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SEQUENCE NOTE

The Spleen Is an HIV-1 Sanctuary During Combined Antiretroviral Therapy

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Abstract

Combined antiretroviral therapy (cART) does not eradicate HIV, which persists for years and can re-establish replication if treatment is stopped. The current challenge is identifying those tissues harboring virus through cART. Here, we used HIV *env-nef* single genome sequencing and HIV *gag* droplet digital PCR (ddPCR) to survey 50 tissues from five subjects on cART with no detectable plasma viral load at death. The spleen most consistently contained multiple proviral and expressed sequences (4/5 participants). Spleen-derived HIV demonstrated two distinct phylogenetic patterns: multiple identical sequences, often from different tissues, as well as diverse viral sequences on long terminal branches. Our results suggested that ddPCR may overestimate the size of the tissue-based viral reservoir. The spleen, a lymphatic organ at the intersection of the immune and circulatory systems, may play a key role in viral persistence.

Keywords: HIV evolution, viral reservoirs, phylogenetics, lymphatic system

Patients infected with human immunodeficiency virus (HIV+) who interrupt suppressive combined antiretroviral therapy (cART) will experience viral rebound. During cART, HIV persists in extensively characterized, circulating resting T cell populations.[1] Recently, increased attention has been focused on HIV+ cells residing in anatomical tissues that have less cART exposure than those in the circulating cell populations.[2] Our previous work suggests that HIV is not ubiquitous among autopsy tissue sites.[3] We also reported that a positive quantitative HIV droplet digital PCR (ddPCR) in tissues does not necessarily result in amplifiable HIV when using single genome sequencing (SGS). Herein, we surveyed 50 tissues from 5 HIV+, cART-suppressed subjects using both ddPCR and SGS approaches, to establish the anatomical locations with the most consistent presence of a large HIV coding region (3,198 base pairs, spanning *env-nef* reading frames).

The autopsy tissues used in this study are from participants in the National Neurological AIDS Bank (NNAB) program, a member of the National NeuroAIDS Tissue Consortium (NNTC), and were obtained through the AIDS and Cancer Specimen Resource (ACSR). Full details about the eligibility criteria, medical histories of study participants, sample collection and storage, and IRB approvals for the NNAB and the ACSR have been published.[3,4] The five participants in this study were cART treated and had an undetectable (<40 HIV copies/mm^3 fluid) viral load at the time of autopsy in the cerebrospinal fluid and/or cardiac aspirate, and had a documented history of viral suppression on cART treatment before and close to the time of death. ddPCR, which detects the HIV *gag* DNA, was performed on all tissue samples as previously described.[3] HIV proviral genomes and transcripts were amplified using an SGS approach.[5] Sequences were assembled and aligned using published methods[2] and final *env* and *nef* alignments were created that spanned from positions 6213–7823 and positions 8797–9411 relative to the HXB2 genome, respectively. Sequences have been submitted to GenBank (GenBank accession nos. *pol*: MF957318–MF957333; *env*: KY270561–KY270659, MG017706–MG017754; *nef*: MF997275–MF997418). Pairwise genetic distances were calculated and maximum-likelihood (ML) phylogenies were estimated under a general time reversible nucleotide substitution model and gamma distributed rate variation among sites as previously described.[2]

A total of 50 tissues from five participants were surveyed for HIV *env* and *nef* sequences using SGS (Table 1). A subset

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TABLE 1. PATIENT TISSUES WITH HIV *ENV* AND *NEF* SEQUENCE TOTALS

	Env *sequences*					Nef *sequences*				
ID	*4175*	*6083*	*5024*	*5025*	*4124*	*4175*	*6083*	*5024*	*5025*	*4124*
Subject	*HC06*	*HC07*	*HC08*	*HC09*	*HC10*	*HC06*	*HC07*	*HC08*	*HC09*	*HC10*
Years infected	1	6	15	13	16	1	6	15	13	16
Age at death	31	54	39	69	58	31	54	39	69	58
Basal ganglia	0	0		0	0	0	0		0	0
Cerebellum	0	0			0	0	0			0
Dura mater	0			0		0			0	
Frontal cortex		0		0	0		0		0	0
Occipital cortex	0	0		0	0	0	0		0	0
Temporal cortex	0	0		0	0	0	0		0	0
Adrenal			1					0		
Aorta	0		0			0		0		
Colon					0					0
Esophagus			1					0		
Heart		0					0			
Kidney	0	0	0	0	0	0	0	0	0	0
Liver	0	0	0	0		0	0	0	0	
Lung			15	11/6	5			12	7/8	5
Lymph node			0	12/21				0	9/18	
Pancreas		0	0				0	0		
Spleen	0	18	6/17	9/19	6	0	22	5/15	19/23	10
Testis/ovary	0				0	0				0
Thyroid			0					0		

Patient's NNAB ID number and subject number for this study are listed, as well as the years infected and age at death. The number of sequences obtained for each tissue is listed. Underline indicates the tissue had a positive ddPCR result (>200 copies/million cell equivalents). RNA- and DNA-derived sequence totals are separated by a forward slash (RNA/DNA) where applicable. A blank field indicates that the tissue for that patient was not available for SGS analysis.

ddPCR, droplet digital PCR; SGS, single genome sequencing.

of tissues that were SGS positive in isolated genomic DNA were also positive for expressed HIV transcripts in the cDNA, although the reverse was not found. Among the 18 different types of tissues assayed, 3 were most likely to harbor HIV using SGS: spleen, lymph node, and lung. Expressed viral RNA was also detected in the spleen of two participants. Of the 50 tissues, 20 were derived from brain. Although 11 of them had a positive ddPCR result, SGS results were all negative. Nine kidney and liver tissue sections were assayed, also with no amplifiable *env-nef*. Because a positive *gag*

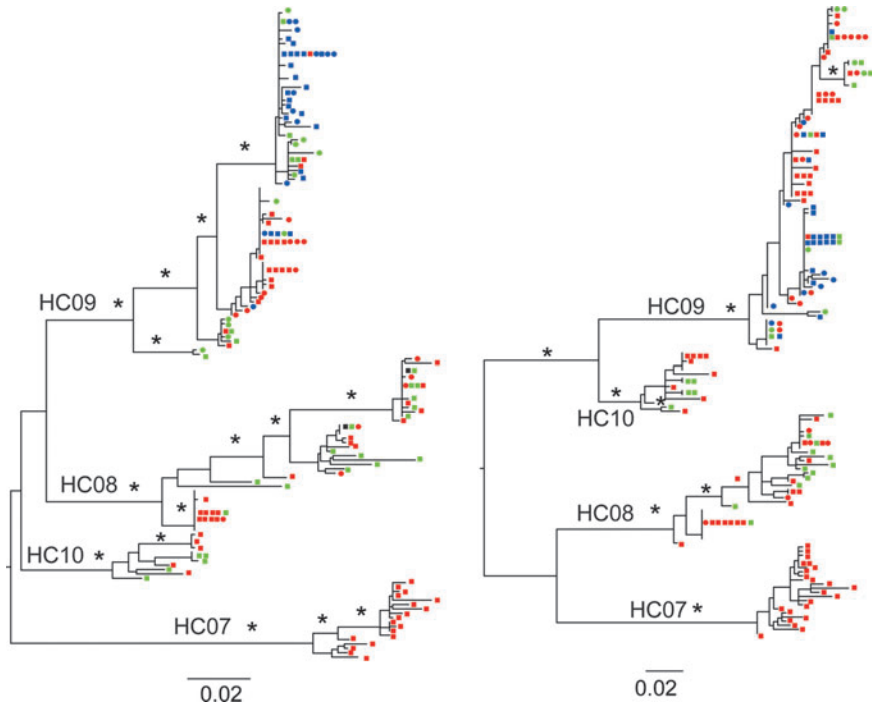

FIG. 1. Maximum likelihood phylogenies for *env* (*left*) and *nef* (*right*). All subjects' sequences were pooled and the branch leading to each subject is indicated. Branch lengths are proportional to the number of substitutions according to the scale on the bottom. *Asterisks* represent branches with >80 bootstrap value. Sequences are represented at the tips with *squares* (DNA) and *circles* (RNA). *Colors* indicate the tissue of origin as follows: *red* = spleen; *blue* = lymph node; *green* = lung; *black* = other peripheral tissues. Color images available online at www.liebertpub.com/aid

ddPCR did not guarantee *env* and *nef* SGS success, the possibility exists that assays using primers specific for small conserved regions of the HIV genome may overestimate viral reservoirs by detecting truncated genomes.[6] Drug resistance mutations were found in *pol* sequences for participants C08 (1 out of 3 or 1/3 sequences) and C09 (4/5), but were not found in participants C07 (0/2) and C10 (0/3).

ML phylogenies of both *env* and *nef* showed considerable viral diversity in the spleen, as well as in lymph node and lung when present (Fig. 1). Two patterns were observed: in some cases, many identical viruses clustered together, suggesting clonally expanding cells with identical proviral genomes; in other cases, distinct viral populations were found on long terminal branches. In participants C08 and C09, RNA virus showed similar patterns and was interspersed with DNA. Infection of multiple cell types may provide an explanation for the two distinct phylogenetic branching patterns found in the sequence populations of the participants, consistent with ongoing evolution in long-lived cells such as macrophages, and clonal expansion in T cell populations.

Reduced drug penetration into lymphatic tissues could be responsible for the persistence and expression of a diverse HIV population during otherwise successful cART.[7] Therefore, focus has shifted to anatomical tissue sites as an important target for novel or more potent cART. However, studying these HIV anatomical sanctuaries is complicated by the difficulty of obtaining tissues from well-defined study participants and the lack of a consistent assay for reliably assessing persistent tissue-based HIV. Here we used two methods for assessing HIV presence in 50 postmortem tissues from five participants, and found that three tissues were most consistently infected: lymph node, lung, and spleen. Furthermore, both DNA and RNA sequences were detected, suggesting an actively expressing virus.

Lymph node has previously been identified as a potential sanctuary with a diverse viral population, and is a natural reservoir given the number and diversity of immune cells.[8] However, spleen and lung tissue have been less frequently studied in the context of cART.[9] The spleen is an interesting organ, as it is the largest secondary lymphoid organ and plays a role in both the circulatory and immune systems. The spleen hosts one quarter of the body's lymphocytes as well as a large and diverse population of macrophages, which are potential targets of HIV.[10] Although efferent lymph vessels carry fluid and immune cells away from the spleen to the splenic lymph nodes, no afferent lymph vessels have been found to carry lymph into the spleen, indicating that transient immune cells arrive in the spleen through the circulatory system but may leave back through the veins or through the lymphatics.[11] The role of the spleen at the intersection of the circulatory and immune system, and the abundance of HIV-susceptible cells, makes it a prime candidate for an HIV tissue sanctuary and a therapeutic target in HIV cure research.

Author Disclosure Statement

No competing financial interests exist.

References

1. Lee GQ, Lichterfeld M: Diversity of HIV-1 reservoirs in CD4+ T-cell subpopulations. Curr Opin HIV AIDS 2016; 11:383–387.
2. Rose R, Lamers SL, Nolan DJ, *et al.*: HIV maintains an evolving and dispersed population among multiple tissues during suppressive cART with periods of rapid expansion corresponding to the onset of cancer. J Virol 2016;90: 8984–8993.
3. Lamers SL, Rose R, Maidji E, *et al.*: HIV DNA is frequently present within pathologic tissues evaluated at autopsy from cART-treated patients with undetectable viral load. J Virol 2016;90:8968–8983.
4. Morgello S, Gelman BB, Kozlowski PB, *et al.*: The National NeuroAIDS Tissue Consortium: A new paradigm in brain banking with an emphasis on infectious disease. Neuropathol Appl Neurobiol 2001;27:326–335.
5. Nolan DJ, Lamers SL, Rose R, Dollar JJ, Salemi M, McGrath MS: Single genome sequencing of expressed and proviral HIV-1 envelope glycoprotein 120 (gp120) and nef Genes. Bio-protocol 2017;7:e2334.
6. Massanella M, Richman DD: Measuring the latent reservoir in vivo. J Clin Invest 2016;126:464–472.
7. Fletcher CV, Staskus K, Wietgrefe SW, *et al.*: Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proc Natl Acad Sci U S A 2014;111:2307–2312.
8. Lorenzo-Redondo R, Fryer HR, Bedford T, *et al.*: Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature 2016;530:51–56.
9. Costiniuk CT, Jenabian MA: The lungs as anatomical reservoirs of HIV infection. Rev Med Virol 2014;24:35–54.
10. Mebius RE, Kraal G: Structure and function of the spleen. Nat Rev Immunol 2005;5:606–616.
11. Cesta MF: Normal structure, function, and histology of the spleen. Toxicol Pathol 2006;34:455–465.

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