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Regulation of IL-32 Expression by a Promoter Polymorphism and MicroRNA29b in HIV-1 patients

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INTRODUCTION

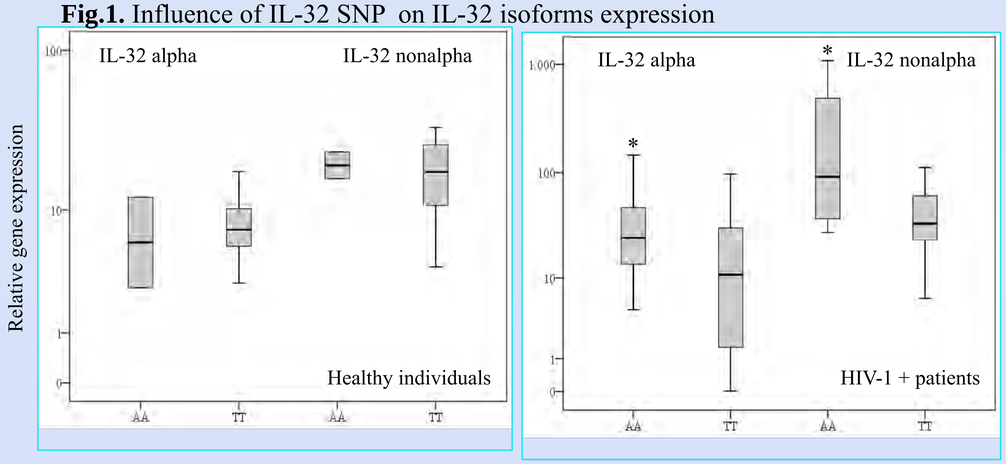
Interleukin-32 (IL-32) is a multi-isoform cytokine that has recently received growing attention as a key component in the antiviral immune response to HIV-1 infection. We previously demonstrated that IL-32 isoforms are highly expressed during HIV-1 infection and that IL-32 is associated with low levels of viremia and can increase the expression of well-established type I IFN induced myxovirus resistance protein 1 (MxA) and apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) 3G and APOBEC3F (Monteleone K et al., Med Microbiol Immunol 2014). Interestingly, microRNA (miRNA-29b) which belongs to anti-HIV miRNA-29 family, was recently found to be involved in regulating the expression of IL-32 by directly targeting the IL-32 mRNA 3'-untranslated (Li Y et al., J Biol Chem. 2013). Since recent reports highlighted that genetic variations in IL-32 are associated with the outcome of infection-associated acute lung injury (Arcaroli JJ et al., 2011 Crit Care) and epithelial cell-derived thyroid carcinoma (Plantinga TS et al., Carcinogenesis 2013), we have extended our previous analysis evaluating whether an IL-32 promoter polymorphism (rs28372698) modulates the mRNA expression of IL-32 alpha and nonalpha isoforms in HIV-1 positive patients and whether it influences the clinical course of HIV-1 infection. Furthermore, the relationship between miRNA-29b levels and the activation rate of IL-32 immune response was also evaluated

Methods: PBMC samples from 104 untreated HIV-1 infected patients (male/female 82/22; median age: 40 years; median viral load: 46580 HIV-RNA copies/ml and median CD4+T cell count: 355 cells/mm^3) were collected at the Sapienza University Hospital (Rome, Italy). Levels of IL-32 isoforms (alpha and nonalpha) mRNA, MxA-mRNA, APOBEC3G/3F-mRNA, microRNA29b and rs28372698 (T/A) IL-32 promoter single nucleotide polymorphisms (SNPs) were evaluated by using distinct TaqMan assays.

RESULTS

1) The genotypes frequencies (%) observed in healthy individuals and in naïve HIV-1 positive patients were the following: healthy donors: AA 5.9, AT 55.9, TT 38.2; naïve HIV-1 positive patients: AA 13.58, AT 58.02, TT 28.4 From the comparison of observed frequencies, no statistically significant difference in the distribution of genotypes was revealed (AA p=0.285; AT p=0.806; TT p=0.805). Our results also showed that transcript levels of both IL-32 alpha and IL-32nonalpha were not different among healthy subjects carrying different IL-32 SNP genotypes (AA vs TT: IL-32 alpha: p=0.734;IL-32 nonalpha: p=0.734). In contrast, we found that IL-32rs28372698 SNP influences IL-32 expression levels in HIV-1 positive patients, suggesting that IL-32 expression could be differently modulated by a genetic host factor during HIV-1 infection (**Fig.1**). In particular HIV-1 positive patients harbouring AA genotypes express higher IL-32 (alpha and nonalpha) isoforms levels compared to those carrying TT genotypes (AA vs TT: IL-32 alpha: p<0.05;IL-32 nonalpha: p<0.05) (Fig.1). We observed a two-fold reduction of HIV RNA levels in HIV-1 positive patients homozygous for A allele compared to patients carrying TT genotype; even though this difference was not statistically significant (**Table 1**, p>0.05). No differences were associated with CD4+ T-cell count (**Table 1**, p=0.268).

Fig.1. Influence of IL-32 SNP on IL-32 isoforms expression

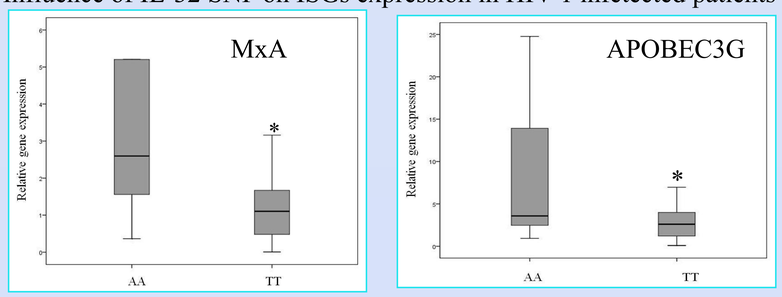


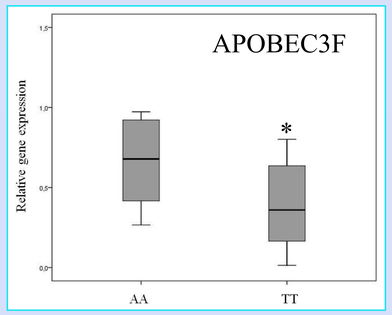
Furthermore, we observed that patients expressing higher levels of IL-32 (AA genotype) also expressed higher levels of MxA (p=0.015) and APOBEC3F (p=0.025) and APOBEC3G (p=0.070), confirming that an axis between IL-32 and IFN signalling pathways does exist (**Fig.2**)

Table 1

	Median value CD4 +T cell count (cells/mm^3)	Median value HIV-RNA copies/ml
AA	**376**	**35940**
TT	**476**	**59499**
p>0.05		

Fig.2 Influence of IL-32 SNP on ISGs expression in HIV-1 infetected patients





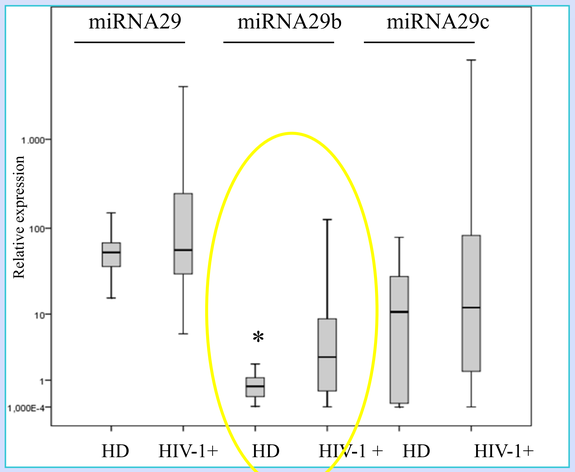
2) To investigate whether the transcript levels of miRNA-29b affect IL-32 expression and, in turn, the antiretroviral response, we measured IL-32 (alpha and nonalpha isoforms) mRNA levels in PBMC collected from HIV-1-infected patients naïve for antiretroviral treatment. Interestingly, we found an inverse weak correlation between miRNA-29b and IL-32nonalpha levels in HIV-1 positive patients (p=0.028; r=-0.298) whereas no significant correlation was found with IL-32 alpha levels (**Table 2**). Furthermore, we found that among miRNA29 family, only miRNA29b was more expressed in HIV-1 + patients compared to healthy controls (**Fig. 3**, p<0.0.5).

Table 2 Relationship between miRNA-29b expression and IL-32 isoforms levels

Item	miR-29b	
	p	*r*
IL-32α	0.318	-0.140
IL-32nonα	**0.028**	**-0.298**

* Spearman's rho test; significant correlations are highlighted in bold
The analysis was performed in 58 HIV-1 + patients

Fig. 3 Comparison of miR-29a/b/c levels between HIV-1-infected patients and gender- and age-matched healthy donors (HD)



CONCLUSION

Our results indicate that the expression of anti HIV-1 IL-32 isoforms are influenced both by the presence of IL32 promoter polymorphism and levels of miRNA-29b, highlighting the importance of host genetic factors and cellular microRNA in the regulation of IL-32 during HIV-1 patients.

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