Whole body clonality analysis in an aggressive STLV-1 associated leukemia (ATLL) reveals an unexpected clonal complexity

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Abstract 188 words

HTLV-1 causes Adult T cell Leukemia/Lymphoma (ATLL) in humans. We describe an ATL-like disease in a 9 year-old female baboon naturally infected with STLV-1 (the simian counterpart of HTLV-1), with a lymphocyte count over 10^{10}/L, lymphocytes with abnormal nuclear morphology, and pulmonary and skin lesions. The animal was treated with a combination of AZT and alpha interferon. Proviral load (PVL) was measured every week. Because the disease continued to progress, the animal was euthanized. Abnormal infiltrates of $CD3^{+}CD25^{+}$ lymphocytes and Tax-positive cells were found by histological analyses in both lymphoid and non-lymphoid organs. PVL was measured and clonal diversity was assessed by LM-PCR (Ligation-Mediated Polymerase Chain Reaction) and high throughput sequencing, in blood during treatment and in 14 different organs. The highest PVL was found in lymph nodes, spleen and lungs. One major clone and a number of intermediate abundance clones were present in blood throughout the course of treatment, and in organs. These results represent the first multi-organ clonality study in ATLL. We demonstrate a previously undescribed clonal complexity in a chronic ATLL. Our data reinforce the usefulness of natural STLV-1 infection as a model of ATLL.

Highlights

- Adult T leukemia in STLV-1 infected non human primate
- Multiple organ clonal distribution of retrovirus in Adult T Leukemia case
- Viral oligoclonality observed in all samples
- Clone specific distribution in the organs
- Difference with the dogma stating oligoclonal proliferation precedes malignancy

Keywords : STLV-1, retrovirus, leukemia/lymphoma, therapy, clonality, LM-PCR

Abbreviations:

ATLL : Adult T cell Leukemia/Lymphoma

AZT: azidothymidine

CUIS : Common unique integration site

HAM/TSP: Associated Myelopathy/Tropical Spastic Paraparesis

HTLV-1: Human T-Lymphotropic Virus type 1

HTS : high-throughput sequencing

IFN-α: interferon-α

LM-PCR: Ligation-Mediated Polymerase Chain Reaction

NHP: Non-Human Primates

OCI : oligoclonality index

PVL: proviral load

STLV-1 : Simian T-Lymphotropic Virus type 1

UIS : unique Integration site

1. Introduction

Human T-Lymphotropic Virus type 1 (HTLV-1) infects 5-10 million people worldwide [1] and causes a malignant lymphoproliferative disease called Adult T-cell Leukemia/Lymphoma (ATLL) [2, 3], as well as a neurological condition named HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) [4, 5]. ATLL is normally characterized by the proliferation of transformed CD4+CD25+ infected lymphocytes [6] and is usually described as a monoclonal clonal infection, i.e. with a single integration of the HTLV-1 proviral genome. Nevertheless, recent studies suggested that HTLV-1 clonality might be more complex in ATL [7]. ATLL has a poor prognosis, with a mean survival time of 6 and 13 months in the acute and lymphoma types respectively (for a review see [8]). A series of reports have however shown that antiviral therapy i.e. the use of azidothymidine (AZT) combined with interferon-α (IFN-α) improves the survival rate of ATL patients suffering from the acute and chronic/smoldering forms [8-10] even if mechanism(s) of action of those compounds is (are) still debated [11, 12].

In vivo, integrated HTLV-1 genomes (i.e. proviruses) are mostly detected in $CD4^+$ T-lymphocytes, although other cell types are susceptible to viral infection [13, 14]. In addition, a high proviral load (PVL), i.e. the percentage of circulating HTLV-1-infected lymphocytes, is strongly associated with a higher risk of developing an HTLV-1-related disease [15-17]. *In vivo*, the virus can propagate and increase the PVL by two routes: first, by *de novo* infection of cells by virions which then undergo reverse transcription and integrate in a new genomic site; second, by clonal expansion of the already infected cells in the absence of viral replication. During the chronic phase of infection, HTLV-1 replicates mostly *via* clonal expansion [18-20] and individual clones of infected cells can persist in patients for many years [19].

Simian T-Lymphotropic Virus type 1 (STLV-1) is the simian counterpart of HTLV-1 [21-25]. HTLV-1 and STLV-1 are almost identical at the nucleotide sequence level [21, 22] and at least 31 old-world Non-Human Primates (NHP) species, including baboons, are naturally infected with STLV-1 [21, 24, 26-30]. After a period of clinical latency, some NHP may develop an STLV-1-associated disease characterized by CD4+ proliferation, which has been considered as STLV-associated lymphoma or as

a simian version of ATLL [23, 31-37]. The most common findings in baboon lymphomas were weight loss, generalized lymphadenopathy, overall weakness, dyspnea, hepato-splenomegaly, pneumonia and nodular skin lesion [33]. Upon necropsy, visceral involvement of lymph nodes was also noted. Histologically, there was infiltration of lymph nodes with sheets of neoplastic lymphocytes. In some animals, large pleomorphic lymphocytes were seen with prominent centrally located nucleoli [31].

The CNRS primate Center hosts a colony of males and females *Papio anubis* that are naturally infected with STLV-1. These animals live in semi-free ranging conditions. Among these animals, one female displayed symptoms that were reminiscent of human ATL and was therefore treated with combination of AZT and interferon-α. Blood samples were taken on a weekly basis for 18 weeks. Because the disease continued to progress and the proviral load remained high, the animal was euthanized. Histopathological analyses confirmed the presence of ATL with the presence of CD4+ T-cells infiltrates in organs, some of which expressed the viral oncoprotein Tax. STLV-1 clonality analysis was then performed in a series of 18 consecutive blood samples as well as in 14 organs. The same major clones and an expected high number of intermediate clones are shared by the blood and the organs but displayed an organ specific distribution These results are interesting because they are different from the well accepted model of ATL. These data also suggest that infected clones of minor size may play a role in ATL pathogenesis.

2. Materials and methods

2.1 Animal

The *Papio anubis* female (animal #V932E), 12kg, belonged to a previously described cohort [38] of STLV-1 naturally infected *P. anubis* housed at the primate center of the Centre National de la Recherche Scientifique in Rousset sur Arc and cared for in compliance with French regulations and guidelines of the Federation of European Laboratory Animal Science Associations (FELASA).

2.2 Treatment

The animal received a combination of AZT that was embedded in food (Combivir, 1 tablet every day). A person working at the animal facility monitored the animal until he ate its food containing AZT. Alpha interferon (PEG-viraferon, 50 µg/week) was injected. The PEG-viraferon dose was even higher than in humans (1.5 µg/kg/week). Fever was not observed during this period. STLV-1 blood proviral load (PVL) was measured every week. Leukocyte count was also measured on a MS9-5V cell analyzer (Melet Schloesing laboratories).

2.3 Flow cytometry

Frozen PBMCs were thawed in complete RPMI and resuspended in PBS-2%FCS. CD3+ (clone SP34-2), CD4+ (clone L200) and CD8+ (clone RPA-T8) antibodies were purchased from BD Bioscience. Flow cytometry was performed using a Facscanto II with the FlowJo (version 9.9.3) software.

2.4 Histological analyses

Lymph node and skin biopsies were collected during treatment, while other organs were collected post-mortem. Formalin-fixed paraffin-embedded sections of each specimen were stained with HE (haematoxylin and eosin) and a panel of antibodies against the following antigens: CD3 (Dako), CD4 (Thermo scientific, 4B12), CD5 (Leica Biosystem, 4C7), CD7 (Leica Biosystem, LP15), CD20 (Dako, L26), CD25 (Leica Biosystem, 4C9), CD30 (Dako, BerH2), Ki67 (Dako, MIB-1), and anti-Tax LT-4 [39].

2.5 DNA Purification

Genomic DNA was extracted, using the Nucleospin® Blood kit for blood samples and the NucleoSpin® Tissue kit for tissue biopsies, according to the manufacturer's instructions (Macherey-Nagel, Düren, Germany).

2.6 STLV-1 proviral load

Proviral load (PVL) was measured using previously described SK43 and SK44 Tax primers [40]. β-actin primers were previously published [41]. Quantitative PCR was performed using FastSYBR mastermix (Life technologies) with the Fast SYBRgreen thermal cycle protocol on a QuantStudio 7 Flex real-time PCR system (Life technologies). A CD4+ clone with a single integrated provirus, isolated from an HTLV-1 infected patient [42], was used as a standard [43].

2.7 Amplification and quantification of proviral integration sites

Identification of integration sites was performed via Ligation-Mediated Polymerase Chain Reaction (LM-PCR) on extracted genomic DNA, followed by high-throughput sequencing (HTS) as described previously [19]. LM-PCR primers (see supplemental Table 1) were designed after sequencing of the *P. anubis* Long Terminal Repeat (accession number : KX683861) as previously described [44]. Libraries were deep-sequenced using an Illumina HiSeq. DNA sequences were aligned to the Baboon genome (papAnu2) and the STLV-1 genome (accession number: JX987040) sequences using an Eland implementation of CASAVA software (Illumina). The relative abundance of a given unique integration site (UIS) was calculated from its number of different shear sites and its absolute abundance (number of copy of the clone per 100 cells) was determined from the measurement of the proviral load. See supplemental Table 2 for details of sequencing results.

2.8 Statistical analyses

Data analysis was carried out using R [45]. The VennDiagram package [46] was used to generate the Venn diagram. To characterize the clonal distribution, the oligoclonality index (OCI; Gini coefficient) index was calculated, as previously described [19], using the package reldist [47]. An oligoclonality index of 1 corresponds to a monoclonal distribution (one clone carries all the load) whereas an oligoclonality index of 0 describes a perfect polyclonal population (all clones have equal abundance).

3. Results

3.1 Aggressive ATL in a STLV-1 naturally infected *Papio anubis*

During the surveillance of our naturally STLV-1-infected *Papio anubis* cohort (n=45), we identified a 9 year-old female baboon (#V932E) exhibiting dyspnea, marked emaciation, no hypercalcemia, a lymphocyte count over the normal range (Figure 1A), and a STLV-1 proviral load of 68 copies per 100 cells in peripheral blood (Figure 1B). Pulmonary metastases and skin lesions similar to those observed in human ATLL patients were also present (supplemental Figures 1A and 1B). Lymphocytes with characteristic abnormal "flower" nuclei were also observed in blood smears (data not shown). Massive lymphocyte infiltrates were detected in an inguinal lymph node biopsy (Figure 1C), with a partial disruption of the architecture associated with interfollicular hyperplasia composed by a mix of small, medium and large CD3+, CD4+ CD25+ CD30+ T cells, and a few scattered eosinophils. Although hypercalcemia was not present at the beginning of the treatment, these results suggest that the animal suffered from a chronic ATL that evolved towards an acute ATL, because of the specific involvement of the lung and the kinetic of the disease progression.

As it is the case for human chronic and acute ATL patients in Europe, the animal received a combination of AZT and alpha interferon (IFN) for 18 weeks, monitored weekly by measurement of blood counts and blood PVL. Despite treatment, the count of total lymphocytes (Figure 1A) as well as CD4+ and CD8+ percentages (supplemental Figure 2) remained above normal values. Of note, the apparent increase in the lymphocytes counts at week 12 was not significantly different from the other values. Due to the absence of health improvement, the animal was euthanized and necropsy was performed.

3.2 Histological and proviral load postmortem analyses

Post-mortem liver biopsy showed a preserved architecture with lobular necrosis and portal CD3+ CD25+ T cell infiltrates, associated with a moderate, focal lobular infiltration, numerous lymphocytes in the sinusoids, and lobular necrosis. Tax protein-positive cells were also detected in several organs (Figure 1D, lymph node) and in mesenteric and axillary lymph nodes and lung, while Tax positive cells were not detected by flow cytometry among PBMC samples from week 0, week 8 after the

treatment started and week 18 before autopsy (data not shown). These histological and immunophenotypical aspects were consistent with a diagnosis of adult T cell leukemia / lymphoma. In addition, lung biopsies showed massive interstitial infiltration by tumor cells, CD3+ CD25+ T cells (supplemental Figure 3A). Skin lesion biopsy showed a massive dermal, hypodermic and muscular cell infiltrate of positive CD3+ CD25+ T cells (supplemental Figure 3B).

STLV-1 PVL was measured in 14 different organs (Figure 2A). Apart from a blood sample obtained during necropsy (Figure 2A, right lane), the highest PVL was found in lymph nodes, spleen and lungs, which is consistent with observations made in human ATL cases [48]. STLV-1 PVL was also high in the buccal wall (59%), which is consistent with studies reporting the apparent acquisition of STLV-1 in humans through severe animal bites [49, 50]

3.3 Unexpected high number of intermediate abundance STLV-1 clones in the blood

ATL is commonly described as a monoclonal proliferation of CD4+ CD25+ HTLV-1-infected cells. We therefore quantified STLV-1 clonality in sequential blood samples and 14 different organs by LM-PCR and HTS, to study both the dynamics of STLV-1 infected cells in blood over time and during the treatment, and the clonal distribution on the whole-body scale. First a series of 18 consecutive blood samples were analyzed. The STLV-1 clonal distribution did not vary significantly over time in blood (Figure 2B and supplemental Figure 4). These results are consistent with the lack of PVL variation observed during antiviral treatment, and demonstrate that AZT+IFN treatment did not affect significantly viral clonality in blood. Unexpectedly, in addition to the major clone, which represented between 22 and 37% of STLV-1 PVL in blood (denoted in all panels by light blue color), a number of abnormally expanded clones were also present in blood throughout the course of treatment (Figure 2B, 2C and supplemental Figures 4 and 5). This number of intermediate abundance STLV-1 clones was much greater than expected from typical cases of ATL in humans. In addition, while 1109 unique integration sites (UIS) were identified per blood sample on average (supplemental table 2), the proviral load was mainly carried by 10 or fewer clones (1 major clone and 9 low/intermediate abundance clones) representing between 68 and 97% of the PVL. Of note, some blood samples had a proviral load higher than 100%, that is greater than 100 proviral copies per 100 cells. The clonal

distribution analysis suggests that multiple integrations might occur in some cells. The overall oligoclonality index (OCI) remained stable during treatment (see Figure 2B), consistent with the lack of effect of drug treatment.

3.4 Organ specific distribution of STLV-1 clones

We then applied the same protocol to DNA samples extracted from 14 different organs. As in blood, the same major clone was found in all samples analyzed representing 19% to 79% of the load in organs, in addition to a number of identical smaller/intermediate clones (Figure 2C). The low- and intermediate-abundance clones were found in organs with a low STLV-1 PVL as well as those with a high PVL and no expanded organ-specific clone could be identified. A total of 212 clones were conserved within all consecutive blood samples; 27 were present in all organs, while 24 were found both in blood samples and in organs (Figure 2D). We also observed a direct correlation between the number of Common Unique Integration Site (CUIS) present in the different organs and in the blood at necropsy and the PVL of the organs (Figure 3A). These CUIS represented at least 85% of the PVL of each organ (data not shown). Altogether these results may suggest that the STLV-1 infected clones emerging in the organs have originated from the blood, although the opposite (i.e origin of these clones in organs followed by their dissemination in blood cannot be excluded). Interestingly, STLV-1 clonal distribution differed between the organs and the blood. This difference was easily observed in organs with lower PVL such as the mesenteric lymph node or pancreas (Figure 3C). Since the animal was not perfused before necropsy (for ethical reason), one could initially hypothesize that STLV-1 clonality in these organs, simply mirrors that in the blood. However, this was not the case. Indeed, certain clones were highly frequent in some organs, but remained almost undetectable in blood sample obtained at necropsy (see Figures 2C, 3B and 3C). These results suggest that organs represent niches where STLV-1 clones proliferate independently from those present in the blood. In some organs with a lower OCI and a low PVL, the major clone may reflect an “early” invasion of these tissues by STLV-1 (Figure 3B).

Finally, we also observed a positive correlation between STLV-1 PVL and the number of unique integration sites (UIS) in all samples, thus suggesting that these clones may play a role in the pathology (Figure 4).

4. Discussion

NHP are naturally infected with STLV-1. This retrovirus causes ATL in a number of animals and represents a unique model for studying retroviral pathogenesis or for developing new therapies [23, 38, 51, 52]. Here, we diagnosed an aggressive CD4+ T-cell proliferation consistent with a diagnosis of chronic ATL in a 9-year old *Papio anubis* female that is likely to have evolved then towards an acute form with visceral and lung involvement. The animal was subsequently treated with a combination of AZT and IFN-α equivalent in term of weekly doses to what is used to treat chronic/smoldering and acute ATL in humans. The animal did not respond to the treatment and had to be euthanized. A previous report demonstrated that human patients that respond to AZT/IFN-α have a wild-type p53 gene [53]. It might be of interest to determine whether the absence of remission was linked to *p53* mutation at the time of treatment initiation.

We took advantage of a series of blood and organ samples to study body-wide STLV-1 clonality using a highly sensitive high-throughput technique [19]. ATL is traditionally considered as a monoclonal proliferation of CD4+CD25+ cells. In most ATL cases, a single copy of HTLV-1 is integrated into the host genome [42]. A recent study using this technique showed that the majority of human ATL cases display a dominant clone with a relative abundance >35%, while a small percentage contain either 2 clones of approximately equal abundance or one dominant clone associated with one or more intermediate clones each of >10% relative abundance [7]. Here, despite the expected presence of a major clone in all blood samples and organs, we also detected not only a large number of intermediate-abundance clones (>5%) in all blood samples and in organs but also, and more surprisingly, a very large number of low-abundance small clones (212 clones found in all blood samples) usually characteristic of non-malignant HTLV-1 infection in humans [19]. These results are different from the dogma that states that oligoclonal proliferation should precede malignant transformation. In the case of human ATLL with one or more intermediate clones, it was reported that these intermediate-sized clones have unique genomic characteristics, whereas small large clones had the same genomic characteristics [7]. It would be of interest to understand the role of these intermediate clones, and more generally the dynamics of proliferation of the different clones (low-, intermediate- and high-abundance) but also their origin, i.e. to understand whether they are emerging in

the blood or the organs or in both. Finally, and contrary to a previous report obtained in an STLV-1-infected Japanese macaque with a single lymphomatous lesion in the brain [23] and even though the distribution in organs differed from the blood, we did not identify any organ-specific expanded clone.

We also demonstrated a positive correlation between the number of unique integration sites and STLV-1 proviral load in this leukemic animal. Such a correlation was previously described for HTLV-1 carriers suffering from non-malignant disease [19]. This observation suggests that minor clones may also play a role in the lymphoproliferation, although we cannot exclude the possibility that ATL-induced immunodeficiency allows the persistence of these clones. A previous report stated that the difference in PVL among HTLV-1 individuals with non-malignant infection depended upon the number of UIS [19]. It would therefore be of interest to determine whether STLV-1 infected asymptomatic baboons have also fewer infected clones.

Finally, the variation observed in the low-abundance clones in blood throughout the course of the treatment suggests that these clones are not transformed, are sensitive to AZT/IFN treatment and may play a role in the microenvironment of major STLV-1 leukemic clones.

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7. Conflict of Interest:

The authors declare no conflicts of interest

8. References

[1] A. Gessain, O. Cassar, Epidemiological Aspects and World Distribution of HTLV-1 Infection, Front Microbiol, 3 (2012) 388.

[2] B.J. Poiesz, F.W. Ruscetti, A.F. Gazdar, P.A. Bunn, J.D. Minna, R.C. Gallo, Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma, Proc Natl Acad Sci U S A, 77 (1980) 7415-7419.

[3] M. Yoshida, I. Miyoshi, Y. Hinuma, Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease, Proc Natl Acad Sci U S A, 79 (1982) 2031-2035.

[4] A. Gessain, F. Barin, J.C. Vernant, O. Gout, L. Maurs, A. Calender, G. de The, Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis, Lancet, 2 (1985) 407-410.

[5] M. Osame, K. Usuku, S. Izumo, N. Ijichi, H. Amitani, A. Igata, M. Matsumoto, M. Tara, HTLV-I associated myelopathy, a new clinical entity, Lancet, 1 (1986) 1031-1032.

[6] K. Yamaguchi, M. Seiki, M. Yoshida, H. Nishimura, F. Kawano, K. Takatsuki, The detection of human T cell leukemia virus proviral DNA and its application for classification and diagnosis of T cell malignancy, Blood, 63 (1984) 1235-1240.

[7] L.B. Cook, A. Melamed, H. Niederer, M. Valganon, D. Laydon, L. Foroni, G.P. Taylor, M. Matsuoka, C.R. Bangham, The role of HTLV-1 clonality, proviral structure, and genomic integration site in adult T-cell leukemia/lymphoma, Blood, 123 (2014) 3925-3931.

[8] A. Bazarbachi, Y. Plumelle, J. Carlos Ramos, P. Tortevoye, Z. Otrock, G. Taylor, A. Gessain, W. Harrington, G. Panelatti, O. Hermine, Meta-analysis on the use of zidovudine and interferon-alfa in adult T-cell leukemia/lymphoma showing improved survival in the leukemic subtypes, J Clin Oncol, 28 (2010) 4177-4183.

[9] A. Bazarbachi, F. Suarez, P. Fields, O. Hermine, How I treat adult T-cell leukemia/lymphoma, Blood, 118 (2011) 1736-1745.

[10] O. Hermine, E. Wattel, A. Gessain, A. Bazarbachi, Adult T cell leukaemia: a review of established and new treatments, BioDrugs, 10 (1998) 447-462.
[11] C. Journo, R. Mahieux, HTLV-1 and innate immunity, Viruses, 3 (2011) 1374-1394.
[12] R. Nasr, H. El Hajj, Y. Kfoury, H. de The, O. Hermine, A. Bazarbachi, Controversies in targeted therapy of adult T cell leukemia/lymphoma: ON target or OFF target effects?, Viruses, 3 (2011) 750-769.
[13] S. Alais, R. Mahieux, H. Dutartre, Viral Source-Independent High Susceptibility of Dendritic Cells to Human T-Cell Leukemia Virus Type 1 Infection Compared to That of T Lymphocytes, J Virol, 89 (2015) 10580-10590.
[14] A. Melamed, D.J. Laydon, H. Al Khatib, A.G. Rowan, G.P. Taylor, C.R. Bangham, HTLV-1 drives vigorous clonal expansion of infected CD8(+) T cells in natural infection, Retrovirology, 12 (2015) 91.
[15] M. Nagai, K. Usuku, W. Matsumoto, D. Kodama, N. Takenouchi, T. Moritoyo, S. Hashiguchi, M. Ichinose, C.R. Bangham, S. Izumo, M. Osame, Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP, J Neurovirol, 4 (1998) 586-593.
[16] A. Okayama, S. Stuver, M. Matsuoka, J. Ishizaki, G. Tanaka, Y. Kubuki, N. Mueller, C.C. Hsieh, N. Tachibana, H. Tsubouchi, Role of HTLV-1 proviral DNA load and clonality in the development of adult T-cell leukemia/lymphoma in asymptomatic carriers, Int J Cancer, 110 (2004) 621-625.
[17] S. Olindo, A. Lezin, P. Cabre, H. Merle, M. Saint-Vil, M. Edimonana Kaptue, A. Signate, R. Cesaire, D. Smadja, HTLV-1 proviral load in peripheral blood mononuclear cells quantified in 100 HAM/TSP patients: a marker of disease progression, J Neurol Sci, 237 (2005) 53-59.
[18] C.R. Bangham, L.B. Cook, A. Melamed, HTLV-1 clonality in adult T-cell leukaemia and non-malignant HTLV-1 infection, Semin Cancer Biol, 26 (2014) 89-98.
[19] N.A. Gillet, N. Malani, A. Melamed, N. Gormley, R. Carter, D. Bentley, C. Berry, F.D. Bushman, G.P. Taylor, C.R. Bangham, The host genomic environment of the provirus determines the abundance of HTLV-1-infected T-cell clones, Blood, 117 (2011) 3113-3122.
[20] E. Wattel, M. Cavrois, A. Gessain, S. Wain-Hobson, Clonal expansion of infected cells: a way of life for HTLV-I, J Acquir Immune Defic Syndr Hum Retrovirol, 13 Suppl 1 (1996) S92-99.

[21] I.J. Koralnik, E. Boeri, W.C. Saxinger, A.L. Monico, J. Fullen, A. Gessain, H.G. Guo, R.C. Gallo, P. Markham, V. Kalyanaraman, et al., Phylogenetic associations of human and simian T-cell leukemia/lymphotropic virus type I strains: evidence for interspecies transmission, J Virol, 68 (1994) 2693-2707.

[22] R. Mahieux, C. Chappey, M.C. Georges-Courbot, G. Dubreuil, P. Mauclere, A. Georges, A. Gessain, 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, 72 (1998) 10316-10322.

[23] M. Miura, J. Yasunaga, J. Tanabe, K. Sugata, T. Zhao, G. Ma, P. Miyazato, K. Ohshima, A. Kaneko, A. Watanabe, A. Saito, H. Akari, M. Matsuoka, Characterization of simian T-cell leukemia virus type 1 in naturally infected Japanese macaques as a model of HTLV-1 infection, Retrovirology, 10 (2013) 118.

[24] J. Mone, E. Whitehead, M. Leland, G. Hubbard, J.S. Allan, Simian T-cell leukemia virus type I infection in captive baboons, AIDS Res Hum Retroviruses, 8 (1992) 1653-1661.

[25] T. Watanabe, M. Seiki, H. Tsujimoto, I. Miyoshi, M. Hayami, M. Yoshida, Sequence homology of the simian retrovirus genome with human T-cell leukemia virus type I, Virology, 144 (1985) 59-65.

[26] R. Mahieux, J. Pecon-Slattery, G.M. Chen, A. Gessain, Evolutionary inferences of novel simian T lymphotropic virus type 1 from wild-caught chacma (Papio ursinus) and olive baboons (Papio anubis), Virology, 251 (1998) 71-84.

[27] N.K. Saksena, V. Herve, J.P. Durand, B. Leguenno, O.M. Diop, J.P. Digouette, C. Mathiot, M.C. Muller, J.L. Love, S. Dube, et al., Seroepidemiologic, molecular, and phylogenetic analyses of simian T-cell leukemia viruses (STLV-I) from various naturally infected monkey species from central and western Africa, Virology, 198 (1994) 297-310.

[28] H. Schatzl, L. Yakovleva, B. Lapin, D. Rose, L. Inzhiia, K. Gaedigk-Nitschko, F. Deinhardt, K. von der Helm, Detection and characterization of T-cell leukemia virus-like proviral sequences in PBL and tissues of baboons by PCR, Leukemia, 6 Suppl 3 (1992) 158S-160S.

[29] D.M. Sintasath, N.D. Wolfe, M. Lebreton, H. Jia, A.D. Garcia, J. Le Doux-Diffo, U. Tamoufe, J.K. Carr, T.M. Folks, E. Mpoudi-Ngole, D.S. Burke, W. Heneine, W.M. Switzer, Simian T-lymphotropic virus diversity among nonhuman primates, Cameroon, Emerg Infect Dis, 15 (2009) 175-184.

[30] S. Van Dooren, E.J. Verschoor, Z. Fagrouch, A.M. Vandamme, Phylogeny of primate T lymphotropic virus type 1 (PTLV-1) including various new Asian and African non-human primate strains, Infect Genet Evol, 7 (2007) 374-381.
[31] J.S. Allan, M. Leland, S. Broussard, J. Mone, G. Hubbard, Simian T-cell lymphotropic Viruses (STLVs) and lymphomas in African nonhuman primates, Cancer Invest, 19 (2001) 383-395.
[32] J.M. d'Offay, R. Eberle, R.F. Wolf, S.D. Kosanke, K.R. Doocy, S. Ayalew, K.G. Mansfeild, G.L. White, Simian T-lymphotropic Virus-associated lymphoma in 2 naturally infected baboons: T-cell clonal expansion and immune response during tumor development, Comp Med, 63 (2013) 288-294.
[33] G.B. Hubbard, J.P. Mone, J.S. Allan, K.J. Davis, 3rd, M.M. Leland, P.M. Banks, B. Smir, Spontaneously generated non-Hodgkin's lymphoma in twenty-seven simian T-cell leukemia virus type 1 antibody-positive baboons (Papio species), Lab Anim Sci, 43 (1993) 301-309.
[34] T.J. McCarthy, J.L. Kennedy, J.R. Blakeslee, B.T. Bennett, Spontaneous malignant lymphoma and leukemia in a simian T-lymphotropic virus type I (STLV-I) antibody positive olive baboon, Lab Anim Sci, 40 (1990) 79-81.
[35] Y. Noda, K. Ishikawa, A. Sasagawa, S. Honjo, S. Mori, H. Tsujimoto, M. Hayami, Hematologic abnormalities similar to the preleukemic state of adult T-cell leukemia in African green monkeys naturally infected with simian T-cell leukemia virus, Jpn J Cancer Res, 77 (1986) 1227-1234.
[36] I. Sakakibara, Y. Sugimoto, A. Sasagawa, S. Honjo, H. Tsujimoto, H. Nakamura, M. Hayami, Spontaneous malignant lymphoma in an African green monkey naturally infected with simian T-lymphotropic virus (STLV), J Med Primatol, 15 (1986) 311-318.
[37] H. Tsujimoto, Y. Noda, K. Ishikawa, H. Nakamura, M. Fukasawa, I. Sakakibara, A. Sasagawa, S. Honjo, M. Hayami, Development of adult T-cell leukemia-like disease in African green monkey associated with clonal integration of simian T-cell leukemia virus type I, Cancer Res, 47 (1987) 269-274.
[38] P.V. Afonso, M. Mekaouche, F. Mortreux, F. Toulza, A. Moriceau, E. Wattel, A. Gessain, C.R. Bangham, G. Dubreuil, Y. Plumelle, O. Hermine, J. Estaquier, R. Mahieux, Highly active antiretroviral treatment against STLV-1 infection combining reverse transcriptase and HDAC inhibitors, Blood, 116 (2010) 3802-3808.
[39] B. Lee, Y. Tanaka, H. Tozawa, Monoclonal antibody defining tax protein of human T-cell leukemia virus type-I, Tohoku J Exp Med, 157 (1989) 1-11.

[40] S. Kwok, G. Ehrlich, B. Poiesz, R. Kalish, J.J. Sninsky, Enzymatic amplification of HTLV-I viral sequences from peripheral blood mononuclear cells and infected tissues, Blood, 72 (1988) 1117-1123.
[41] J. Villaudy, M. Wencker, N. Gadot, N.A. Gillet, J.Y. Scoazec, L. Gazzolo, M.G. Manz, C.R. Bangham, M.D. Dodon, HTLV-1 propels thymic human T cell development in "human immune system" Rag2(-)/(-) gamma c(-)/(-) mice, PLoS Pathog, 7 (2011) e1002231.
[42] L.B. Cook, A.G. Rowan, A. Melamed, G.P. Taylor, C.R. Bangham, HTLV-1-infected T cells contain a single integrated provirus in natural infection, Blood, 120 (2012) 3488-3490.
[43] K. Manivannan, A.G. Rowan, Y. Tanaka, G.P. Taylor, C.R. Bangham, CADM1/TSLC1 Identifies HTLV-1-Infected Cells and Determines Their Susceptibility to CTL-Mediated Lysis, PLoS Pathog, 12 (2016) e1005560.
[44] R. Mahieux, F. Ibrahim, P. Mauclere, V. Herve, P. Michel, F. Tekaia, C. Chappey, B. Garin, E. Van Der Ryst, B. Guillemain, E. Ledru, E. Delaporte, G. de The, A. Gessain, 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, 71 (1997) 1317-1333.
[45] R development core team, R: a language and environment for statistical computing, R Foundation for statisitical computing, Vienna, Austria, 2011.
[46] H. Chen, P.C. Boutros, VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R, BMC Bioinformatics, 12 (2011) 35.
[47] M.S. Handcock, M. Morris, Relative distribution methods in the social sciences., Statistics for social sciences and public policy, Springer-Verlag Inc., New York, 1999.
[48] K. Tamura, T. Yokota, R. Mashita, S. Tamura, Pulmonary manifestations in adult T-cell leukemia at the time of diagnosis, Respiration, 60 (1993) 115-119.
[49] C. Filippone, E. Betsem, P. Tortevoye, O. Cassar, S. Bassot, A. Froment, A. Fontanet, A. Gessain, A Severe Bite From a Nonhuman Primate Is a Major Risk Factor for HTLV-1 Infection in Hunters From Central Africa, Clin Infect Dis, 60 (2015) 1667-1676.
[50] M. Kazanji, A. Mouinga-Ondeme, S. Lekana-Douki-Etenna, M. Caron, M. Makuwa, R. Mahieux, A. Gessain, Origin of HTLV-1 in hunters of nonhuman primates in Central Africa, J Infect Dis, 211 (2015) 361-365.

[51] I. Castro, T.M. Giret, D.M. Magnani, H.S. Maxwell, O. Umland, J.K. Perry, J.K. Pecotte, K.M. Brasky, G.N. Barber, R.C. Desrosiers, D.I. Watkins, Cellular Immune Responses against Simian T-Lymphotropic Virus Type 1 Target Tax in Infected Baboons, J Virol, 90 (2016) 5280-5291.

[52] A.R. Panfil, J.J. Al-Saleem, P.L. Green, Animal Models Utilized in HTLV-1 Research, Virology (Auckl), 4 (2013) 49-59.

[53] A. Datta, M. Bellon, U. Sinha-Datta, A. Bazarbachi, Y. Lepelletier, D. Canioni, T.A. Waldmann, O. Hermine, C. Nicot, Persistent inhibition of telomerase reprograms adult T-cell leukemia to p53-dependent senescence, Blood, 108 (2006) 1021-1029.

1. Figure legends

Figure 1: ATL diagnosis in a *Papio anubis* naturally infected with STLV-1.
(A) Lymphocyte counts and (B) STLV-1 proviral load were measured in 18 blood samples over the 21 weeks of the study. The dashed line below the graphs indicates the 18 weeks of treatment (A): normal lymphocyte count in baboon is indicated with hatched lines. (B): STLV-1 proviral load (PVL) was measured in genomic DNA extracted from 18 blood samples and is given as the number of STLV-1 provirus per 100 cells. Lymphocyte PVL was calculated by normalizing PVL obtained in blood by the percentage of lymphocytes. (C): immunohistochemistry was performed on an inguinal lymph node biopsy obtained at week 3 after AZT/IFN treatment was initiated. The sample was stained with hematoxylin/eosin (magnification x12.5 and x400) (D): inguinal lymph node biopsy obtained at necropsy was hybridized with anti-Tax antibody (LT4- at 20 µg/ml). Circle indicates a positive Tax signal. Bar, 20 µm. (C-D) Samples were processed and observed as described in Materials and Methods.

Figure 2: STLV-1 proviral load and clonality analysis in organs and blood from the leukemic *P. anubis*.
(A): STLV-1 proviral load (PVL) was measured in 14 different organs obtained at necropsy as well as in a blood sample from the same day. (B-C): relative abundance of STLV-1 clones identified by their integration sites and quantified by HTS in (B) blood before and during AZT/IFN treatment or (C) in 14 different organs ranked by increasing PVL. Each slice of the pie represents a single observed clone, and the width of the slice is proportional to its relative abundance. The clones with a relative abundance over 5% in at least one of the sample were labelled by a specific color while others are in grey. The color code is the same for all the samples. (D): Venn diagram of the clones conserved within the blood samples (212-red), within the organs (27-green) and within organs and blood (24).

Figure 3: Diversity in clone distribution between organs.
(A): The number of unique integration sites shared by a given organ and the blood at the necropsy, named common unique integration sites (CUIS) are directly

proportional to the PVL of the organ (P =0.0001, R^2 = 0.7122). The 95% confidence interval is indicated by dotted lines. (B): The eleven major and intermediate (over 5% in at least one organ) clones, described in figure 2B and 2C are shown according to their absolute abundance (number of copy per 100 cells) in the 14 different organs as well as in blood before treatment, at the end of the treatment and at the necropsy. Black line indicates STLV-1 PVL in each sample. (C): Organ specific distribution of two different STLV-1 clones (Chr06:142959565 and Chr03:95395495) as defined by their integration site. Relative abundance of these clones is represented for each of the 14 organs and the 18 blood samples. These two clones are proportionally overrepresented in the mesenteric lymph node and the pancreas whose pie charts of their clonal distribution is compared to the distribution in the blood at necropsy.

Figure 4: Linear correlation between STLV-1 proviral load and the number of Unique Integration Sites (UIS).

The total number of UIS detected by HTS in all samples (blood and organs) are directly correlated with their PVL (P <0.0001, R^2 = 0.7591) and the 95% confidence interval is indicated by dotted lines.

Supplemental Figure 1: ATL diagnosis in a STLV-1 naturally infected *P. anubis*.

(A): Chest X-ray examinations before treatment shows bilateral, multifocal pulmonary opacities (red arrows) on both lungs. (B): Nodular skin lesion (red arrow) collected at the beginning of the treatment.

Supplemental Figure 2: **CD4 and CD8 percentages during the course of AZT/IFN treatment.**

Blood samples were obtained on a weekly basis. PBMCs were purified after ficoll and frozen in FCS/10%DMSO. After thawing, CD3+ cells were analyzed for the presence of CD4+ or CD8+ marker. As a control, PBMCs from three STLV-1 positive *P. anubis* or from three STLV-1 negative baboons were used. Results are represented as the percentage of CD4 and CD8 positive cells among the total number of CD3+ cells.

Supplemental Figure 3: Massive lymphoproliferation in the lung and dermis.

(A): Post-mortem lung biopsy showed massive interstitial infiltration (black arrow) by tumor T-cells that are CD3+CD25+ and Tax+ (black circle). (B): Biopsy of a nodular

skin lesion collected at the beginning of the treatment. The analysis showed massive infiltrates of CD3+CD25+CD7+ T cells in muscle (white arrow), dermis, and hypodermis but not in the epidermis (black arrow). Magnification x12.5 and x400, for Tax image: bar = 20 µm

Supplemental Figure 4: STLV-1 clonality analysis in 18 blood samples.

Blood samples were obtained before AZT/IFN treatment was initiated (week 0), during treatment (week 1 to week 18) and after treatment was stopped (week 21). They were then processed as in Figure 2.

Supplemental Figure 5: Major and intermediate clones color code.

The color codes used in Figures 2 and 3 to characterize the major and intermediate clones are indicated by the pie chart. The clones are defined the genomic position of their integration site on the baboon genome.

Supplemental Table 1: LM-PCR and HTS primers.

Supplemental Table 2: Identified integration sites.