Check for updates

Brief report

HTLV-1–infected T cells contain a single integrated provirus in natural infection

*Lucy B. Cook,[1] *Aileen G. Rowan,[1] Anat Melamed,[1] Graham P. Taylor,[2] and Charles R. M. Bangham[1]

[1]Department of Immunology, Wright Fleming Institute, Imperial College London, United Kingdom; and [2]Department of Genito-Urinary Medicine and Communicable Disease, Imperial College London, London, United Kingdom

Human T lymphotropic virus type 1 (HTLV-1) appears to persist in the chronic phase of infection by driving oligoclonal proliferation of infected T cells. Our recent high-throughput sequencing study revealed a large number (often > 10^4) of distinct proviral integration sites of HTLV-1 in each host that is greatly in excess of previous estimates. Here we use the highly sensitive, quantitative high-throughput sequencing protocol to show that circulating HTLV-1$^+$ clones in natural infection each contain a single integrated proviral copy. We conclude that a typical host possesses a large number of distinct HTLV-1–infected T-cell clones. (*Blood*. 2012;120(17):3488-3490)

Downloaded from http://ashpublications.net/blood/article-pdf/120/17/3488/1358654/zh804312003488.pdf by guest on 06 May 2024

Introduction

Human T lymphotropic virus type 1 (HTLV-1) infection results in lifelong carriage. More than 90% of infected persons remain asymptomatic carriers; the remainder develop either adult T-cell leukemia/lymphoma (ATLL) or a progressive neurologic disorder known as HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) or other inflammatory conditions, such as HTLV-1–associated polymyositis or uveitis.[1-3]

In each infected person, a stable proviral load (PVL), the number of proviral copies per 100 PBMCs appears to be maintained by proliferation chiefly of HTLV-1$^+$ CD4$^+$ T cells. The PVL remains relatively stable within 1 host but varies by > 1000-fold between hosts. A high PVL is associated with oligoclonal proliferation of HTLV-1$^+$ T cells, with several clones reaching a high abundance in the peripheral blood. However, the dynamics of HTLV-1 replication in vivo remain poorly understood: there is no reliable estimate of the number of HTLV-1–infected T-cell clones, the number and abundance of infected T-cell clones, or the relative contributions of T-cell proliferation and infectious spread to the maintenance of the PVL. The reason for selective expansion of individual T-cell clones also remains unexplained.

Detection of a single genomic integration site by Southern blot is characteristic of malignant disease,[4,5] but this technique lacks the sensitivity to detect minor populations in polyclonal HTLV-1–infected PBMCs. Inverse PCR and linker–mediated PCR have been used to map integration sites in patients with malignant or nonmalignant HTLV-1 infection.[6-9] However, these techniques have 2 major biases that prevent accurate mapping and quantification. First, the use of restriction enzyme digestion leads to preferential detection of proviruses that lie near the restriction sites. Second, PCR amplification strongly favors short DNA fragments.[10,11] We therefore recently developed a novel high-throughput protocol to map and accurately quantify proviral integration sites in the host cell genome.[12] The results demonstrated that the previous estimate of ~ 100 proviral integrations in 1 host was grossly inaccurate: the true number is frequently > 10^4. The question arises: how many distinct HTLV-1–infected T-cell clones are present in each host? That is, what is the average number of proviral copies per infected cell?

Here, we generated T-cell clones from CD4$^+$CD25$^+$ PBMCs of 10 persons with different clinical manifestations of HTLV-1 infection and used unbiased linker–mediated PCR and high-throughput sequencing to accurately quantify the abundance of unique integration sites within each T-cell clone.

Methods

Blood samples

Ten persons (7 female, 3 male; median age, 55 years) infected with HTLV-1 attending the National Center for Human Retrovirology (Imperial College Healthcare NHS Trust, London) provided blood samples with written consent in accordance with the Declaration of Helsinki. This study was approved by the United Kingdom National Research Ethics Service (NRES). The subjects represented each major clinical manifestation of HTLV-1 infection: 2 asymptomatic carriers (median PVL, 6.2%), 5 HAM/TSP patients (median PVL, 13.6%), 2 ATLL patients (median PVL, 57.5%), and 1 patient with polymyositis (PVL, 18.3%). PBMCs were isolated using standard techniques and cryopreserved in 90% FCS (Invitrogen) with 10% DMSO (Sigma-Aldrich).

Isolation of T-cell clones by limiting dilution

Because cells expressing the activation/regulatory marker CD25 are more frequently infected than other PBMC subsets,[13] CD4$^+$25$^+$ cells were isolated by magnetic activated cell sorting (CD4$^+$CD25$^+$ isolation kit, Miltenyi Biotec). Cells were subsequently cloned by limiting dilution in RPMI containing 10% human AB serum (Invitrogen) in the presence of 50 IU/mL IL-2 (Promocell), 1 μg/mL PHA (Sigma-Aldrich), 10μM raltegravir (Selleck Chemicals), and 0.5 × 10^6/mL γ-irradiated feeder cells (mixed PBMCs from 3 uninfected donors). Clones were expanded with feeder cells and PHA every 14 days and fed with IL-2 twice weekly. The integrase inhibitor raltegravir was present throughout the in vitro culture, to minimize secondary infectious spread of HTLV-1.[14] Clones were cultured

Submitted July 26, 2012; accepted August 24, 2012. Prepublished online as *Blood* First Edition paper, September 6, 2012; DOI 10.1182/blood-2012-07-445593.

*L.B.C. and A.G.R. contributed equally to this study.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 USC section 1734.

© 2012 by The American Society of Hematology

Table 1. Characteristics of isolated CD4+CD25+ T-cell clones

Clone	Clone derived from	Genomic location of dominant integration site, hg 18*	Proviral load composed of dominant integration site, %
1	Asymptomatic carrier 1	Chromosome 6: 84613188	98.95
2	Asymptomatic carrier 1	Chromosome 1: 184745603	99.95
3	HAM/TSP patient 1	Chromosome 16: 52158560	99.9
4	HAM/TSP patient 1	Chromosome X: 114257179	99.9
5	HAM/TSP patient 1	Chromosome 10: 2277787	100
6	HAM/TSP patient 1	Chromosome 13: 74997821	98.88
7	HAM/TSP patient 2	Chromosome 10: 80828281	99.96
8	HAM/TSP patient 2	Chromosome 5: 1997980	99.9
9	HAM/TSP patient 2	Chromosome 4: 70601874	95.2
10	HAM/TSP patient 2	Chromosome X: 129048967	100
11	HAM/TSP patient 2	Chromosome 14: 45274700	100
12	HAM/TSP patient 3	Chromosome 4: 107219655	99.9
13	HAM/TSP patient 3	Chromosome 12: 40920384	100
14	HAM/TSP patient 4	Chromosome 5: 50609660	98.4
15	HAM/TSP patient 4	Chromosome 3: 76576960	99.9
16	HAM/TSP patient 4	Chromosome 19: 32974427	100
17	HAM/TSP patient 4	Chromosome 19: 38521388	100
18	HAM/TSP patient 4	Chromosome 14: 80728657	95.5
19	HAM/TSP patient 4	Chromosome 4: 169472575	100
20	HAM/TSP patient 5	Chromosome 3:75952018	100
21	Polymyositis patient 1	Chromosome 2: 214080658	99.95
22	Polymyositis patient 1	Chromosome 6: 167451858	99.9
23	Polymyositis patient 1	Chromosome 22: 31885379	100
24	Polymyositis patient 1	Chromosome 3: 32582626	98.6
25	ATLL patient 1	Chromosome 22: 42654531	99.89
26	ATLL patient 2	Chromosome 4: 9905297	99.8
27	Asymptomatic carrier 2	Chromosome 8: 87708389	52
27	Asymptomatic carrier 2	Chromosome 9: 11420980	42

hg 18 indicates human genome build 18 (NCBI build 36).

*This column shows the genomic location of the dominant integration site by chromosome and position using hg18 assembly as reference.

for 4-6 weeks before genomic DNA extraction (DNeasy Blood and Tissue Kit; QIAGEN).

PCR detection of HTLV-1

T-cell cultures were screened for the presence of HTLV-1 by PCR amplification of the *tax* gene using the primers: 5′-CGGATACCCAGTCTACGTGT-3′ and 5- GAGCCGATAACGCGTCCATCG-3′[15] (Phusion blood direct PCR kit, Finnzymes). Cycling conditions were as follows: 98°C 5 minutes, 35 cycles each of 98°C 5 seconds, 65°C 5 seconds, 72°C 30 seconds; and final extension 72°C for 5 minutes. The PCR products were separated on a 2% agarose gel and visualized under UV light.

Integration site mapping and quantification

The high-throughput protocol was used as previously described.[12] Fifty base-pair paired-end reads were acquired on an Illumina analyzer (either GAII or HiSeq 2000) and the insertion sites and shear sites deduced as described.[12]

TCR gene rearrangement

TCR gene rearrangements were analyzed in the Molecular Diagnostic Department, Imperial College Healthcare NHS Trust, London using the established BIOMED-2 protocol followed by analysis using GeneMapper 4.1 software (Applied Biosystems).

Results and discussion

Twenty-seven HTLV-1–infected, CD4+ cultures were successfully expanded in vitro (Table 1). In 26 of these, a unique integration site constituted 99.9% (range, 95.5%-100%) of integrated provirus detected, with a median of 206 197 sequence reads (range, 6621-2 599 892), indicating that we had cultured 26 clones, each with a single dominant integration site. The remainder of the integration sites detected within each clone were of low frequency (median, 3 sequence reads): we attribute these rare integration events to the incomplete inhibition by raltegravir of HTLV-1 infectious spread during in vitro culture.

One HTLV-1+ culture, derived from asymptomatic carrier 2, contained cells with 2 equally abundant integration sites. This observation suggested either the presence of 2 HTLV-1 proviruses in a single T-cell clone or that 2 infected T-cell clones were cloned in the same well. Analysis of the TCRG gene rearrangement revealed 2 distinct gene rearrangements in the Vγ1-8 region (not illustrated), consistent with the presence of 2 T-cell clones.

The CD4+CD25+ T-cell clones isolated from the 2 patients with acute ATLL were distinct from the abnormally expanded, putatively malignant clone but were small clones contributing 0.12% and 1.6% of the PVL in PBMCs, respectively. This study was not designed to compare the mean proviral copy number between different disease states: such a study will require a larger number of persons with each respective condition.

In conclusion, the majority of nontransformed cells naturally infected with HTLV-1 contain a single copy of the HTLV-1 provirus in the host genome. In HIV infection, despite the high frequency of viral recombination observed and efficient cell-to-cell spread of multiple viruses, Josefsson et al[17] recently estimated that > 85% of CD4+ HIV-1–infected cells contain a single integrated provirus. One possible explanation for the observed low mean proviral copy number is superinfection resistance (SIR). SIR

Downloaded from http://ashpublications.net/blood/article-pdf/120/17/3488/1358654/zh804312003488.pdf by guest on 06 May 2024

is well documented in retroviruses,[18] but the precise molecular mechanisms have not been unequivocally identified. In HIV-1 infection, down-regulation of CD4 from the infected cell surface by the HIV Nef protein may contribute to SIR. However, SIR becomes evident substantially earlier than CD4 down-regulation, suggesting that additional mechanisms may contribute to SIR in HIV-1 infection, including interference by host proteins with transport of the preintegration complex and blocking the HIV receptor.[18-20] The mechanisms of SIR in HTLV-1 infection have not been identified.

Because the number of distinct proviral integration sites in 1 host[12] often exceeds 10^4, the conclusion that each clone contains a single provirus implies that a typical host carries a very large number of HTLV-1–infected T-cell clones.

Acknowledgments

The authors thank the members of the Genomics Laboratory of the MRC Clinical Sciences Center, Hammersmith, London (Laurence Game, Adam Giess, Nathalie Lambie, and Ivan Andrew), the Molecular Diagnostic Department, Imperial College Healthcare NHS Trust (Mikel Valganon), and the patient donors in the HTLV-1 clinic at the National Center for Human Retrovirology, Imperial College Healthcare NHS Trust (St Mary's Campus).

This work was supported by Leukemia and Lymphoma Research and the Wellcome Trust.

Authorship

Contribution: L.B.C., A.G.R., and C.R.M.B. conceived and designed the research and wrote the manuscript; L.B.C. and A.G.R. performed the experiments and analyzed the data; A.M. developed essential bioinformatic data analysis tools; and G.P.T. recruited patients.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Charles R. M. Bangham, Section of Immunology, Department of Medicine, Imperial College London, St Mary's Campus, Wright-Fleming Institute, Norfolk Place, London W2 1PG, United Kingdom; e-mail: c.bangham@imperial.ac.uk.

References

1. Osame M, Usuku K, Izumo S, et al. HTLV-I associated myelopathy, a new clinical entity. *Lancet.* 1986;1(8488):1031-1032.
2. Arisawa K, Soda M, Endo S, et al. Evaluation of adult T-cell leukemia/lymphoma incidence and its impact on non-Hodgkin lymphoma incidence in southwestern Japan. *Int J Cancer.* 2000;85(3): 319-324.
3. Mochizuki M, Yamaguchi K, Takatsuki K, Watanabe T, Mori S, Tajima K. HTLV-I and uveitis. *Lancet.* 1992;339(8801):1110.
4. Yoshida M, Seiki M, Yamaguchi K, Takatsuki K. Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult T-cell leukemia suggests causative role of human T-cell leukemia virus in the disease. *Proc Natl Acad Sci U S A.* 1984;81(8):2534-2537.
5. Yamaguchi K, Takatsuki K, Seiki M, Yoshida M. [Adult T-cell leukemia virus (ATLV or HTLV) proviral DNA for the classification of T-cell malignancies]. *Rinsho Ketsueki.* 1984;25(4):547-553.
6. Wattel E, Vartanian JP, Pannetier C, Wain-Hobson S. Clonal expansion of human T-cell leukemia virus type I–infected cells in asymptomatic and symptomatic carriers without malignancy. *J Virol.* 1995;69(5): 2863-2868.
7. Etoh K, Tamiya S, Yamaguchi K, et al. Persistent clonal proliferation of human T-lymphotropic virus type I–infected cells in vivo. *Cancer Res.* 1997; 57(21):4862-4867.
8. Doi K, Wu X, Taniguchi Y, et al. Preferential selection of human T-cell leukemia virus type I provirus integration sites in leukemic versus carrier states. *Blood.* 2005;106(3):1048-1053.
9. Meekings KN, Leipzig J, Bushman FD, Taylor GP, Bangham CR. HTLV-1 integration into transcriptionally active genomic regions is associated with proviral expression and with HAM/TSP. *PLoS Pathog.* 2008;4(3):e1000027.
10. Wang GP, Ciuffi A, Leipzig J, Berry CC, Bushman FD. HIV integration site selection: analysis by massively parallel pyrosequencing reveals association with epigenetic modifications. *Genome Res.* 2007;17(8): 1186-1194.
11. Wang GP, Garrigue A, Ciuffi A, et al. DNA bar coding and pyrosequencing to analyze adverse events in therapeutic gene transfer. *Nucleic Acids Res.* 2008;36(9):e49.
12. Gillet NA, Malani N, Melamed A, et al. The host genomic environment of the provirus determines the abundance of HTLV-1–infected T-cell clones. *Blood.* 2011;117(11):3113-3122.
13. Toulza F, Heaps A, Tanaka Y, Taylor GP, Bangham CR. High frequency of CD4$^+$FoxP3$^+$ cells in HTLV-1 infection: inverse correlation with HTLV-1-specific CTL response. *Blood.* 2008; 111(10):5047-5053.
14. Seegulam ME, Ratner L. Integrase inhibitors effective against human T-cell leukemia virus type 1. *Antimicrob Agents Chemother.* 2011;55(5): 2011-2017.
15. Kwok S, Ehrlich G, Poiesz B, Kalish R, Sninsky JJ. Enzymatic amplification of HTLV-I viral sequences from peripheral blood mononuclear cells and infected tissues. *Blood.* 1988;72(4):1117-1123.
16. van Dongen JJ, Langerak AW, Bruggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98–3936. *Leukemia.* 2003;17(12):2257-2317.
17. Josefsson L, King MS, Makitalo B, et al. Majority of CD4$^+$ T cells from peripheral blood of HIV-1–infected individuals contain only one HIV DNA molecule. *Proc Natl Acad Sci U S A.* 2011; 108(27):11199-11204.
18. Nethe M, Berkhout B, van der Kuyl AC. Retroviral superinfection resistance. *Retrovirology.* 2005;2:52.
19. Lama J. The physiological relevance of CD4 receptor down-modulation during HIV infection. *Curr HIV Res.* 2003;1(2):167-184.
20. Volsky DJ, Simm M, Shahabuddin M, Li G, Chao W, Potash MJ. Interference to human immunodeficiency virus type 1 infection in the absence of downmodulation of the principal virus receptor, CD4. *J Virol.* 1996;70(6):3823-3833.

Downloaded from http://ashpublications.net/blood/article-pdf/120/17/3488/1358654/zh804312003488.pdf by guest on 06 May 2024