likely not to spread HTLVs outside the Pygmy community.

Where Did the Bakola Pygmies Acquire HTLV-2?

It is unlikely that interspecies transmission of STLV-2/HTLV-2 to Bakola happened in Cameroon. In fact, despite intensive searching, no STLV-2–infected monkeys have ever been found in Cameroon. By contrast, in regions where HTLV-1 and HTLV-3 infection were found, studies performed on bush-meat samples or wild-caught live animals have revealed the presence of STLV-1 and STLV-3 [40–42]. Moreover, despite screening >15,000 individuals from Cameroon, no investigators have described HTLV-2 infection apart from in the Bakola [3, 43, 44].

Genetic studies showed that most Pygmy groups located in Cameroon/Gabon form a homogeneous population, whose ancestors separated from the ancestors of farming populations around 60,000 years ago [38]. However, the origin of the Bakola population remains unclear. On the basis of their oral tradition, they might originate from the northwestern part of DRC, where

Downloaded from jid.oxfordjournals.org at Institut Pasteur, M?diath?que scientifique on May 6, 2011

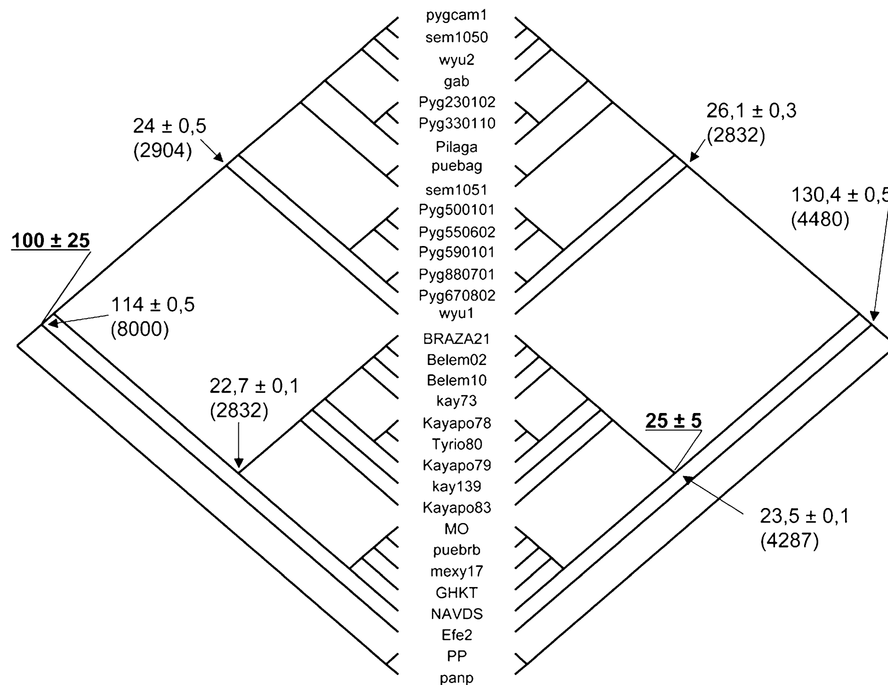

Figure 5. Molecular clock analyses and evolution times of human T-lymphotropic virus type 2 (HTLV-2). Molecular-clock analysis was performed on an alignment of long terminal repeat sequence from HTLV-2 previously obtained using the general time-reversible substitution model implemented with BEAST software from the University of Oxford, Oxford, UK. We used 2 different hypotheses for the calibration of the time scale (shown in boldface, in thousands of years). On the left, we supposed that the HTLV-2 group might have radiated when the population spread out of Africa. On the right, we supposed that the divergence between HTLV-2A and -2C corresponds to the migration over the Bering Strait. The values are given in thousands of years (values in parentheses correspond to the effective sampling size). For graphical convenience, the lengths of the branches are not strictly proportional to the divergence times, and the tree has been rooted with simian sequences (even though these were not considered in the molecular clock per se).

the few epidemiological surveys that have been performed showed sporadic cases of HTLV-2 infection [22, 45]. Thus, the ancestors of the present-day Bakola could have acquired HTLV-2 either in their region of origin or during their migration to their present-day region of living. Such a hypothesis has been proposed to explain how Aboriginal groups of Melanesian origin acquired HTLV-1, although no nonhuman primates have ever lived in Melanesia [46]. Their ancestors certainly acquired the virus through zoonotic interspecies transmission from STLV-1–infected macaques in southeast Asia prior to their migration [47].

How Can HTLV-2B in Pygmies Be So Similar to Amerindian Strains?

Until now, the HTLV-2A/C and -2B subtypes were supposed to have originated from the Americans. Indeed, these strains were found to be endemic in Native American populations scattered all over the Americas [4, 8–11]. The few previous reports of sporadic HTLV-2 infection in Africa were thus supposed to result mainly from recent viral introduction either by infected Native Americans or, rather, by individuals previously in contact with infected Native Americans.

Previous studies indeed suggested that the separation between the original Bakola strain Pygcam-1 and the Seminole Amerindian strain SEM1050 had occurred only 400 years ago and that the separation from the Wayu strain WY100 (from a Colombian Amerindian) occurred even more recently [18, 28]. In 1 study, Salemi et al calibrated the molecular clock analysis by considering the evolution rate from all PTLV strains originating from individuals who do not use intravenous drugs to be identical [18]. However, as mentioned previously and revealed in that very study, the first hypothesis seems to be inadequate. In the other study, the authors considered the HTLV-2A and -2B subtypes to be all of Amerindian origin [28]. In such a model, the divergence for the whole HTLV-2 group (-2A, -2B, and 2D) occurs around 40,000 years ago, although, because HTLV-2D is supposed to be African, the date should be prior to the "out of Africa" period. The authors proposed then that the virus might just reflect a recent acquisition from a simian host. However, even if there is no proof for the ancestral presence of HTLV-2D among humans, there is also no argument to support the opposite hypothesis.

We demonstrate, however, that HTLV-2B is endemic among Bakola Pygmies. Interestingly enough, HTLV-2B strains were also sporadically found in adults living in different unconnected rural areas, located hundreds or even a few thousand miles apart in DRC and Gabon [22, 45]. This indicates clearly that HTLV-2B is spread throughout Central Africa, thus challenging the dogma about the Native American origin of the HTLV-2B subtype.

Downloaded from jid.oxfordjournals.org at Institut Pasteur, M?diath?que scientifique on May 6, 2011

We propose instead that the HTLV-2B group has an African origin. This hypothesis has been challenged by a molecular clock calibrated with 2 different but likely calibration dates using HTLV-2 sequences: (1) the origin of the HTLV-2 type corresponds to the "out of Africa" event, estimated at 100,000 years ago, as it contains the HTLV-2D African Efe sequence; and (2) the HTLV-2 A/C group, as only composed of Native Amerindian sequences, may have diverged after 25,000 years. Both hypotheses gave concordant dates for the HTLV-2B group divergence, about 25,000 years ago. In this hypothesis, the evolution rate for HTLV-2 strains is about $(4.7–1.9) \times 10^{-7}$ mutations/site/year. This rate is very low but may also explain the fact that nearly identical strains were found in several Amerindian individuals originating from different populations, including Wayuus from Colombia [48], Mapuche from Chile [49], Seminole from Florida [50], and Guyami from Panama [10]; these people are believed not to have had contact with each other for at least several centuries. The molecular mechanisms of such a phenomenon are unknown but could be in part linked to the major clonal expansion of HTLV-2–infected cells, as demonstrated in endemic populations [36].

With these data, we propose the following model/hypothesis for HTLV-2 history. The genuine HTLV-2 appeared in Africa. Some of them left Africa and became the ancestors of HTLV-2A/C or HTLV-2B. The remaining virus became the HTLV-2D strains. HTLV-2B–bearing populations may have migrated through Asia and then separated into several subgroups: some went to America, and others returned to Africa.

This model has 2 major limitations. We first suppose that HTLV-2D is a genuine human retrovirus group and did not arise from recent zoonoses [26]. Although our molecular clock studies seem to argue in favor of such an idea, it is not certain.

Second, this model would predict endemic foci of HTLV-2 throughout Asia, which has not been demonstrated as of yet. The absence of detected endemic HTLV-2 could, however, be explained. The discrete distribution of HTLV-2 makes it difficult to make a definitive statement about the absence of infection in a given area. Therefore, it is unlikely that the presence of populations in Asia in which HTLV-2 is endemic would be revealed after only a few studies. Moreover, the absence of the virus in current populations does not mean that the virus was not present at a previous time. Additional studies searching for other HTLV-2 strains endemic in African populations and for their simian counterparts will be crucial to yield new insights into the origin, evolution, and modes of dissemination of these retroviruses.

Supplementary Data

Supplementary data are available at http://jid.oxfordjournals.org/ online.

Funding

This work was supported by the Institut Pasteur, by Agence Nationale de Recherches sur le SIDA (ANRS). S.C. was supported by fellowships from la Ligue Contre le Cancer and from the Association Virus Cancer Prevention. R.M. is supported by INSERM. P.V.A. is supported by the Ministere de la Recherche and the Pasteur Weizmann Association. We are grateful to Sanofi Diagnostics Pasteur, which provided the ELISA tests.

References

1. Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Golde D, Gallo RC. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science **1982**; 218:571–3.
2. Calattini S, Chevalier SA, Duprez R, et al. Discovery of a new human T-cell lymphotropic virus (HTLV-3) in Central Africa. Retrovirology **2005**; 2:30.
3. Wolfe ND, Heneine W, Carr JK, et al. Emergence of unique primate T-lymphotropic viruses among central African bushmeat hunters. Proc Natl Acad Sci U S A **2005**; 102:7994–9.
4. Roucoux DF, Murphy EL. The epidemiology and disease outcomes of human T-lymphotropic virus type II. AIDS Rev **2004**; 6:144–54.
5. Bartman MT, Kaidarova Z, Hirschkorn D, et al. Long-term increases in lymphocytes and platelets in human T-lymphotropic virus type II infection. Blood **2008**; 112:3995–4002.
6. Proietti FA, Carneiro-Proietti AB, Catalan-Soares BC, Murphy EL. Global epidemiology of HTLV-I infection and associated diseases. Oncogene **2005**; 24:6058–68.
7. Mahieux R, Ibrahim F, Mauclere P, et al. Molecular epidemiology of 58 new African human T-cell leukemia virus type 1 (HTLV-1) strains: identification of a new and distinct HTLV-1 molecular subtype in Central Africa and in Pygmies. J Virol **1997**; 71:1317–3.
8. Maloney EM, Biggar RJ, Neel JV, et al. Endemic human T cell lymphotropic virus type II infection among isolated Brazilian Amerindians. J Infect Dis **1992**; 166:100–7.
9. Dube DK, Sherman MP, Saksena NK, et al. Genetic heterogeneity in human T-cell leukemia/lymphoma virus type II. J Virol **1993**; 67:1175–84.
10. Pardi D, Switzer WM, Hadlock KG, Kaplan JE, Lal RB, Folks TM. Complete nucleotide sequence of an Amerindian human T-cell lymphotropic virus type II (HTLV-II) isolate: identification of a variant HTLV-II subtype b from a Guaymi Indian. J Virol **1993**; 67:4659–64.
11. Hjelle B, Zhu SW, Takahashi H, Ijichi S, Hall WW. Endemic human T cell leukemia virus type II infection in southwestern US Indians involves two prototype variants of virus. J Infect Dis **1993**; 168:737–40.
12. Lee H, Swanson P, Shorty VS, Zack JA, Rosenblatt JD, Chen IS. High rate of HTLV-II infection in seropositive i.v. drug abusers in New Orleans. Science **1989**; 244:471–5.
13. Chironna M, Calabro ML, Quarto M, et al. HTLV-I and HTLV-II infections in subjects at risk for HIV-I infection from southeastern Italy (Apulia region). Int J Cancer **1996**; 65:746–50.
14. Egan JF, O'Leary B, Lewis MJ, et al. High rate of human T lymphotropic virus type IIa infection in HIV type 1-infected intravenous drug abusers in Ireland. AIDS Res Hum Retroviruses **1999**; 15:699–705.
15. Vandamme AM, Salemi M, Desmyter J. The simian origins of the pathogenic human T-cell lymphotropic virus type I. Trends Microbiol **1998**; 6:477–83.
16. Mahieux R, Chappey C, Georges-Courbot MC, et al. Simian T-cell lymphotropic virus type 1 from Mandrillus sphinx as a simian counterpart of human T-cell lymphotropic virus type 1 subtype D. J Virol **1998**; 72:10316–22.
17. Slattery JP, Franchini G, Gessain A. Genomic evolution, patterns of global dissemination, and interspecies transmission of human and simian T-cell leukemia/lymphotropic viruses. Genome Res **1999**; 9:525–40.
18. Salemi M, Desmyter J, Vandamme AM. Tempo and mode of human and simian T-lymphotropic virus (HTLV/STLV) evolution revealed

Downloaded from jid.oxfordjournals.org at Institut Pasteur, M?diath?que scientifique on May 6, 2011

by analyses of full-genome sequences. Mol Biol Evol **2000**; 17: 374–86.
19. Biglione M, Vidan O, Mahieux R, et al. Seroepidemiological and molecular studies of human T cell lymphotropic virus type II, subtype b, in isolated groups of Mataco and Toba Indians of northern Argentina. AIDS Res Hum Retroviruses **1999**; 15:407–17.
20. Switzer WM, Pieniazek D, Swanson P, et al. Phylogenetic relationship and geographic distribution of multiple human T-cell lymphotropic virus type II subtypes. J Virol **1995**; 69:621–32.
21. Eiraku N, Novoa P, da Costa Ferreira M, et al. Identification and characterization of a new and distinct molecular subtype of human T-cell lymphotropic virus type 2. J Virol **1996**; 70:1481–92.
22. Tuppin P, Gessain A, Kazanji M, et al. Evidence in Gabon for an intrafamilial clustering with mother-to-child and sexual transmission of a new molecular variant of human T-lymphotropic virus type-II subtype B. J Med Virol **1996**; 48:22–32.
23. Goubau P, Liu HF, De Lange GG, Vandamme AM, Desmyter J. HTLV-II seroprevalence in pygmies across Africa since 1970. AIDS Res Hum Retroviruses **1993**; 9:709–13.
24. Igarashi T, Yamashita M, Miura T, et al. Isolation and genomic analysis of human T lymphotropic virus type II from Ghana. AIDS Res Hum Retroviruses **1993**; 9:1039–42.
25. Mauclere P, Gessain A, Garcia-Calleja JM, et al. HTLV-II in African prostitutes from Cameroon. AIDS **1993**; 7:1394–5.
26. Vandamme AM, Salemi M, Van Brussel M, et al. African origin of human T-lymphotropic virus type 2 (HTLV-2) supported by a potential new HTLV-2d subtype in Congolese Bambuti Efe Pygmies. J Virol **1998**; 72:4327–40.
27. Gessain A, Mauclere P, Froment A, et al. Isolation and molecular characterization of a human T-cell lymphotropic virus type II (HTLV-II), subtype B, from a healthy Pygmy living in a remote area of Cameroon: an ancient origin for HTLV-II in Africa. Proc Natl Acad Sci U S A **1995**; 92:4041–5.
28. Salemi M, Lewis M, Egan JF, Hall WW, Desmyter J, Vandamme AM. Different population dynamics of human T cell lymphotropic virus type II in intravenous drug users compared with endemically infected tribes. Proc Natl Acad Sci U S A **1999**; 96:13253–8.
29. Koppert G, Dounais E, Froment A, Pasquet P. Food consumption in three forest populations of the southern coastal Cameroon. In Hladik CM, Hladik A, Linares O, Pagezy H, Semple A, Hadley M eds. Tropical forests, people and food. Vol. 13. Paris: Parthénon-UNESCO, 1993; 295–310.
30. Gessain A, Saal F, Gout O, et al. High human T-cell lymphotropic virus type I proviral DNA load with polyclonal integration in peripheral blood mononuclear cells of French West Indian, Guianese, and African patients with tropical spastic paraparesis. Blood **1990**; 75:428–33.
31. Mahieux R, Horal P, Mauclere P, et al. Human T-cell lymphotropic virus type 1 gag indeterminate Western blot patterns in Central Africa: relationship to *Plasmodium falciparum* infection. J Clin Microbiol **2000**; 38:4049–57.
32. Murphy EL, Mahieux R, de The G, et al. Molecular epidemiology of HTLV-II among United States blood donors and intravenous drug users: an age-cohort effect for HTLV-II RFLP type aO. Virology **1998**; 242:425–34.
33. Drummond AJ, Ho SY, Phillips MJ, Rambaut A. Relaxed phylogenetics and dating with confidence. PLoS Biol **2006**; 4:e88.
34. Letourneur F, d'Auriol L, Dazza MC, et al. Complete nucleotide sequence of an African human T-lymphotropic virus type II subtype b isolate (HTLV-II-Gab): molecular and phylogenetic analysis. J Gen Virol **1998**; 79:269–77.
35. Li WH, Tanimura M. The molecular clock runs more slowly in man than in apes and monkeys. Nature **1987**; 326:93–6.
36. Gabet AS, Moules V, Sibon D, et al. Endemic versus epidemic viral spreads display distinct patterns of HTLV-2b replication. Virology **2006**; 345:13–21.
37. Feigal E, Murphy E, Vranizan K, et al. Human T cell lymphotropic virus types I and II in intravenous drug users in San Francisco: risk factors associated with seropositivity. J Infect Dis **1991**; 164:36–42.
38. Quintana-Murci L, Quach H, Harmant C, et al. Maternal traces of deep common ancestry and asymmetric gene flow between Pygmy hunter-gatherers and Bantu-speaking farmers. Proc Natl Acad Sci U S A **2008**; 105:1596–601.
39. Kajiyama W, Kashiwagi S, Hayashi J, et al. Study of seroconversion of antibody to human T-cell lymphotropic virus type-I in children of Okinawa, Japan. Microbiol Immunol **1990**; 34:259–67.
40. Nerrienet E, Meertens L, Kfutwah A, Foupouapouognigni Y, Ayouba A, Gessain A. Simian T cell leukaemia virus type I subtype B in a wild-caught gorilla (*Gorilla gorilla gorilla*) and chimpanzee (*Pan troglodytes vellerosus*) from Cameroon. J Gen Virol **2004**; 85:25–9.
41. Nerrienet E, Meertens L, Kfutwah A, Foupouapouognigni Y, Gessain A. Molecular epidemiology of simian T-lymphotropic virus (STLV) in wild-caught monkeys and apes from Cameroon: a new STLV-1, related to human T-lymphotropic virus subtype F, in a *Cercocebus agilis*. J Gen Virol **2001**; 82:2973–7.
42. Liegeois F, Lafay B, Switzer WM, et al. Identification and molecular characterization of new STLV-1 and STLV-3 strains in wild-caught nonhuman primates in Cameroon. Virology **2008**; 371:405–17.
43. Mauclere P, Le Hesran JY, Mahieux R, et al. Demographic, ethnic, and geographic differences between human T cell lymphotropic virus (HTLV) type I-seropositive carriers and persons with HTLV-I Gag-indeterminate Western blots in Central Africa. J Infect Dis **1997**; 176:505–9.
44. Calattini S, Betsem E, Bassot S, et al. New strain of human T lymphotropic virus (HTLV) type 3 in a Pygmy from Cameroon with peculiar HTLV serologic results. J Infect Dis **2009**; 199:561–4.
45. Dube DK, Dube S, Erensoy S, et al. Serological and nucleic acid analyses for HIV and HTLV infection on archival human plasma samples from Zaire. Virology **1994**; 202:379–89.
46. Cassar O, Capuano C, Bassot S, et al. Human T lymphotropic virus type 1 subtype C Melanesian genetic variants of the Vanuatu Archipelago and Solomon Islands share a common ancestor. J Infect Dis **2007**; 196:510–21.
47. Ibrahim F, de The G, Gessain A. Isolation and characterization of a new simian T-cell leukemia virus type 1 from naturally infected celebes macaques (*Macaca tonkeana*): complete nucleotide sequence and phylogenetic relationship with the Australo-Melanesian human T-cell leukemia virus type 1. J Virol **1995**; 69:6980–93.
48. Switzer WM, Owen SM, Pieniazek DA, et al. Molecular analysis of human T-cell lymphotropic virus type II from Wayuu Indians of Colombia demonstrates two subtypes of HTLV-IIb. irus Genes **1995**; 10:153–62.
49. Miura T, Yamashita M, Zaninovic V, et al. Molecular phylogeny of human T-cell leukemia virus type I and II of Amerindians in Colombia and Chile. J Mol Evol **1997**; 44(suppl);S76–82.
50. Levine PH, Jacobson S, Elliott R, et al. HTLV-II infection in Florida Indians. AIDS Res Hum Retroviruses **1993**; 9:123–7.

Downloaded from jid.oxfordjournals.org at Institut Pasteur, M?diath?que scientifique on May 6, 2011