



Interleukin-15 Enhances the Natural Killer Activity according to HAART Suppression of HIV-1 Replication



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ABSTRACT

Background: NK cells are important effectors of innate immune response with antiviral functions. Many evidences have been reported about natural killer (NK) frequency and cytotoxicity impairment in HIV-infected subjects and the partial normalization during highly active antiretroviral therapy (HAART). In a previous study we have reported the decrease of production of IL-15 by PBMC in naive and in HAART treated viremic patients. On the contrary, in patients with response to HAART IL-15 production was comparable to healthy donors. In present study we have evaluated the effect of exogenous IL15 administration on NK cytotoxicity in HIV HAART treated patients with suppressed or detectable plasma RNA.

Results: NK cell cytolytic activity was increased by IL-15 stimulation as well in Group B patients (medium =24.5 LU₂₀/10⁷ ; medium+IL-15 =125.8, p<0.0005) as in Group A patients (medium = 27.8 LU₂₀/10⁷ ; medium+IL-15 = 70.6 p<0.0005). No significative increase was observed in Group C subjects. We have compared the ratios of LU₂₀/10⁷ (medium+IL-15 LU₂₀/10⁷ / medium LU₂₀/10⁷) of HAART treated patients and we observed that IL-15 was able to induce a stronger stimulation in Group B patients respect the Group A ones (p<0.02).

Conclusions: Our data suggest that IL-15 is capable of priming cytolytic activity of NK cells in HIV infected patients during HAART suggesting a possible role in immune therapy. In particular the observed high values of IL15 primed NK cells LU₂₀/10⁷ in viremic HAART patients underline the ability of IL-15 in abolishing the natural immune impairment despite HIV replication.

INTRODUCTION

NK cells are important effectors of innate immune response with antiviral functions.

Many evidences have been reported about natural killer (NK) frequency and cytotoxicity impairment in HIV-infected subjects despite the presence of normal NK numbers and the partial normalization during highly active antiretroviral therapy (HAART).

In a previous study we reported the decrease in IL-15 production by PBMC in naive and HAART treated viremic patients. On the contrary, in virologic responders to HAART IL-15 production was comparable to that observed in healthy donors.

In the present study we have evaluated the effect of exogenous IL15 administration on NK cytotoxicity in HIV positive HAART treated patients with suppressed or detectable plasma viremia.

METHODS

Study population:

19 HAART treated patients with RNA <1.7 log (Group A); 15 HAART treated viremic patients (3.4 log mean RNA; Group B); 12 HIV negative healthy donors (Group C). Epidemiologic and clinical dat are reported in table 1

TABLE 1

	AGE (years)	SEX f/m	PRE HAART RNA (mean log copies/ml)	PRE HAART CD4 (mean cells/mm ³)	HAART duration (mean months)	HIV RNA (mean log copies/ml)	CD4 (mean cells/mm ³)
GROUP A: HAART SUPPRESSED (n. 19)	45.2	9/10	4.96	424.4	37.5	1.69	550
GROUP B: HAART VIREMIC (n. 15)	42.4	6/9	5.116	305.6	48.84	3.42	435
GROUP C: HEALTHY CONTROLS (n. 12)	46.3	5/7	-	-	-	-	820

Nk activity assay:

PBMC were cultured at concentration of 2x10⁶ cells/ml over night with medium alone and in presence of recombinant IL-15 (10 ng/ml, PeproTech, Rocky Hill, New Jersey, USA). The cell line used as target cells in Cytotoxicity assay was K562, an erythroleukemic cell line. K562 CELLS were radiolabelled by incubating cell pellets with 100 uCi of 51Cr-sodium chomate (NEN/DuPont Canada) at 37°C for 1 hr, with intermittent shaking. After washing four times, 5,000 radiolabelled target cells were cocultured in triplicate with PBMC at EFFECTOR-TARGET ratios ranging from 3.5:1 to 100:1 in the wells of a U-bottomed microculture plate in a total volume of 200 ul.

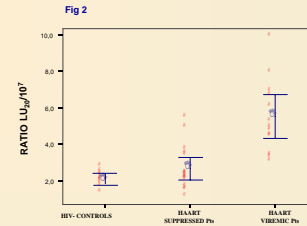
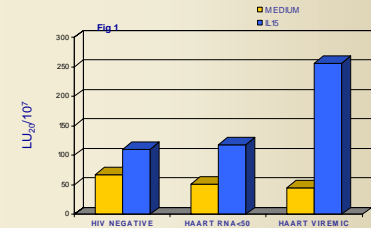
The plates were incubated for 4h and supernatant radioactivity was measured in a gamma counter (Topcount, Packard).

Results are expressed as LU₂₀ (Lytic Units) calculate like the number of effector cells required to lyse the 20% of 5000 target cells; the number of LU contained in 10⁷ effector cells represent the LU₂₀/10⁷.

RESULTS

NK cell cytolytic activity in the absence of cytokine stimulation was 68.7 LU₂₀/10⁷ in HIV- control subjects, 51.6 LU₂₀/10⁷ in HAART patients with RNA <50 copies/ml and 45.5 LU₂₀/10⁷ in viremic HAART subjects. HIV negative controls showed NK activity comparable to HIV infected patients with suppressed viremia; the difference respect to HAART viremic patients showed a trend towards statistical significance.

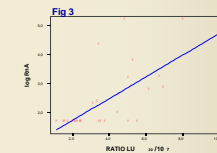
Priming PBMCs with IL-15 induced an increase of NK activity respect to the Medium condition in all three groups of subjects: 110.5 LU₂₀/10⁷ in HIV negative donors, 118.4 LU₂₀/10⁷ in HAART patients with RNA <50 copies/ml and 256.5 LU₂₀/10⁷ in viremic HAART subjects. For the last two groups the increases were statistically significant (p<0.01). Fig 1



To verify if the IL-15 induced increment of NK activity was similar in the three study groups, we have compared the mean ratios of LU₂₀/10⁷ (medium+IL-15 LU₂₀/10⁷ / medium LU₂₀/10⁷). Fig 2

The mean of LU₂₀/10⁷ ratio was similar in HIV negative controls and in HAART suppressed patients. IL-15 was able to induce a stronger NK activity stimulation in HAART viremic patients than in aviremic ones (p<0.02).

In Fig 3 the HIV-RNAcopies/ml (log) versus LU₂₀/10⁷ ratios for HIV patients are plotted. Despite the presence of RNA values <50 the Pearson Correlation test has shown a significant positive correlation between plasma viremia and IL-15 ability to augment the NK cytolytic activity (p<0.01)



CONCLUSIONS

Our data confirm that in HIV infected patients with suppressed levels of plasma viremia there is no significant difference in cytolytic activity of NK cells in comparison to the activity in HIV negative controls.

IL-15 is capable of priming cytolytic activity of NK cells in PBMC from HIV infected patients during HAART suggesting a possible role of this cytokine in immune therapy.

In particular the observed high LU₂₀/10⁷ ratio values in viremic HAART patients underline the ability of IL-15 to abolish the impairment of NK activity in the presence of HIV replication. The ability of IL-15 to induce an increase in NK activity seems to be positively correlated to RNA plasma viremia.