Detection of Anti-ASP Antibodies in Sera of HIV+ Individuals

N. 171

[1]Mancarella A., [2]De Crignis E., [3]Corradin G.P., [1]Fenwick C., [1]Perreau M., [1]Pantaleo G., and [1]C. Graziosi

[1]Division of Immunology and Allergy, Lausanne University Hospital, Switzerland, [2]Department of Biochemistry, Erasmus Medical Center, Rotterdam, The Neetherlads, [3]Department of Biochemistry, University of Lausanne, Switzerland

Background

HIV-1 Antisense protein (ASP) is an antisense open reading frame (ORF) encoding for a putative protein of 20 kDa encompassing env at the junction gp120-gp41. Although initially suggested in 1988 (1), the existence of the ASP protein remains controversial. Previous studies (2) show that sera from HIV+ individuals can recognize ASP, suggesting actual production of this protein during infection. In these experiments however, the same sera were used for both immunoprecipitations and immunoblots. This, together with the fact that immune response against defective ribosome products has been reported for HIV+ patients, may have allowed for cross-reactivity leading to false positives. In this study we report the detection of anti-ASP antibodies in sera of HIV patients by ELISA assay.

Methods

Anti-ASP antibodies against 7 overlapping synthetic peptides (P1 to P-7) spanning the whole ASP ORF were determined by ELISA in sera of 45 HIV+ individuals. 96-well flat-bottom plates were coated overnight with the synthetic peptides (1 peptide/well). Sera were diluted 1:200 prior to screening. Goat anti-human AP-conjugated secondary antibodies were diluted 1:2000. Results are the mean optical density (OD) of duplicate wells. Threshold for positivity was calculated as the mean OD plus trice the standard deviation (dotted lines represent the cut-off values) of 20 HIV- donors. GraphPad Prism was used for statistical analyses. Differences between groups were analyzed by the Mann-Whitney U test. Correlation between variables were examined using Spearman's rank correlation analysis ($p<0.05$).

Results

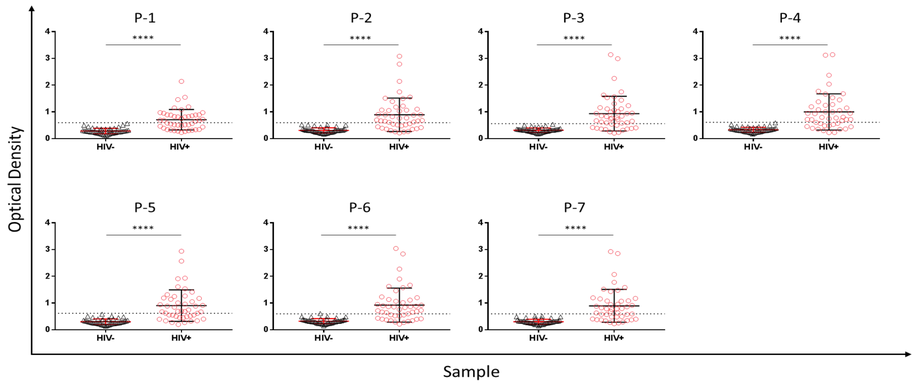
Anti-ASP Antibodies are Present in Serum of HIV+ Individuals. Antibody response against all seven peptides (P-1 to P-7) was detected in the majority of the HIV-infected patients (Figure 1). Specifically, 24 patients (53.3%) showed immunoreactivity against P-1, 29 patients (64.4%) showed immunoreactivity against P-2, P-5, P-6 and P-7, 32 patients (71.7%) showed immunoreactivity against P-3 and 30 patients (66.7%) showed immunoreactivity against P-4 (Table 1).

Anti-ASP Antibodies are Mostly Found in Patients Infected With Subtype B. The majority (≥ 60%) of the patients in which an immune response to ASP was detected were infected with HIV-1 subtype B. The remaining patients were infected with AG and BG recombinants. Although the synthetic peptides were indeed derived from HXB2, the reference sequence for clade B, this finding is consistent with previous studies on the distribution and conservation of the ASP ORF in the various subtypes (3) (Table 1).

Anti-ASP Antibodies Correlate Directly With Levels of Viremia. Anti-ASP antibodies were found to directly correlate with the presence of detectable viremia. High OD values corresponded to patients characterized by high viremia, whereas ODs around or below cut-off corresponded to patients with low to undetectable viremia (Figure 2).

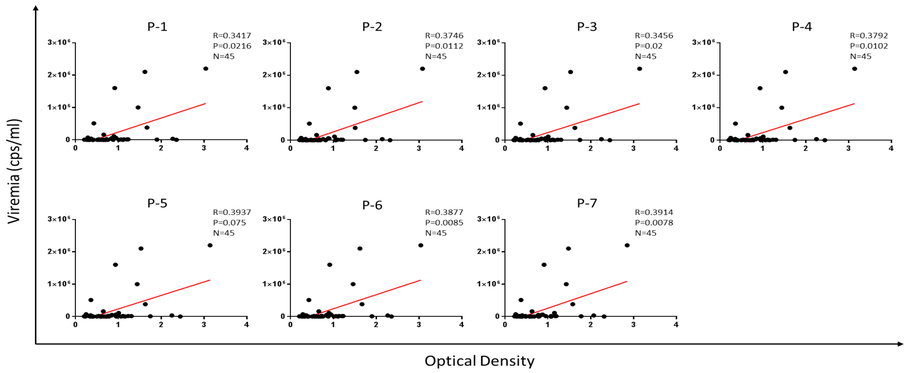
An Inverse Correlation Is Found Between Anti-ASP Antibodies and Years of ART. Interestingly, in patients undergoing suppressive antiretroviral therapy, an inverse correlation between the OD and the years of ART was found, so that the longer the patient had been in therapy, the weaker the antibody response to ASP (Figure 3).

Figure 1. Detection of Anti-ASP Antibodies in Sera From HIV Patients.



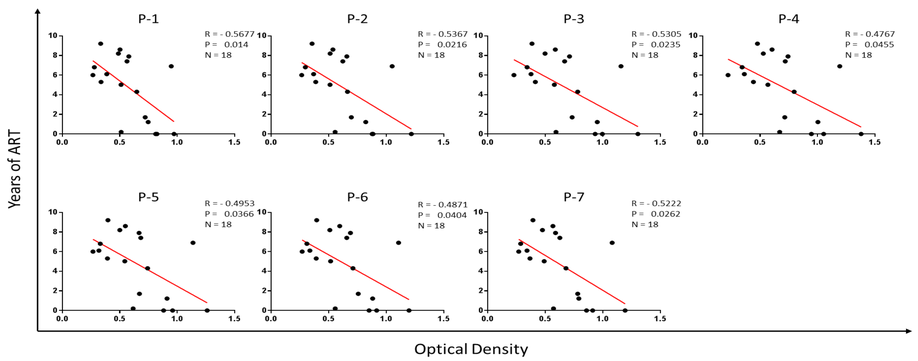
Comparison of ASP-specific antibody response against 7 overlapping peptides spanning the whole ASP ORF length, in serum of 45 HIV+ patients and of 20 HIV- individuals by Mann-Whitney U test ($p<0.05$).

Figure 2. Correlation Between Anti-ASP Abs and Presence of Viremia



Direct correlation between viral load and ASP-specific humoral response in 45 HIV-infected individuals, by Spearman's correlation test ($p<0.05$)

Figure 3. Inverse Correlation Between Anti-ASP Abs and Years of Therapy



Inverse correlation between years of antiretroviral suppressive therapy and anti-ASP antibody response in serum of 18 HIV-infected individuals undergoing ART, by Spearman's correlation test ($p<0.05$)

Table 1. Immune Response to Individual ASP Synthetic Peptides

Peptide	Ab+ patients (N)	Ab+ patients (%)	Subtype B (%)
P-1	24/45	53.3	58.3
P-2	29/45	64.4	62.07
P-3	32/45	71.7	65.63
P-4	30/45	66.7	63.33
P-5	29/45	64.4	65.52
P-6	29/45	64.4	65.52
P-7	29/45	64.4	75.86

Relative number of clade B-infected patients with OD above cut-off for each of the 7 synthetic peptides analyzed

Conclusions

- Anti-ASP antibodies against each overlapping peptide are present in sera of HIV+ individuals.
- The majority (≥60%) of patients in which a humoral antibody response against ASP is detectable are infected with HIV-1 subtype B.
- A direct correlation exists between the presence of anti-ASP antibodies and detectable viremia.
- Anti-ASP antibodies correlate inversely with the years of suppressive antiretroviral therapy.
- The fact that antibodies can be elicited against each overlapping peptide strongly indicates that the observed immune response may be triggered by the whole protein and not by incomplete ribosome products.
- Disappearance of the ASP-specific response during suppressive therapy is consistent with ART impacting the longevity of the HIV-specific antibody response, strongly suggesting that a functional ASP protein is indeed produced during HIV infection.

References

1) Miller, R.H., Human immunodeficiency virus may encode a novel protein on the genomic DNA plus strand. Science, 1988. 239(4846):1420-2.

2) Vanhee-Brossollet, C., et al., A Natural Antisense Rna Derived from the Hiv-1 Env Gene Encodes a Protein Which Is Recognized by Circulating Antibodies of Hiv+ Individuals. Virology, 1995. 206(1):196-202.

3) Cassan, E., et al., Concomitant emergence of the antisense protein gene of HIV-1 and of the pandemic. Proceedings of the National Academy of Sciences of the United States of America, 2016. 113(41):11537-11542.