

Detection of HIV in Liver Biopsies and Intrahepatic Lymphocytes

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Background: Among persons with hepatitis C virus (HCV), HIV co-infection results in increased HCV RNA levels, more rapid progression to cirrhosis and end-stage liver disease and reduced HCV treatment response rates. Given their principle sites of replication, the opportunity for direct viral interactions between HIV and HCV would appear to be limited. Nonetheless, several lines of evidence suggest that multiple liver cell types can be productively infected with HIV *in vitro*. We therefore investigated the detection of HIV RNA in liver biopsy tissues from persons with HIV/HCV co-infection.

Methods: Liver tissues were obtained by core needle biopsy from 8 individuals with HIV/HCV co-infection (Table 1). For three of these individuals, one-half of the biopsy tissue was used for *in vitro* expansion of intrahepatic lymphocytes (IHLs) in the presence of anti-CD3 and IL-2 for approximately 3 weeks (Figure 1). Viral RNA was extracted from all biopsy tissues, as well as from expanded liver tissues, cell culture supernatants, IHLs, and the corresponding plasma when available. The presence of HIV RNA was detected by nested RT-PCR for a 485-nucleotide fragment of gag (p24) or a 337-nucleotide fragment of envelope (env). PCR products were then cloned and at least 10 clones per tissue/cell type were sequenced. Phylogenetic analysis was performed to examine clustering of viral sequences in different compartments.

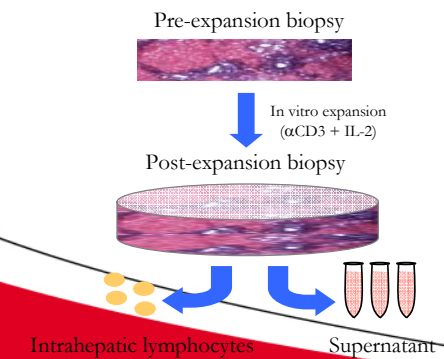


Figure 1. Expansion of liver biopsy tissues

Table 1. Patient demographics

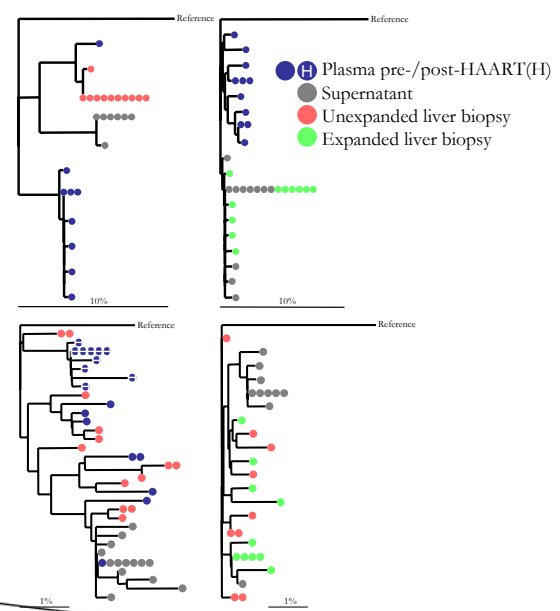
Patient	HIV viral load (copies/mL)	HIV treatment	HCV status
Cin 01	56,335	No	Positive
Cin 02	218,106	No	Positive
1724	Undetectable	Yes	Positive
1344	Undetectable	Yes	Positive
1370	5,481	Unknown	Positive
1493	6,126	Unknown	Positive
1580	1,200	No	Positive
1627	463	Unknown	Positive

Results: HIV RNA was detected in fresh liver biopsy tissue from 4 of 8 (50%) individuals (Cin 01, Cin 02, 1724, and 1580). HIV RNA was detected after *in vitro* expansion in 2 of 3 biopsies (Cin 02 and 1724). HIV RNA was also detected in IHLs (1724) and the corresponding cell culture supernatant (Cin 01 and Cin 02) after *in vitro* expansion (Table 2). For two individuals with available sequence data, the consensus p24 nucleotide sequences were distinct for each compartment (data not shown), while clonal analysis further demonstrated segregation by tissue source in several instances (Figure 2). Inpatient genetic distances were generally higher for serum and liver biopsy samples compared to cell culture supernatants (Table 2).

Table 2. Detection of HIV RNA by RT-PCR, average genetic distances (G.D.), and entropy

		Plasma pre-HAART	Plasma post-HAART	Liver biopsy pre-expansion	Liver biopsy post-expansion	Supernatant
Cin 01 env	PCR	Positive	Not tested	Positive	Negative	Positive
	G.D.	2.19		0.05		0.09
	Entropy	0.80		0.13		0.18
Cin 01 gag	PCR	Positive	Positive	Positive	Negative	Positive
	G.D.	2.93	0.36	2.66		0.49
	Entropy	0.83	0.65	0.98		0.81
Cin 02 env	PCR	Positive	Not tested	Negative	Positive	Positive
	G.D.	1.89			0.34	0.33
	Entropy	0.86			0.62	0.50
Cin 02 gag	PCR	Negative	Not tested	Positive	Positive	Positive
	G.D.			0.80	1.02	0.42
	Entropy			0.82	0.76	0.65

Figure 2. Phylogenetic trees of HIV env (upper panel) and HIV gag (lower panel) for Cin 01 (left) and Cin 02 (right)



Conclusions: Our data suggest that HIV RNA can be detected in liver biopsies from persons with HIV/HCV co-infection. The detection of HIV RNA in intrahepatic lymphocytes (and cell culture supernatants) after *in vitro* expansion suggests that this cell type may serve as a reservoir for HIV replication in the liver. Further investigation is underway to determine the clinical variables associated with HIV detection in the liver and its pathogenic consequences on hepatic function and HCV replication.

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