

# AUTOLOGOUS NEF RNA HIV-1 ELECTROPORATED DC ENHANCES THE DETECTION OF CD8-SPECIFIC PROLIFERATIVE RESPONSES

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## Abstract

**Background:** Early studies demonstrated that CD8<sup>+</sup> T lymphocytes from HIV-1-infected individuals could suppress HIV-1 replication in autologous CD4<sup>+</sup> T lymphocytes *in vitro* and that CD8<sup>+</sup> responses evoked by NEF gene had the highest inhibition effect on HIV-1 replication. The major problem in the design of a vaccine against HIV is due to the diversity of this virus as well as to the usual techniques to monitor the breadth of CTL epitopes recognized by CD8<sup>+</sup> cytotoxic T cells. The main objective of this study is to compare HIV-specific CD8<sup>+</sup> T-cell immune responses to autologous viral NEF RNA transfected into dendritic cells (DC) and to compare it to reference virus sequences.

**Methods:** We recruited 10 chronically aviremic treated HAART HIV infected patients. We generated autologous NEF mRNA from viral RNA isolated from the patient plasma collected prior to treatment. DCs are then electroporated with RNA encoding viral proteins and induced to mature for 24 hours. DCs transfected with RNA encoding the NEF HIV-1 protein were then co-cultured with autologous PBMCs labelled using CFSE dye for 6 days in ratio 1:40 respectively and after were assessed by flow cytometry. For characteristics of CD8<sup>+</sup> T-cell receptor (TCR) repertoire we used DNA heteroduplex mobility assay (HMA) analysis.

**Results:** Our results indicated that we were able to induce HIV-specific CD8<sup>+</sup> T cell proliferation when we electroporated DCs with the autologous NEF RNA. This proliferation occurred in the absence of any detectable CD4<sup>+</sup> response. We further compared the differences in the proliferative response induced by autologous mRNA to that induced by the reference virus sequences or to consensus peptides.

PBMCs stimulated with autologous NEF mRNA showed a massive proliferative dose-dependent response in all patients tested (higher amount of RNA electroporated increased HIV-specific CD8<sup>+</sup> T cell proliferation). In contrast, a small proliferative response was observed using the reference virus sequences or the consensus peptides. Using HMA for TCR repertoire analysis we estimated a significant increase in total CD8<sup>+</sup> clonal expansion after co-culturing PBMCs with DCs electroporated with autologous NEF RNA compare to reference virus sequences or consensus peptides.

**Conclusions:** We show that the use of autologous NEF mRNA permits the detection of the proliferative capacity of specific CD8<sup>+</sup> T-cells, a significant factor in HIV vaccine design and development.

## Introduction

Control of the worldwide AIDS epidemic requires an effective vaccine. However, the development of an AIDS vaccine has proven a colossal scientific challenge. The major problem in the design of vaccine against HIV-1 is due to the diversity of this virus. Early studies demonstrated that CD8<sup>+</sup> T lymphocytes from HIV-1-infected individuals could suppress HIV-1 replication in autologous CD4<sup>+</sup> T lymphocytes *in vitro*. The main objective of this study is to compare HIV-specific CD8<sup>+</sup> T-cell immune responses to autologous viral NEF mRNA transfected into dendritic cells (DC) and to compare it to reference virus sequences. Our results indicated that we were able to induce an HIV-specific CD8<sup>+</sup> T cells proliferation when we electroporated DC with the autologous NEF mRNA. This proliferation occurred in the absence of any detectable CD4<sup>+</sup> response. In conclusion, we show that the use of autologous NEF mRNA permits the detection of the proliferative capacity of specific CD8<sup>+</sup> T-cells, a significant factor in HIV vaccine design and development.

## Methods

- Isolation viral RNA from plasma and RT PCR.
- DC preparation.
- Autologous viral genes amplification.
- Electroporation DC.
- The intracellular cytokine staining (ICS) technique.
- The 5-(and-6)-carboxyfluorescein diacetate-succinimidyl ester (CFSE)-based proliferation assay.
- Heteroduplex mobility assay analysis of T-cell receptor β-chain repertoires.

Table1. Characteristics of the patients involved in the study

Patient #	Treated	Time between infection and treatment	Time under treatment	Viral load during treatment	Viral load prior treatment (copies/ml)
HTM 330	Yes	50 days	22 months	No	23155
HND 037	Yes	40 days	10 months	No	53725
HDM 011	Yes	85 days	3 months	No	883110
HDM 001	Yes	45 days	30 months	No	883110
HDM 004	Yes	256 days	38 months	No	86221
HDM 007	Yes	195 days	10 months	No	118560
HTM 318	Yes	106 days	30 months	No	1145
HTM 349	Yes	210 days	29 months	No	513000
HND DR 026	No	NA	NA	NA	280000

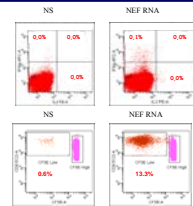


Figure 1. Examples of ICS and CFSE staining of antigen-specific T cells. (A) Example of the IFN $\gamma$  and IL-2 responses from CD8<sup>+</sup> T cells of donor HND DR 026's PBMC, stimulated with DC electroporated by NEF RNA. (B) Example of the % CFSE low CD8<sup>+</sup> T cells detected after 6 days in vitro proliferation with DC transfected by NEF RNA.

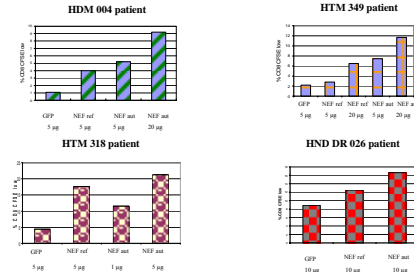


Figure 3. CFSE dilution in CD8<sup>+</sup> T cells following stimulation with DC transfected with autologous NEF mRNA vs reference NEF mRNA.

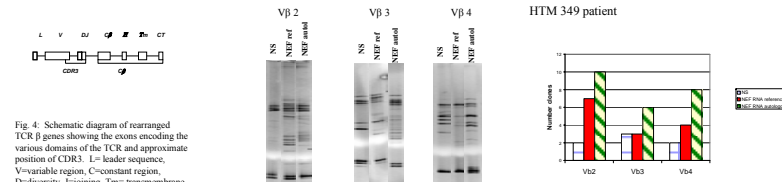


Fig 4. Schematic diagram of rearranged TCR β genes showing the exons encoding the various domains of the TCR and approximate position of CDRE3. L= leader sequence, V=variable region, C=constant region, D=diversity, J=joining, T=transmembrane region, CT= cytoplasmic tail.

Fig 5. The total number TCR clonal expansions in CD8<sup>+</sup> T cells after co-culture with DC transfected by NEF autologous and reference mRNA which was determined using a TCR Vβ chain third complement-determining region (CDR3) DNA heteroduplex mobility assay (HMA).

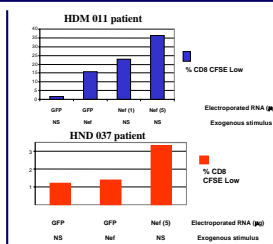


Figure 2. CFSE dilution in CD8<sup>+</sup> T cells following stimulation with DC transfected with RNA encoding GFP or NEF genes.

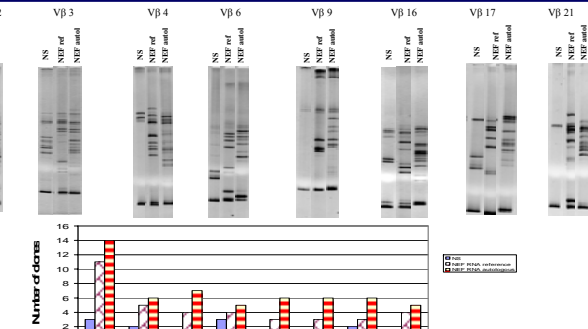
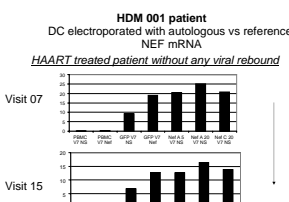


Figure 6. Clonotype analysis of TCR V $\beta$  expression in anti-NEF vaccine-induced CD8<sup>+</sup> specific T-cell responses from HDM 004 patient.

## Results

- We have demonstrated the advantages of using CFSE staining technique for detection of immunopotency of NEF RNA-transfected DC compare to ICS technique (Fig. 1).
- Initial results presented in Fig. 2 indicate for the presence CD8<sup>+</sup> T cells response after co-culture PBMCs with DC transfected by NEF autologous RNA. The level of CD8<sup>+</sup> T cells proliferative responses varied from 2 to 40% in HAART treated HIV-infected patients.
- We shown the increase of proliferation response of CD8<sup>+</sup> T cells after activation with DC transfected by autologous NEF RNA compare to the reference NEF RNA as in HAART treated patients as in untreated HIV-infected individual (Fig.3).
- We detected by HMA the significant clonal expansions TCR in CD8<sup>+</sup> T cells after stimulation with autologous NEF RNA compare to reference RNA(Fig.5-6).

## Conclusions

- Most of patients used are able to show a significant HIV-specific CD8<sup>+</sup> responses when cultured in presence of DC transfected by NEF autologous RNA.
- PBMC stimulated with DC electroporated with autologous HIV-1 NEF mRNA showed a massive CD8<sup>+</sup> T cells proliferative dose-dependent response when compared to that induced by the reference NEF RNA in all patients.
- The breadth of repertoire of CD8<sup>+</sup> T-cell receptors is increasing after co-culture with DC electroporated with autologous NEF mRNA.