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IFN-ALPHA RECEPTOR-1 UPREGULATION IN PBMC FROM HCV NAÏVE PATIENTS CARRYING CC GENOTYPE. POSSIBLE ROLE OF IFN-LAMBDA

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Background

A group of recently discovered cytokines (IFN-λ1/interleukin-29 [IL-29], IFN-λ2/IL-28A, and IFN-λ3/IL-28B), assigned to a new type of IFN (type III IFN) gained increased attention in the Hepatitis C virus (HCV) field [1]. Moreover, genome-wide association studies (GWAS) identified several single-nucleotide polymorphisms (SNPs) in IL-28B gene region, that were strongly related to therapy-induced HCV clearance rate in chronic hepatitis C (CHC) patients [2,3,4]. Among the identified SNPs, rs12979860, located 3 kb upstream of the IL-28B gene, appeared as the most relevant, being the rs12979860-favorable CC genotype associated with a more than two-fold increased rate of sustained virologic response (SVR) with respect to hapless (CT or TT) genotypes [5]. However, despite the existing clear evidence of association of IL-28B polymorphisms on spontaneous or therapy-induced resolution of the infection, the mechanisms triggered by these polymorphisms and their real biological consequences remain unexplained, and attract intensive investigation. In a previous study we have shown that reduced expression of the IFN-alpha receptor -1 (IFNAR-1) may represent the biological basis for reduced response to standard of care (SOC) in poorly performing patients [6], such as HIV-coinfected patients, hampering their ability to mount an appropriate response to exogenously administered IFN-α. Moreover, higher expression of IFN-λ has been reported in patients with the rs12979860 CC favourable genotype [3,4].

Aim

The aim of this study was to establish the possible relationships between IL-28B rs12979860 genotypes and expression of IFNAR-1 in naïve HCV patients, and to explore the possible role of IFN-λ.

Results

➢**Levels of IFNAR-1 in PBMC from naïve HCV-infected patients carrying different IL-28B rs12979860 genotypes, and *in vitro* response to IFN-α:** Patients carrying CC genotype showed IFNAR-1 mRNA basal levels significantly higher than patients with CT/TT genotype patients [median values: 1.420 (IQR: 0.875-1.655) vs 0.629 (IQR: 0.504-1.005); p=0.0142]; in addition, a significantly higher expression in CC vs CT/TT genotypes was observed after exposure to IFN-α [median values: 2.220 (IQR: 0.908-3.647) vs 0.6280 (IQR: 0.395-1.522); p=0.0149]. More in detail, the most prominent difference was observed between CC and TT groups, both at basal level **(Fig. 1, Panel A)** [median values: 1.420 (IQR: 0.875-1.655) vs 0.629 (IQR: 0.532-0.925); p=0.0135] and after exposure to IFN-α **(Fig. 1, Panel B)** [median values: 2.220 (IQR: 0.908-3.647) vs 0.461 (IQR: 0.353-1.048); p=0.0373], while between CC and CT groups a borderline significant difference was observed both at basal level **(Fig. 1, Panel A)** [median values: 1.420 (IQR: 0.875-1.655) vs 0.676 (IQR: 0.468-1.137); p=0.0500] and after exposure to IFN-α **(Fig. 1, Panel B)** [median values: 2.220 (IQR: 0.908-3.647) vs 0.840 (IQR: 0.397-1.599); p=0.0500].

➢**Role of IFN-λ on IFNAR-1 mRNA expression in PBMC from healthy donors carrying different IL-28B rs12979860 genotypes:** The median basal levels of IFNAR-1 mRNA in healthy donors were significantly higher than those observed in HCV patients [median values: 6.516 (IQR: 4.888-7.556) vs 0.732 (IQR: 0.393-1.829); p=0.0004], and seemed to be independent from IL-28B genotype. The dose dependent response after 3h exposure to IFN-λ is shown in **Fig. 2**, were up-regulation of IFNAR-1 mRNA was observed in both genotypes, being 10ng/ml the most effective dose. More interestingly, the IFN-λ-driven stimulation was more pronounced in subjects carrying the CC genotype at both 10 (CC mean value: 13.86±0.966; TT mean value: 8.886±0.550; p=0.0209) and 100ng/ml (CC mean value: 13.79±0.955; TT mean value: 7.100±1.290; p=0.0500) of IFN-λ. In **Fig. 3** time- and dose-dependent response from two representative subjects (CC and TT) is shown, confirming 10ng/ml of IFN-λ as optimal dose, and showing, again, a better stimulation in CC genotype. In particular, at 10 ng/ml peak stimulation in CC genotype occurred earlier (12h) and was more extensive than in TT genotype (24h).

Methods

PBMC and plasma samples from 40 treatment-naïve patients with CHC for whom IL-28B genotype at locus rs12979860 was known, were retrospectively selected, in order to include a sufficient number of patients harboring the CC genotype to perform a comparative analysis: 9 CC (22.5%), 18 CT (45%), 13 TT (32.5%). Clinical characteristics of patients are shown in **Table 1**. IL-28B rs12979860 CC/CT/TT genotype was established on genomic DNA, using a custom made TaqMan assay. PBMC from CHC patients were cultured for 3h in the absence or presence of 10^3IU/ml human recombinant IFN-α2b, while PBMC from healthy donors were exposed either to 10ng/ml or 100ng/ml human recombinant IL-28/IFN-λ2 for different time points. Total cellular RNA was extracted by Trizol. The quantification of IFNAR-1 mRNA was performed by a Taqman real-time RT-PCR, using β-actin as housekeeping gene. Basal mRNA levels for IFN-λ were measured in freshly thawed PBMC by quantitative Real-time RT-PCR. Plasma levels of IFN-λ protein were measured by ELISA.

Table 1. Characteristics of 40 treatment naive HCV infected patients included in the study

Age [median(range)]	53 (30-81)
Sex (Male/Female)	30/10
AST [median(range)] IU/L	45 (17-162)
ALT [median(range)] IU/L	62 (13-310)
γ-GT [median(range)] IU/L	40 (9-599)
HCV load [median(range)] Log_{10} IU/ml	6.12 (4.35-6.84)
HCV genotypes (gt1, gt4)	27 gt1, 13 gt4
rs 12979860 IL28B genotypes (CC/CT/TT)	9/18/13

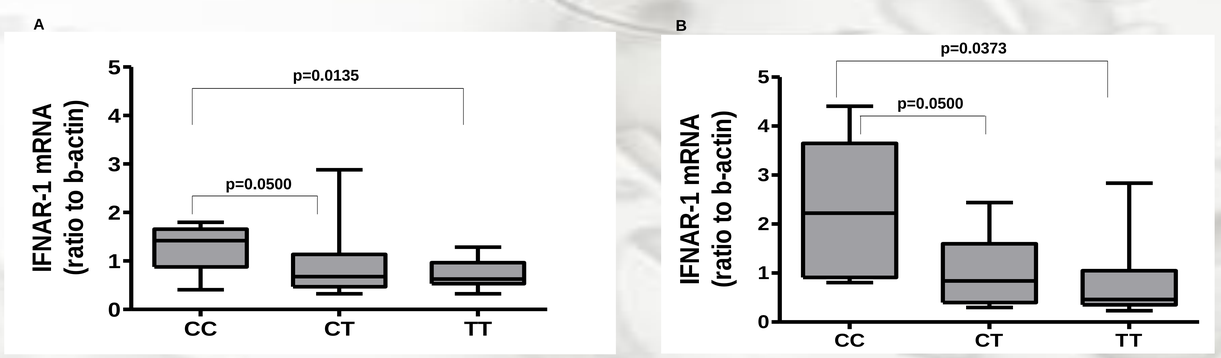

Fig. 1

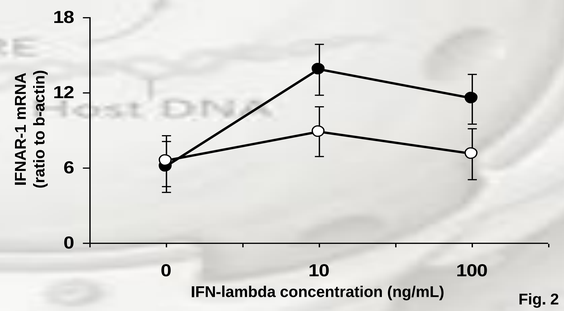

Fig. 2

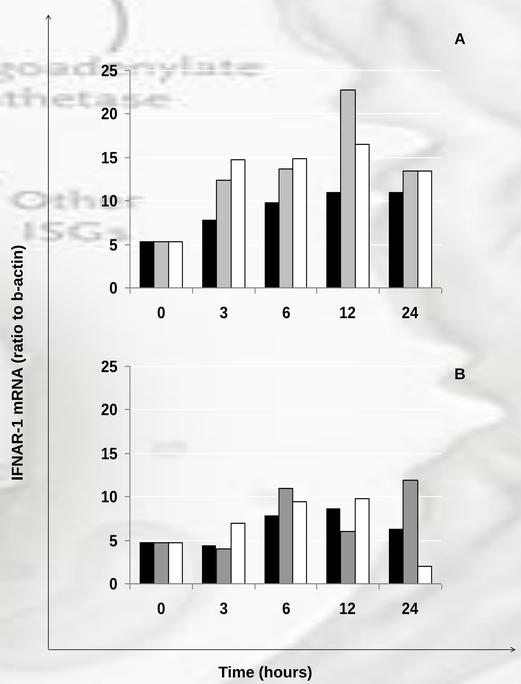

Fig. 3

Conclusion

Our findings suggest that IFN-λ could play a crucial role in the modulation of IFNAR-1 expression, and that endogenous levels of IL-28 may be responsible for partial restoration of IFNAR-1 expression in HCV patients with favourable IL-28B genotype. This, in turn, may confer to CC carriers a response advantage to either endogenous or exogenous IFN-α, representing the biological basis for the observed association between CC genotype and favourable outcome of either natural infection (clearance vs chronicization) or IFN therapy. In summary, although the findings from the present study derive from a limited number of patients and might benefit from larger studies, they provide novel informations, contributing to elucidate the mechanisms underlying the strong predictive value of IL-28B polymorphisms on the natural history and on the response to IFN therapy in HCV infection.

[1] Donnelly RP, Kotenko SV. (2010) Interferon-lambda: a new addition to an old family. J Interferon Cytokine Res 30:555-64
[2] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV et al. (2009) Genetic variation in IL-28B predicts hepatitis C treatment-induced viral clearance. Nature 461:399-401.
[3] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K et al. (2009) Genome-wide association of IL-28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105-9.
[4] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M et al. (2009) IL-28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41:1100-4.
[5] Lange CM, Zeuzem S. (2011) IL-28B single nucleotide polymorphisms in the treatment of hepatitis C. J Hepatol 55:692-701.
[6] Abbate I, Romano M, Longo R, Cappiello G, Lo Iacono O et al. (2003) Endogenous levels of mRNA for IFNs and IFN-related genes in hepatic biopsies of chronic HCV-infected and non-alcoholic steatohepatitis patients. J Med Virol 70(4): 581-7.