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Expansion of Naive and Central Memory CD4 T Cells, and Decreases levels of Immune Activation, Apoptosis and Coreceptor Expression

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ABSTRACT

Background: Enfuvirtide (ENF) is a synthetic peptide that binds to HIV-1 gp41, blocking the fusion of viral and cellular membranes. While its potent antiviral activity against HIV-1 has been demonstrated both *in vitro* and *in vivo*, its impact on T cell homeostasis and immune activation is unknown. We report longitudinal immunological analyses of T cells in patients treated for 12 months with ENF as salvage therapy.

Methods: 18 HAART-experienced patients with multidrug resistant virus received ENF (90 mg, SC, bid) in combination with at least three ARV drugs, according to genotype testing. Median ENF treatment duration was 6 months (range: 1-12 months). At baseline (BL), median CD4 T cells was 290 cells/ml and median VL $4.40 \pm 1.59 \log_{10}$ copies/ml. VL decreased to $2.49 \pm 0.71 \log_{10}$ copies/ml, 2.56 ± 1.17 and $2.42 \pm 1.33 \log_{10}$ copies/ml after 4-, 12 and 24 weeks of therapy respectively (VL < 50 copies/ml for 27.7% and 30% of the patients at W12 and W24 respectively). Considering all patients, the mean increase in CD4 T cells was 97 cells/ml at W24 and 112 cells/ml at W48 of therapy. For 9 of these patients, blood samples were collected at BL, W4, W12, W24 and W48 and phenotyping of naive (N), central memory (CM), effector memory (EM) and effector (E) CD4 and CD8 T cells, expression of activation markers, and expression of HIV co-receptors were analyzed on total blood by multicolor FACS analysis (mAbs specific for CD45RA, CD25, CD28, CD45RO, CD38, HLA-DR, CCR5, CXCR4). T cell priming for spontaneous apoptosis was tested in a short-term culture in the absence of stimulus and cell death was quantified with the annexin-V/PI assay.

Results: Responders to ENF (7/9 patients) (VL reduction > 1 \log_{10} copies/ml at W4 of therapy) exhibited a rapid and sustained increase in N and CM CD4 and CD8 T cells. This was concomitant with a reduction in the priming for apoptosis in these subsets. Moreover, a significant decrease in the activation level of these subsets was observed, as shown by the diminished expression of the activation markers CD38 and HLA-DR. Interestingly, virological response to ENF was found associated with the decrease in the number of CD4 T cells expressing CCR5, and CD4 T cells expressing CXCR4 as well.

Conclusions: Virological response to ENF is associated with a rapid and sustained increase in naive and central memory CD4 and CD8 T cells, concomitant with decreased immune activation and apoptosis. In addition, ENF therapy induces a reduction in the number of T cells expressing HIV co-receptors.

Table 1: Characteristics of the Study Population

Patient	Sex	BL (CD4)	BL (VL)	Start ENF (W)	Completed HAART
1 (F)	M	7	4.40	1	2NRTI + 1PI
2 (M)	M	82	4.00	1	2NRTI + 1PI
3 (M)	M	388	4.05	1	2NRTI + 1PI
4 (M)	M	187	4.30	1	2NRTI + 1PI
5 (M)	M	88	4.30	1	2NRTI + 1PI
6 (M)	M	62	4.15	1	2NRTI + 1PI
7 (F)	F	152	3.34	1	2NRTI + 1PI
8 (M)	M	243	1	1	2NRTI + 1PI
9 (M)	M	232	0.1	1	2NRTI + 1PI
10 (M)	M	210	0.1	1	2NRTI + 1PI
11 (M)	M	291	0.14	1	2NRTI + 1PI
12 (M)	M	244	0.16	1	2NRTI + 1PI
13 (M)	M	339	0.05	1	2NRTI + 1PI
14 (M)	M	127	4.16	1	2NRTI + 1PI
15 (M)	M	324	3.34	1	2NRTI + 1PI
16 (M)	M	194	4.1	1	2NRTI + 1PI
18 (M)	M	67	5.09	1	2NRTI + 1PI

This study included 18 male patients who documented resistance to all classes of antiviral drug. The median age was 43 years (range, 17–57), average duration of HIV-1 infection 14.4 years (range, 1–20), and they were multi-class experienced: mean of 8.4 drugs regimen. One patient was therapy naive and had a history of an AIDS defining event. Background regimen included a median of two antiretroviral drugs (range, 2–4) and for 10 patients 2NRTI and 1PI. Median duration of ENF therapy was 6 months.

INTRODUCTION

Enfuvirtide (ENF) is a synthetic peptide that binds to HIV-1 gp41, blocking viral entry into targets by inhibiting the fusion of viral and cellular membranes. When added to background antiretroviral therapy, ENF significantly decreases viral load and increases CD4 T cell counts in patients with previous treatment failure (1). While its potent antiviral activity against HIV-1 has been demonstrated both *in vitro* and *in vivo*, its impact on T cell homeostasis and immune activation is less known.

The state of chronic immune activation induced by HIV infection is thought to be an important factor of disease progression, contributing to the priming of patient's lymphocytes for premature apoptosis, thus inducing continuous T cell depletion (2). In non pathogenic models of HIV-infection, lack of chronic immune activation correlates with the resistance of T cells to spontaneous and activation-induced apoptosis (3). In addition, blocking viral replication and reducing immune activation by HAART in HIV-1 infected patients results in lymphocyte survival and partial normalization of T cell homeostasis (2). Therefore, the question addressed in the present study is whether the antiviral activity of ENF is associated with the reduction of both immune activation and apoptosis on CD4 and CD8 T cells at various stages of maturation. In addition, the influence of ENF administration on the expression of co-receptors CCR5 and CXCR4 has been analyzed.

STUDY DESIGN AND PATIENTS

Eighteen patients attending the Archet Hospital in Nice (France), starting ENF combined with HAART, were recruited for this study. Written informed consent was obtained from all participants. Virological and immunological status of patients was monitored at W0, W4, W12, W24 and W48. Clinical characteristics of the patients are shown in Table 1.

Identification of CD4 and CD8 T cell differentiation stage, expression of the activation markers CD38 and HLA-DR, and HIV coreceptors CXCR4 and CCR5, were analyzed by multicolor FACS on whole blood. Priming for spontaneous apoptosis was measured on PBMC following overnight culture in culture medium and subsequent analysis on a flow cytometer (FACS) following cell staining with annexin-V combined to mAbs identifying CD4 and CD8 naive and memory subsets.

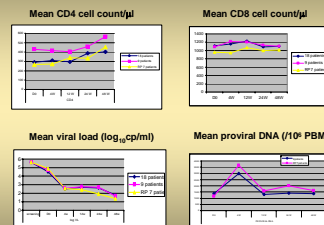
Longitudinal evolution of virological and clinical parameters with ENF

Considering the 18 patients, baseline mean plasma HIV-1 RNA was $4.40 \pm 1.59 \log_{10}$ copies/ml and decreased to 2.49 ± 0.71 , 2.56 ± 1.17 and $2.42 \pm 1.33 \log_{10}$ copies/ml at W4, W12 and W24 of therapy, respectively. No major changes were observed for CD8 cell count and proviral DNA (Figure 1).

A detailed immunological follow-up was performed for 9 patients. They were separated according to changes in viral load from baseline to week 24 as: RP (responders, n=7) i.e. reduction in plasma HIV-RNA >1.0 log copies/ml, and PRP (poor responders, n=2) i.e. reduction in plasma HIV-RNA <1.0 log copies/ml.

At baseline, median CD4 cell count for the 9 patients was 290.35 cells/ml (range, 7–1944), increase in CD4 cells/ml was 97 at W24 of ENF therapy and 112 at W48.

Figure 1: Longitudinal evolution of CD4 and CD8 counts, VL and proviral DNA in ENF-treated patients



Expansion of naive, and central memory CD4 T cells in RP

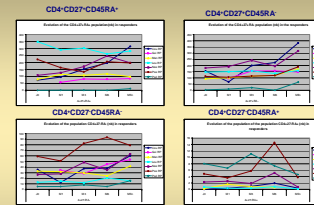


Figure 2: Impact of ENF treatment on longitudinal evolution of naive and memory CD4 T cell subsets.

Decreased spontaneous apoptosis of memory CD4 T cells

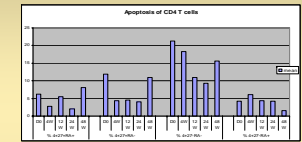


Figure 4: Apoptosis was quantified by FACS in indicated subsets following overnight incubation with or without stimulation of PBMC from RP. Similar data were obtained for CD8 T cell subsets.

Decreased immune activation in CD4 T cells from RP

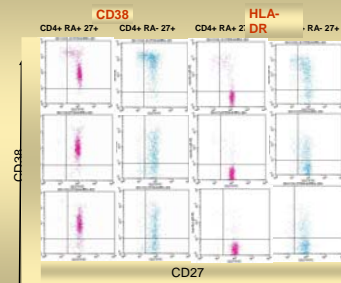


Figure 3: decreased expression of CD38 and HLA-DR on naive and memory CD4 and CD8 T cells

Decreased HIV coreceptor expression with ENF treatment

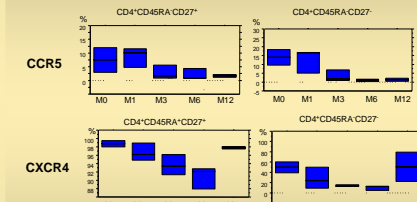
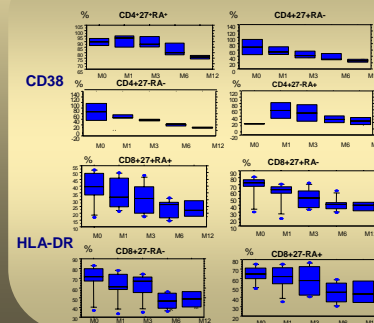


Figure 5: CCR5 and CXCR4 expression on indicated CD4 T cell subsets was analyzed ex-vivo on whole blood from RP patients at indicated time points.

Decreased immune activation in T cells from RP



CONCLUSIONS

This study shows that virological response to ENF is associated with the expansion of naive and central memory CD4 T cells, and a rapid and sustained decrease in T cell activation, as evidenced by the decreased of CD38 and HLA-DR expression in all T cell subsets and a reduction in the level of spontaneous apoptosis. These data confirm and extend previous observations (4).

Interestingly, ENF treatment was found to be associated with a reduction in coreceptor (CCR5 and CXCR4) expression on CD4 T cells, possibly linked to reduced immune activation and/or due to an effect of ENF on HIV entry prior to gp41 interaction.

References

- 1- Lalezari et al. Enfuvirtide, an HIV fusion inhibitor, for drug resistant HIV infection in North and South America. *N Engl J Med* 2003, 13:13.
- 2- Gougeon M-L. Apoptosis as an HIV strategy to escape immune attack. *Nature Rev Immunol* 2003, 3:392-405.
- 3- Gougeon M-L et al. Lack of chronic immune activation in HIV-infected chimpanzees correlates with the resistance of T cells to Fas/Apo-1 (CD95)-induced apoptosis and preservation of a Th1 phenotype. *J Immunol* 1997, 158:2964.
- 4- Barreale et al. Immunological and virological study of enfuvirtide-treated HIV-positive patients. *AIDS* 2004, 18:16783-1682.