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AIDS Alters the Commensal Plasma Virome

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We compared the plasma viromes of HIV-infected subjects with low versus high CD4$^+$ T cell counts from the United States and Uganda by using deep sequencing and detected HIV, hepatitis C virus, hepatitis B virus, GB virus C, anellovirus, and human endogenous retrovirus (HERV) reads. An increase in the proportion of reads for anelloviruses, a family of highly prevalent and genetically diverse human viruses, was seen in subjects with AIDS from both countries. The proportion of endogenous human retrovirus reads was increased in AIDS subjects from Uganda but not the United States. Progression to AIDS is therefore associated with changes in the plasma concentration of commensal viruses.

A characteristic feature of HIV and pathogenic simian immunodeficiency virus (SIV) infections is the loss of CD4$^+$ T lymphocytes, which coordinate the adaptive T and B cell responses against infections (1). The malfunction or failure of the immune system can result in an increased diversity of plasma and enteric viral and microbial communities (2, 3). A recent study showed that pathogenic SIV infection was associated with a significant expansion of the enteric virome, based on 454 pyrosequencing of the RNA and DNA viruses in feces. Pathogenic SIV infection resulted in more than a 10-fold increase in the number of virus reads in feces, including those of multiple enteric adenoviruses, caliciviruses, parvoviruses, picornaviruses, and polyomaviruses (2). Another study that used Illumina sequencing to compare the plasma microbiome of HIV/AIDS subjects and healthy adults reported a different bacterial profile and a higher abundance of bacterial DNA in plasma of people with HIV/AIDS (3).

Here we analyzed the DNA and RNA virome in the plasma of HIV-positive subjects with low (<20 cells/μl) versus high (>700 cells/μl) CD4$^+$ T cell counts from the United States and Uganda. Stored frozen plasma samples from 35 subjects were selected based on high or low CD4$^+$ cell counts. Viral nucleic acids were enriched by filtration and nuclease treatment and extracted from plasma as previously described (4), and libraries were constructed by using the ScriptSeq library preparation kit (Epicentre, Madison, WI). Nucleic acids from each plasma sample were labeled with a different primer bar code. The DNA library was sequenced using MiSeq (Illumina, San Diego, CA), which generated ~33 million paired-end 250-bp reads. The sequence reads were de-barcoded, clonal reads were removed, and low-sequencing-quality tails and adaptors were trimmed, leaving ~6 million unique usable sequence reads, which were then *de novo* assembled separately for each sample by using SOAPdenovo2. The resulting contigs and singlets (>50 bp) were then translated and analyzed via a protein similarity search (using BLASTx), with an E value cutoff of 1×10^{-10}. In this analysis, we focused on known human viruses, including human endogenous retrovirus (HERV). Other viral reads observed included sequences encoding the reverse transcriptase used to generate the cDNA libraries as well as sequences related to small circular and linear single-stranded DNA (ssDNA) genomes and iridoviruses that have been reported to contaminate the nucleic acid extraction columns that we used to purify nucleic acids (5). Fourteen sequence reads were also derived from a recently described rhabdovirus (6) that was being resequenced on the same MiSeq machine we used.

The subjects' information, including gender, age, virus load, and standard antiretroviral therapy (ART) at time of collection, is shown in Table 1. The 12 U.S. subjects were 45 years old on average and 100% male, and 92% were on ART, while the 23 Uganda subjects were 32 years old on average and 35% male, and 43% were on ART.

The plasma viromes of HIV subjects contained viral sequences from HIV, GB virus C (GBV-C), hepatitis B virus (HBV), hepatitis C virus (HCV), anellovirus, and HERV (Table 1). HIV sequences were identified in 13 samples from all but one subject with HIV RNA viral loads of greater than 1×10^5 copies/ml (another subject's viral load was not available), reflecting the approximate limit of detection for ssRNA viruses with the sample and nucleic acid processing method and the depth of Illumina sequencing used here. HIV subtyping was performed by using the automatic subtyping tool RIP (http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html). The three U.S. positives were all subtype B, while five Ugandans carried subtype A1, two were subtype D, and three had too few reads to achieve subtyping. The subtyping results were consistent with the reported geographical distributions of HIV subtypes.

GBV-C belongs to the *Flaviviridae* family and has been classified into 7 genotypes with distinct geographical distributions (7). Coinfection with GBV-C is common among HIV-infected people and has been associated with slower disease progression, a lower mortality rate, and longer survival (8, 9). GBV-C was detected in 5/35 samples. Both GBV-C isolates from the U.S. subjects were genotype 2, which is mostly reported in Europe and the United

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TABLE 1 Summary of sample information and blood viromes of the 35 HIV-infected patients

Subject identifier[a]	$CD4^+$ count (cells/μl)	ART	HIV load (RNA copies/ml)[b]	Age	Gender[c]	Total no. of reads	No. of unique reads	Standardized no. of unique viral reads						
								Anellovirus	HIV	GBV-C	HBV	HCV	HERV-K	HERV
AS10-10508	4	No	970,687	53	M	1,102,080	159,535	81	63	0	0	0	10	68
AS04-01159	4	Yes	400,000	35	M	819,508	133,880	3	19	0	0	0	0	56
AS06-06468	4	Yes	NA	52	M	512,620	74,264	5	32	0	0	0	0	15
AS11-16494	4	Yes	NA	54	M	691,430	99,329	75	0	0	0	4	4	157
AS03-00205	5	Yes	11,814	45	M	1,438,722	310,233	566	0	331	0	0	8	313
AS03-05969	6	Yes	489	44	M	1,016,996	169,319	210	0	0	0	0	2	39
AS12-08878	803	Yes	NA	43	M	754,466	206,029	2	0	0	0	0	9	484
AS08-00064	817	Yes	647	37	M	488,736	80,360	2	0	368	0	0	0	23
AS08-03339	837	Yes	NA	38	M	131,746	25,991	0	0	0	0	0	0	56
AS07-00787	838	Yes	<75	44	M	483,636	169,728	0	0	0	0	0	6	204
AS12-08598	844	Yes	<40	46	M	389,108	115,211	0	0	0	0	0	0	129
AS06-10195	856	Yes	NA	52	M	355,520	56,070	0	0	0	0	0	10	58
MBA1378	2	No	241,276	28	F	1,543,528	184,369	24	28	0	0	0	4	100
MBA1404	5	No	1,004,047	24	F	470,322	21,265	0	94	0	9	0	9	77
MBA1265	6	No	542,049	39	M	1,084,908	34,541	47	0	0	0	0	11	158
MBA1294	7	No	835,575	25	F	1,577,950	76,529	5	19	0	0	0	0	88
MBA1179	8	No	153,031	28	M	476,346	10,272	230	124	0	0	0	0	71
MBA1307	8	No	155,097	38	M	764,620	24,929	146	66	0	0	0	0	131
MBA1441	8	No	645,520	24	M	1,895,934	197,255	0	311	0	0	0	17	150
MBA1119	9	Yes	<400	38	M	962,922	154,475	18	0	0	0	0	7	121
MBA1124	9	No	750,000	34	F	556,908	59,605	658	131	0	1,137	0	0	104
MBA1465	10	Yes	339,818	22	F	1,143,242	638,245	220	4	0[d]	0	0	31	506
MBA1120	11	No	197,542	31	F	935,006	622,898	5	1	0	0	0	27	448
MBA1516	11	No	20,823	32	M	412,166	117,610	136	0	0	0	0	2	23
MBA1089	12	No	29,829	27	F	728,982	306,788	107	0	0	0	0	21	384
MBA1470	12	No	16,416	30	F	780,228	427,903	1	0	0	0	0	39	525
MBA1478	13	No	110,896	28	F	1,801,678	221,667	85	13	0	0	0	6	158
MBA1549	720	Yes	<400	24	M	697,384	220,305	62	0	9	0	0	0	0
MBA1581	740	Yes	<400	40	M	447,492	121,894	0	0	0	0	0	0	3
MBA1218	743	Yes	<400	49	F	272,730	67,843	0	0	0	0	0	0	0
MBA1548	747	Yes	<400	29	F	1,132,556	152,119	20	0	0	0	0	2	54
MBA1256	748	Yes	103	37	F	292,0126	999,030	4	0	0	0	0	9	195
MBA1005	751	Yes	<400	35	F	149,336	44,719	4	0	0	0	0	0	0
MBA1169	801	Yes	<400	36	F	128,2614	28,669	0	0	0	0	0	0	0
MBA1650	811	Yes	<100	27	F	481,096	23,679	0	0	253	0	0	0	0

[a] Identifiers that begin with AS indicate U.S. subjects, and those that begin with MBA indicate Ugandan subjects.
[b] NA, not available.
[c] M, male; F, female.
[d] For the sample from this subject, there was one original read before the standardization.

States (10); the three positives among Ugandan samples had too few reads to be genotyped. One HCV-positive plasma sample (too few reads to be genotyped) and one HBV-positive (genotype A) plasma sample were detected in one U.S. and one Ugandan subject, respectively.

The *Anelloviridae* family consists of nonenveloped, circular, single-stranded DNA viruses that are genetically extremely diverse in humans, including multiple genera that each consist of multiple species and genotypes (11). Multiple mammalian species can also be chronically infected. The reported Torque teno virus prevalence in HIV subjects varies, possibly due to the use of different PCR primers for detection, but has been reported to be between 50% and 100% (12–16). Transmission is thought to initially occur very early after birth, resulting in chronic viremia. Anelloviruses have not been associated with human disease (17). In our study, by using deep sequencing we detected anelloviruses in 71% (25/35) of the HIV-positive subjects. In some samples, multiple species/genotypes of anelloviruses were observed, consistent with prior reports of mixed infections (18, 19). The viral read numbers were standardized to adjust for the different numbers of unique reads from different plasma samples by using the following equation: number of standardized viral reads = [(number of unique reads in a given sample/average number of unique reads per sample) × number of unique viral reads] (Table 1). The Wilcoxon rank-sum exact test was used for all statistical analyses. The low-$CD4^+$ group from the United States had a significantly higher number of anellovirus reads than the high-$CD4^+$ United States group ($P = 0.001$). The Uganda low-$CD4^+$ group showed a trend toward more anellovirus reads than the high-$CD4^+$ Uganda group ($P = 0.118$), but the difference did not reach statistical significance. These results indicated that AIDS was associated with reduced control of anellovirus replication and higher viral loads (20).

HERVs are remnants of germ line retroviral integration. Approximately 3,900 full-length HERVs with two long terminal repeats have been estimated to reside in the human genome (21). HERVs are generally considered functionally defective, and in cases of autoimmune disorders, malignancies, and HIV infection, higher levels of HERV transcription and RNA levels in plasma have been observed (22, 23). HERV-K represents a more recently integrated and active group and the association of HERV-K with HIV infection has been extensively studied (23–26). A significant increase of HERV-K RNA but not HERV-H RNA has been reported in the plasma of HIV-infected subjects (26, 27).

Here, HERV sequences were detected in 30/35 samples. The majority of HERV reads were matched to the class I ERV group, which includes HERV-H and HERV-W (28), while HERV-K (class II ERV group) only represented ~5% of the total HERV reads. Both overall HERV and HERV-K standardized read num-

bers were significantly higher in the Uganda low-CD4$^+$ group than the Ugandan high-CD4$^+$ group (HERV, $P = 0.015$; HERV-K, $P = 0.015$). Unexpectedly the same phenomenon of higher HERV read numbers in low-CD4$^+$ versus high-CD4$^+$ subjects was not observed in the U.S. groups (HERV, $P = 0.96$; HERV-K, $P = 0.98$). The gender distribution and ART prevalence differences between the U.S. and Ugandan subjects and their possible association with HERV expression were also analyzed. Gender was not associated with a difference in HERV ($P = 0.63$) or HERV-K levels ($P = 0.63$). Subjects on ART showed lower, although not statistically supported, levels of HERV ($P = 0.13$) and HERV-K ($P = 0.13$), possibly related to their higher CD4$^+$ T cell counts (ART-treated average CD4$^+$ count of 528 cells/μl, while those not ART treated had an average CD4$^+$ count of 8 cells/μl).

In conclusion, our study provides an initial survey of viral sequences in the plasma of HIV-infected subjects. HIV, HCV, GBV-C, HBV, anelloviruses, and HERV were detected. It is possible that other viruses went undetected, but at least for ssRNA genomes these would likely be at lower viral loads than those ssRNA viruses detected here or have very low protein sequence identity to any already-sequenced viral proteins and thus be undetectable by BLASTx analysis. A higher level of anelloviruses was found in subjects with lower CD4$^+$ counts from the U.S. group ($P = 0.001$) and the Ugandan group ($P = 0.118$). Increased anellovirus levels with lower CD4$^+$ counts may be due to reduced immunological controls on replication of these viruses. The very high genetic diversity of anelloviruses (11) complicates studies of their possible pathogenicity (29), and no generally accepted link to disease has yet been identified for these very highly prevalent human infections (11). Besides possibly further stimulating the immune system (30), the health consequences of increased anellovirus replication during AIDS are unknown.

HERV sequences were detected at higher levels in Ugandan AIDS subjects than HIV-infected Ugandans with high CD4$^+$ counts. The majority of HERV reads found in our study matched HERV-H and HERV-W reads. Upregulation of both HERV-H and HERV-W levels has been reported in subjects with rheumatoid arthritis or multiple sclerosis, but not in HIV-1-infected subject blood plasma samples (26, 31). Increased levels of plasma HERV in low- versus high-CD4$^+$ count subjects were not detected in U.S. subjects.

Recent studies in immunodeficient mice have shown the generation of replication-competent endogenous retrovirus through recombination, which results in higher rates of lymphoma (32, 33). Surprisingly, such ERV activation requires the presence of a full intestinal microbiota (32). It is interesting to speculate on a similar phenomenon occurring in AIDS patients and the possible influence of different microbiota in the U.S. and Ugandan populations.

Nucleotide sequence accession number. The sequence data from each plasma sample were deposited in the DNA Data Bank of Japan under accession number SRA091349.

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