DETECTION AND SEQUENCING OF ASP RNA IN HIV+ individuals N.0179

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Background

Here we report detection and kinetics of expression of ASP RNA in CD4+ T cells from individuals infected with HIV-1 at different stages of the disease and undergoing different therapeutic regimens.

Methods

CD4+ T cells isolated from patients' PBMCs were stimulated with anti-CD3/CD28. RNA was extracted and reverse transcribed using a biotinylated version of the specific antisense primer. cDNA was purified onto streptavidin-coated magnetic beads, and amplified with patient-specific ASP primers. Amplified products were cloned into pCR2.1 and sequenced. ASP expression in the various patients was quantified by qPCR.

Patient ID	Age	Sex	Stage of HIV infection	Clade	Viral load (copies/ml)	CD4 Count (cells/μ)	ART status
MP135	44	M	C3	B	$1.6x10^5$	176	Untreated
MP140	23	M	A2	B	$3.6x10^4$	427	Naïve
MP148	37	M	A1	B	$2.0x10^4$	717	Naïve
MP069	42	M	A1	B	<20	1309	Treated
MP071	47	M	C3	B	<20	167	Treated
MP146	59	M	C3	B	<20	385	Treated

Table 1. Patients' features, clinical information and antiretroviral therapy status

Results

- ASP transcripts are detected in CD4+ T cells isolated from viremic, untreated patients stimulated with anti-CD3/CD28

Patient ID	Number of clones		Region of stop codon	ORF length (aa)
	CD4 (ASP)	Serum (env)		
MP135	1/10	0/8	C3*	192
	3/10	2/8	V5	115
	6/10	6/8	C3	212
MP140	4/8	4/9	C3*	187
	4/8	5/9	V5	104
MP148	8/8	13/13	V4	172

Table 2. Localization of ASP stop codons in gp120. Asterisks indicate the canonical stop codon at the junction C3/V4. Clones carrying the canonical ORF are in bold

- Differences in the expression of ASP RNA in CD4+ T cells from treated aviremic and untreated viremic patients after stimulation with anti-CD3/CD28.

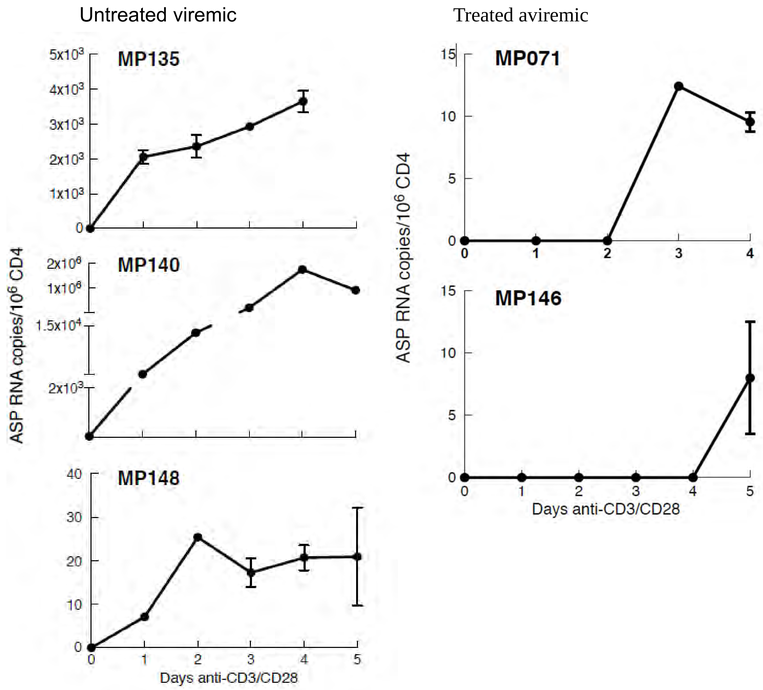


Figure 1. Quantitative analysis of ASP RNA expression in CD4+ T cells from viremic and aviremic patients after stimulation with anti-CD3/28.

- Similar patterns of expression of ASP and env RNA can be observed in one untreated, viremic patient.

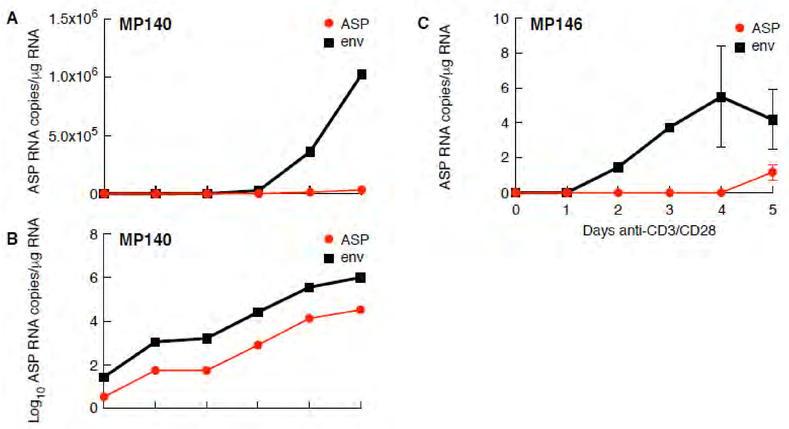


Figure 2. qPCR of ASP and env RNA in CD4+T cells stimulated with anti-CD3/CD28 in untreated (MP140) and treated (MP146) patients.

- ASP RNA transcription is inhibited by low doses (20 ug/ml) of α-amanitin.

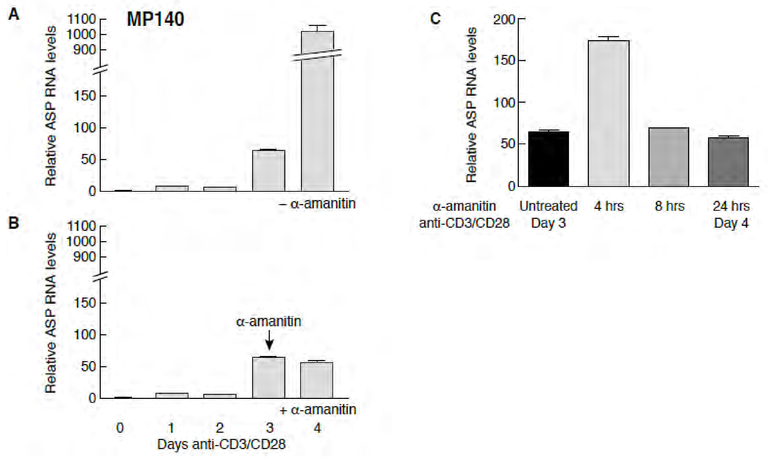


Figure 3. Inhibition of ASP expression by α-amanitin in anti-CD3/CD28-stimulated CD4+ T cells from one untreated, viremic patient. A) Time-course of ASP expression in absence of α-amanitin; B) Inhibitory effect α-amanitin at Day 3 post-stimulation; C) Time-course of ASP RNA inhibition.

Conclusions

- Infected CD4+ T cells can express ASP regardless of the stage of the disease.
- In order to express ASP cells need to be stimulated. Even cells from ART-treated patient are able to express ASP, although to a lesser extent than cells from untreated patients.
- The length of ASP ORF is variable due to a shift of the stop codon upstream or downstream its canonical position in HXB2.
- In untreated patients, patterns of expression of ASP and env are very similar, with levels of ASP RNA markedly lower than those of env.
- ASP expression is inhibited by low doses of α-amanitin, suggesting that it is likely associated to RNAP II, the enzyme responsible for mRNA synthesis during the transcription of protein-coding genes.