Interleukin-32: Expression, Interaction with IFN and Clinical Significance in HIV-1-Infection

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INTRODUCTION

Interleukin-32 (IL-32) is a multi-isoform cytokine (IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε and IL-32ζ) that has received growing attention as an important component in numerous autoimmune and inflammatory disorders. Recent reports have highlighted that IL-32 is regulated during viral infections. Few studies have investigated the role of IL-32 in HIV-1 infection. Elevated serum levels of IL-32 and increased IL-32 expression in both gut and lymphatic tissue were found in patients infected with HIV-1 compared to healthy individuals. In addition, Nold and colleagues reported that IL-32 interferes *in vitro* with viral function. However, IL-32 has also been viewed as a double-edged sword during HIV-1 infection, suppressing immune activation and the antiviral immune response, thereby supporting HIV-1 replication and viral persistence. Moreover, Zepp et al. showed that the IL-32 promoter contains consensus sequences recognized by IFN regulatory factors; although the functionality of these sites remains to be determined The relationship between IL-32 and the IFN system has been further complicated by the demonstration that elevated IL-32 levels during viral infection mediate antiviral effects by stimulating the expression of IFNλ1, which belongs to the type III IFNs.

AIM

This study aimed to characterize both the anti-HIV properties of IL-32 and the cytokine's relationship with host antiviral innate immune responses.

Table 1. Demographic and clinical characteristics of chronically

Item*	HIV-1 infected patients (n=83) Value	Healthy donors (n=36) Value	*p-value*
Male gender	61/83 (73.49%)	20/36 (55.55%)	0.087
Age (years)	40.71 ± 11.03	44.86 ± 15.21	0.097
Virus subtype	B		NA**
HIV-RNA (copies/ml)	33590 (143 – 1,405,000)		NA
$CD4^+$ count (cells/mm^3)	450 (22-1,200)		NA

*Data are expressed as mean ± SD, median (range) or percentage. Differences in demographic characteristics between HIV-1 infected patients and HIV-seronegative healthy individuals were evaluated using Student's t and Chi-squared tests. **NA=not applicable.

PATIENTS AND METHODS

Patients

Peripheral blood samples from 83 HIV-1-infected patients of both genders attending the Policlinico Umberto I Hospital in Rome over the seven year period 2005–2012 were included in this study. All patients were infected with the HIV-1 subtype B strain and were naïve for antiretroviral treatment. No patients had any concurrent acute illness or infection including CMV disease, mycobacterium tubercolosisis, hepatitis B and C virus infections. Blood samples from 36 gender- and age-matched HIV-seronegative healthy individuals were also included in this study to compare the gene expression of IL-32 (α and nonα) isoforms in HIV-1 positive patients and healthy subjects (Table 1).

TaqMan-based real-time RT-PCR technique for mRNA expression evaluation

mRNA levels of IL-32α, IL-32nonα, MxA, APOBEC3G and APOBEC-3F were assessed by quantitative RT-real-time PCR using the LightCycler480 instrument (Roche, Basel, Switzerland). Briefly, total RNA was extracted from PBMC collected from HIV-1 positive patients and healthy individuals using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol, and was retro-transcribed as previously specified. The housekeeping gene β-glucuronidase was used as an internal control. Gene expression values were calculated by employing the comparative threshold cycle (Ct) method.

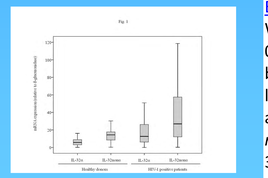
Ex vivo treatment of PBMC with IL-32γ protein

PBMC from five healthy donors were isolated by density centrifugation using Ficoll/Hypaque (Sigma-Aldrich, St. Louis, MO, USA). After removal of the mononuclear cell layer, PBMC were washed twice with cold PBS, resuspended in RPMI 1640 medium supplemented with 10% FCS (heat-inactivated), 0.5% gentamycine and 1% L-glutamine and then seeded in six-well plates in a 1.0 ml volume. PBMC were either untreated or treated with 100 ng/ml of rIL-32γ (R&D Systems, Minneapolis, MN, USA) or 1000 international units (IU)/ml of IFNα2b (Schering-Plough, Kenilworth, NJ, USA) for 24 h at 37°C/5% CO_2. Subsequently, PBMC were harvested by centrifugation and pellets were immediately used for RNA extraction and evaluation of MxA, APOBEC3G and APOBEC3F mRNA expression (see above).

RESULTS and CONCLUSIONS

Elevated IL-32 levels among HIV-1-infected individuals

Results indicated that IL-32 (α and nonα) mRNA levels varied widely among untreated chronically HIV-1-infected patients [coefficient of variation (CV) >100)]. Furthermore, as shown in Fig.1, mRNA levels of both IL-32α and IL-32nonα were significantly higher in chronically HIV-1-infected patients than those measured in the control group (IL-32α: p=0.018; IL-32nonα p<0.0001). The IL-32nonα mRNA levels were also higher than those of IL-32α in both chronically HIV-1-infected patients and healthy individuals (HIV positive patients: p<0.0001; healthy donors: p<0.0001). In addition, we found that age and gender were not associated with transcript levels of IL-32α and IL-32nonα in both groups analyzed (data not shown).



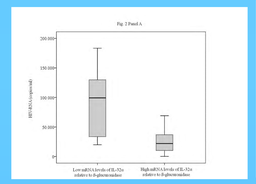
Evaluation of the relationship between IL-32 mRNA levels and plasma viral load or $CD4^+$ T cells count

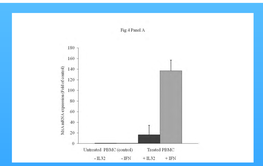
We observed a negative correlation between the gene expression of IL-32 isoforms (α and nonα) and HIV-RNA levels measured in plasma (IL-32α: p=0.002, r= -0.407; IL-32nonα: p=0.027, r=-0.281). The influence of IL-32 isoforms on the viral load was also appreciated dividing the chronically HIV-1-infected patients on the basis of the median IL-32 (α and nonα) mRNA values (Fig.1). Lower levels of HIV-RNA were observed in chronically HIV-1-infected patients expressing higher mRNA levels of IL-32 isoforms compared to those with lower transcript levels of both IL-32 isoforms (IL-32α: p<0.0001, IL-32nonα: p<0.0001; Fig. 2). We failed to detect any significant association between IL-32 (α and nonα) gene expression levels and $CD4^+$ T count in our studied group (IL-32α: p=0.534, r=0.076; IL-32nonα p=0.175, r= 0.155). In addition, when we grouped HIV-1-positive patients according to their $CD4^+$ T-cell count, the subset with <200 CD4 cells/mm^3 had similar levels of IL-32α and IL-32nonα with respect to the subsets with CD4 cell counts of between 200 and 600 and above 600 cells/mm^3 (IL-32α: p=0.905 and IL-32nonα p=0.564;

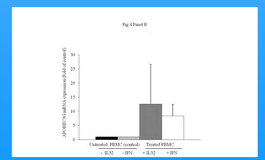
Ex vivo and *in vivo* relationship between IL-32 and the type I IFN pathways

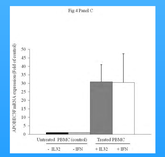
To better characterize the antiviral properties of IL-32 and the cytokine's relationship with the IFN system, we first examined *ex vivo* the ability of IL-32γ to regulate the expression of some ISGs in IL-32γ or IFNα2b treated PBMC from 5 healthy donors .

As expected, all ISGs analysed were significantly induced after IFNα treatment (Fig.4 Panel A and Panel B, MxA: +IFN, p<0.0001; APOBEC3G: +IFN, p<0.0001; APOBEC3F: +IFN, p<0.0001). However, we found that MxA, APOBEC3G and APOBEC3F mRNA levels were also increased after PBMC exposure to IL-32γ (Fig.4 Panel A and Panel B, MxA: +IL-32, p=0.008; APOBEC3G: +IL-32, p=0.018; APOBEC3F: +IL-32, p=0.028).

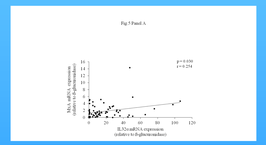


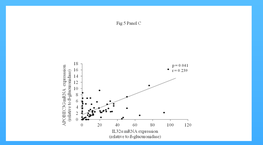


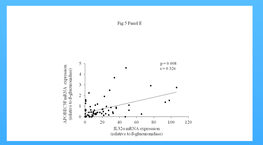


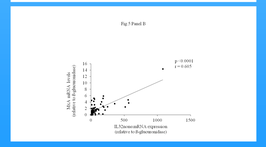


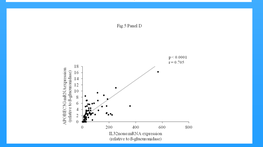
Our results also indicated the existence of weak correlations between transcript levels of IL-32α and those of MxA (IL-32α: p= 0.030, r=0.254) APOBEC3G (IL-32α: p=0.041, r=0.239) and APOBEC3F (IL-32α: p=0.008, r=0.326;) measured in PBMC collected from HIV-1-infected patients (Fig. 5, Panel A, C and E). Differently the transcript levels of IL-32nonα were more strongly correlated to the gene expression of MxA (p<0.0001, r=0.605) APOBEC3G (p<0.0001, r=0.705) and APOBEC3F (p<0.0001, r=0.727) (Fig. 5, Panel B, D and F).

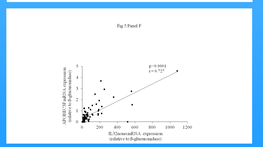












In conclusion, this study provides novel evidence that IL-32 participates in the antiviral immune responses during HIV-1infection *in vivo*. The modulation of IL-32 isoforms on type I and III IFNs might merit further investigation to better understand the host immune response in HIV-1-infected patients.