

Evolution of pathogenic HIV-1 variants within Gut-Associated Lymphoid Tissue

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ABSTRACT

Gut-associated lymphoid tissue (GALT) represents more than half of all body lymphoid tissue, and exists in a heightened state of activation, thus offering a favorable milieu for viral replication. Despite its importance to transmission and pathogenesis, HIV evolution within GALT has rarely been studied. We hypothesize that GALT is an important site for the evolution of pathogenic genotypes including drug resistance and CXCR4 coreceptor usage.

We sampled blood and rectosigmoid mucosal biopsy material at one or more time points in six subjects with chronic HIV-1 infection. Viral nucleic acids were extracted from plasma (RNA), PBMC (DNA), and GALT (DNA and RNA), reverse transcribed as necessary, PCR-amplified, cloned and sequenced by standard methods. 393 HIV-1 env and 48 pol sequences were aligned, and neighbor-joining and maximum-likelihood phylogenetic trees were reconstructed. Drug resistance was determined using the Stanford Drug Resistance Database. Env gene coreceptor usage was deduced using a previously described position-specific scoring matrix (PSSM).

All sequences clustered according to subject by phylogenetic analysis, reflecting lack of cross-contamination. Among the 48 pol sequences isolated from one subject, there was significant compartmentalization between mucosal DNA and PBMC. 15/29 PBMC-derived sequences were intermediately resistant to one or more nucleoside reverse transcriptase inhibitors (NRTIs), and one was highly resistant. 14/19 GALT-derived sequences were intermediately resistant to one or more NRTIs and 4 were highly resistant to one or more drugs. High-level resistance occurred predominantly within GALT (Fisher's Exact test $P = 0.07$), and was inferred to arise from a GALT ancestral sequence. In one subject, there was no apparent association between X4 usage and tissue site. In the second subject, X4 usage occurred only within GALT and to a lesser extent in PBMC, but never in plasma (Fisher's Exact test $P = 0.064$).

In our analysis of HIV-1 pol sequences, resistance to NRTI drugs appears to be more highly represented in GALT than in blood. Appearance of the X4 coreceptor usage phenotype in GALT and PBMC may precede its appearance in plasma. X4-tropic viruses appear to evolve from rapidly replicating viral subpopulations under diversifying selective pressure. GALT may be an important site for the evolution of pathogenic viral phenotypes. Further studies will be required to evaluate these observations.

INTRODUCTION

Studies of AIDS pathogenesis and vaccine development have primarily focused on systemic sites. However, gut-associated lymphoid tissue (GALT) represents more than half of all lymphoid tissue present within the body (2,3), and is constitutively in a state of heightened activation (10,12,13), thus favoring viral replication (1,5,6,8). For these reasons, GALT is probably one of the most important sites of HIV replication and evolution. However, few studies have examined the evolution of drug resistance and pathogenic viral genotypes within GALT (7,9,11). We hypothesize that throughout infection, viral replication within GALT greatly influences the effective size and composition of the overall viral population and is a predominant site for the evolution of CXCR4-tropic viruses and drug resistant variants.

SUBJECTS

Six HIV-1 infected subjects from the greater Los Angeles metropolitan area have presently been enrolled in this study. Clinical characteristics are as follows (Table 1):

Subj.	Sample Date	Viral Load (copies/ml)	CD4 count	Transmission	Treatment History	Env Seqs	RT Seqs
159	2/18/00	<200	207	Sexual	Unknown	7	48
148	7/31/98	<200	379	Sexual	3TC, D4T, NVP, SAQ, RIT	23	0
173	4/21/98	<200	550	Unknown	SAQ, RIT, D4T	35	0
	11/17/98	<200	788		SAQ, ABC, AZT, D4T	37	0
	12/19/00	83,383	560		ABC, 3TC, D4T	55	0
	10/4/02	227,807	338		None	74	0
178	2/9/99	<200	285	Unknown	3TC, NEL, NVP	28	0
520	2/2/01	8,556	340	Sexual	None	62	0
517	3/6/01	182,283	92	Sexual	Unknown	70	0

Table 1. Cohort clinical and demographic data

METHODS

We sampled blood and rectosigmoid mucosal biopsy material at one or more time points in six subjects with chronic HIV-1 infection (see table 1). Tissue samples were obtained from circumferential biopsies of the rectosigmoid colon obtained by flexible sigmoidoscopy. Plasma and PBMC samples were obtained by phlebotomy followed by Ficoll centrifugation. Viral nucleic acids were extracted from plasma (RNA), PBMC (DNA), and GALT (DNA and RNA) using Qiagen nucleic extraction kits, and reverse transcribed as necessary. Extracted DNA and cDNA samples were amplified using a nested polymerase chain reaction protocol with primer pairs POL3/RT2 and POL1/RTB (*reverse transcriptase*) or PE1/PE2 and ED5/ED12 (*env*) (Figure 1). Limiting dilution was performed as necessary for low-copy samples to avoid resampling. PCR products were cloned and sequenced by standard methods.

A total of 393 HIV-1 *env* and 48 *pol* sequences (table 2) were aligned using ClustalX and edited in MacClade 4.06. Neighbor-joining and maximum-likelihood phylogenetic trees were reconstructed using Modeltest 3.6 and Paup* 4.0. Drug resistance was determined using the Stanford Drug Resistance Database (<http://hivdb.stanford.edu/>). Coreceptor usage was deduced using a previously described position-specific scoring matrix (4).



Fig 1. PCR amplification strategy. Green arrows indicate nested primer pairs.

RESULTS

Reverse Transcriptase Analyses: A total of 48 *reverse transcriptase* sequences were available from one subject (table 1). No outlying sequences were observed to suggest contamination by sequences from another subject. RT sequences were observed to cluster by tissue type, suggesting some degree of compartmentalization between GALT and PBMC (Figure 2). The most common resistance mutations seen were M41I, T69N, K70R, and M184I. 13 of 29 PBMC-derived sequences were wild-type with respect to all reverse transcriptase inhibitors, 15 sequences were intermediately resistant to one or more nucleoside reverse transcriptase inhibitors (NRTIs), and one was highly resistant. One of 19 GALT-derived sequences was sensitive to all NRTIs, whereas 14 sequences were intermediately resistant to one or more NRTIs and 4 were highly resistant to one or more drugs. High-level resistance was seen only to 3TC, occurred predominantly within GALT (Fisher's Exact test $P = 0.07$), and was inferred to arise from a GALT ancestral sequence.

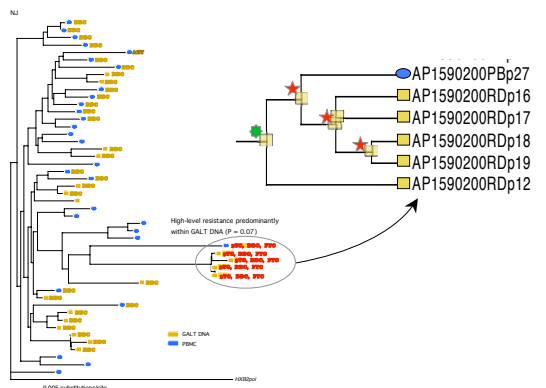


Fig 2. Rooted neighbor-joining phylogenetic tree of protease sequences derived from PBMC (blue) and GALT (yellow). Inset: cladogram of highly resistant sequences, demonstrating ancestral states and inferred resistance properties. High-level resistance genotypes are indicated in red letters (e.g. 3TC) or symbols (▲). Wild-type sequence indicated by (●).

Env Analyses: A total of 393 *env* sequences were available from six subjects. Phylogenetic analysis of all sequences collectively revealed no inter-patient contamination. However, subject 173 was noted to have two highly divergent clades suggestive of dual infection (data not shown). CXCR4-tropic genotypes were seen in two subjects (178 and 517). In subject 178, there was no apparent association between X4 usage and tissue site. In subject 517, X4 usage occurred within GALT and to a lesser extent in PBMC, but was not seen in plasma-derived sequences (Fisher's Exact test $P = 0.064$). In this subject, X4 clade branch lengths were significantly longer than other sequences present ($P < 0.01$) suggesting diversifying selection in this clade (Figure 3).

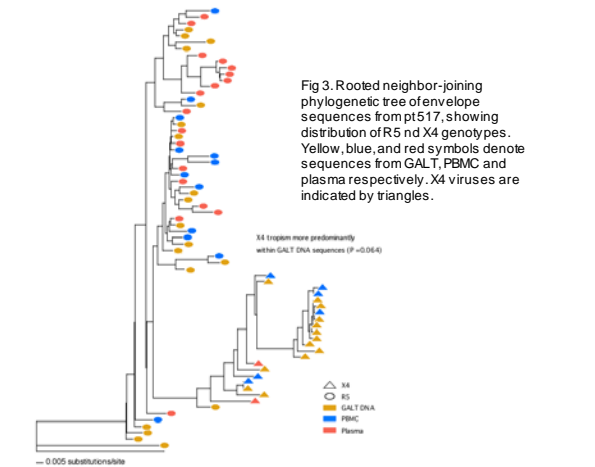


Fig 3. Rooted neighbor-joining phylogenetic tree of envelope sequences from pt 517, showing distribution of R5 nd X4 genotypes. Yellow, blue, and red symbols denote sequences from GALT, PBMC and plasma respectively. X4 viruses are indicated by triangles.

CONCLUSIONS

In our analysis of HIV-1 pol sequences, resistance to NRTI drugs appears to be more highly represented in GALT than in blood.

High-level resistance to 3TC was inferred to originate from a GALT ancestral sequence with subsequent dissemination to PBMC.

Appearance of the X4 coreceptor usage phenotype in GALT and PBMC may precede its appearance in plasma.

X4-tropic viruses appear to evolve from rapidly replicating viral subpopulations under diversifying selective pressure.

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