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Short communication

IL-21 is associated with natural resistance to HIV-1 infection in a Colombian HIV exposed seronegative cohort

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ABSTRACT

Higher IL-21 levels were associated with natural resistance to HIV infection in an Italian cohort. Thus we wanted to confirm such association in HIV exposed seronegative individuals (HESN) from Colombia. Cells from HESN were less susceptible to infection and expressed higher IL-21 mRNA levels than healthy controls at both baseline and 7-days post-infection; similar results were observed for IL-6, perforin, and granzyme. These results suggest that IL-21/IL-6 increase may be a distinctive quality in the profile of HIV-1 resistance, at least during sexual exposure. However, further studies are necessary to confirm the specific protective mechanisms of these cytokines.

Despite global efforts, there is no cure for Human Immunodeficiency Virus (HIV) infection yet [1]. Interestingly, HIV Exposed Seronegative individuals (HESN) are subjects who despite being repeatedly exposed to the virus, remain HIV seronegative, suggesting the existence of mechanisms that lead to natural resistance [2]. However, these mechanisms are not fully identified yet.

We recently observed an augmented production of IL-21, IL-17, miRNA 29a, b, and c in sexually-exposed HESN from an Italian cohort compared to healthy controls (HC) suggesting that IL-21/miRNA 29 axis is involved in natural resistance to HIV-infection [3]. The mechanisms behind this association could be the suppression of viral replication by direct viral mRNA degradation and by inhibition of cyclin T1; thus the positive transcription elongation factor b (p-TEFb) required for Tat-dependent transactivation of viral gene expression [4,5]. To confirm this association, we evaluate whether the IL-21 axis could be reproduced in a different HESN cohort with the same type of exposition but a different ethnic origin and genetic background.

1. Materials and methods

1.1. Population

Blood samples were collected from 7 HESN and 4 HIV-1 positive partners from a serodiscordant cohort of heterosexual couples recruited at the HIV-1 care program HERES in Santa Marta, Colombia. Inclusion criteria for HESN were: seronegative at the time of inclusion, with a history of unprotected sexual intercourse with HIV positive partners with detectable viral loads, 12 or more unprotected sexual episodes in at least 3 consecutive months within 1 year of study entry.

The 7 HESN included were 83% female with age: mean [range] 36.6 [18–50] years and the 4 HIV-1 seropositive partners were 25% female with age: 27.5 [20–40] years, viral load: 1200 [50–180,790] copies/ml (1 cART naïve, 1 on suppressive cART and 2 on cART with low adherence), CD4 count: 344 [134–804] cells/ul and length of HIV infection since diagnosis: 9.5 [3.6–14.4] years. The couples had an average of 13 unprotected sexual intercourses per month during 6.9 [2–11.5] years, with the last unprotected intercourse taking place between 2 days and 6 months before sampling.

Samples from 7 HC with low risk for HIV infection matched by sex and age with HESN were also included.

The study was designed and conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the hospital involved in the study. After thoroughly explaining

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the project and clarifying any doubt concerning the research, all subjects signed an informed consent, and the biological material collected was anonymized to ensure the privacy of the individuals.

1.2. HIV-1 infections assays

PBMCs obtained by Ficoll gradient were treated with 8 μg/mL phytohemagglutinin and 100 IU/mL IL-2 (Sigma–Aldrich) for 72 h. In vitro infections were done by spinoculation [6] using 13 ng of X4-tropic p24 (obtained from the cell line H9-HTLV-IIIB) with 10 mg/mL polybrene (Sigma–Aldrich) for 3 h. Cells and supernatants were harvested 7 days post-infection for mRNA extraction and p24 quantification (QuickTiter™ Lentivirus-associated p24 ELISA kit).

1.3. Gene expression quantification by real time PCR

RNA was extracted from basal and *in vitro* HIV-1 infected PBMC using Trizol (ZYMO RESEARCH), then was DNase-treated and retro-transcribed (Applied Biosystem™). Gene expression was quantified by real time PCR on a CFX-96 PCR equipment (BIORAD, Hercules) using Maxima SYBR green qPCR master mix (Thermo Scientific) with specific primers for IL-21 (Fwd-5′-TATGTGAATGACTTGGTCCCTAG-3′ Rev-5′-AGGAAAAAGCTGACCACTCACAG-3′), IL-6 (Fwd-5′-ATTCGG-TACATCCTCGAC-3′ Rev-5′-GGGGTGGTTATTGCATC-3′), Perforin (Fwd-5′- CCGCTTCTACAGTTTCCATGT-3′ Rev-5′-GTGCCGTAGTTGGA-GATAAGC-3′), Granzyme (Fwd-5′-CACTGTTGGGGAAGCTCCAT-3′ Rev-5′-TGGGGGATGGGTCTTTTCAC-3′). The relative mRNA

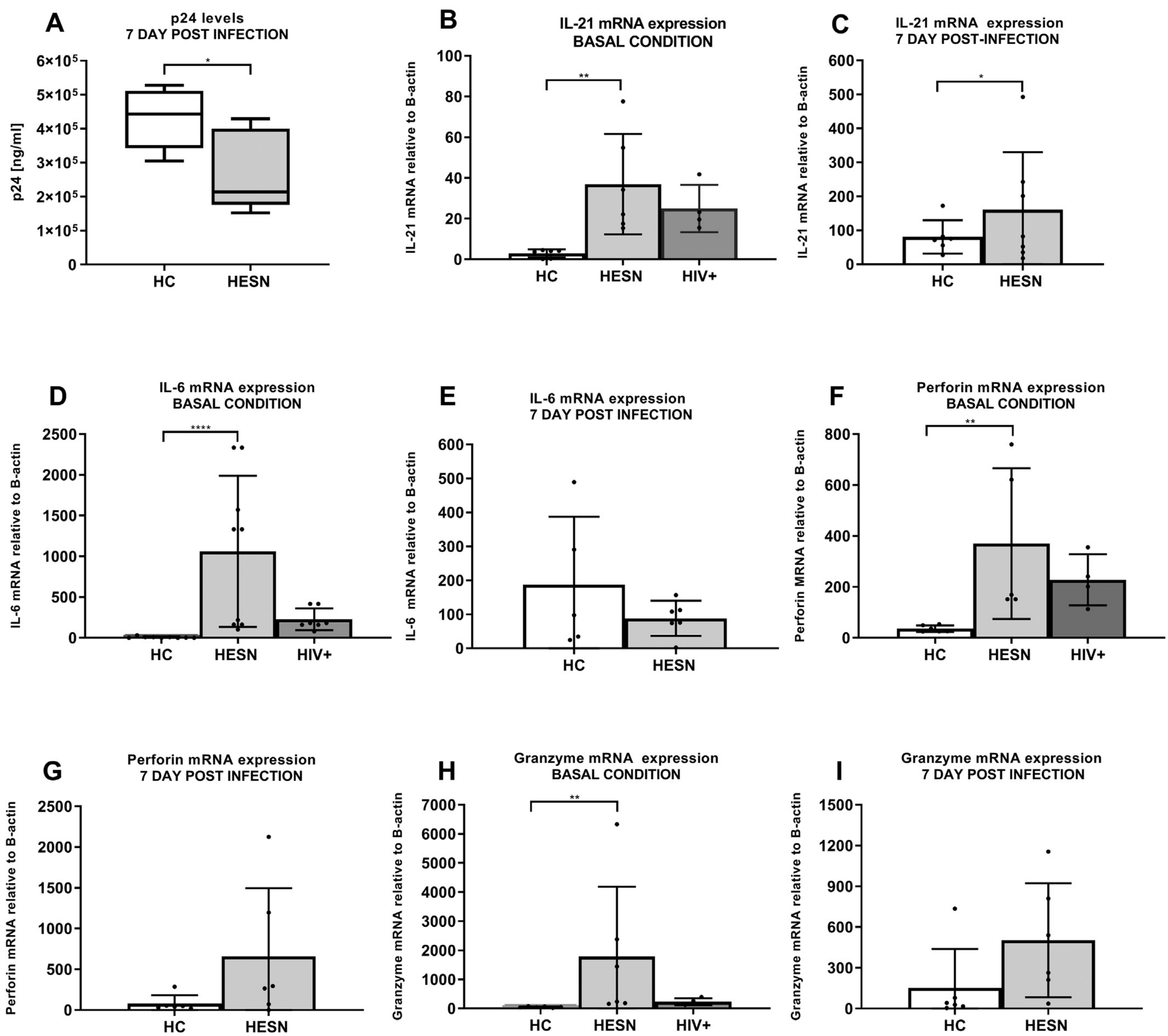

Fig. 1. p24 levels were significantly lower in PBMCs from HESN than HC 7 days post HIV-1 *in vitro* infection **(A)**. IL-21 mRNA levels in PBMCs at baseline **(B)** and 7 days post HIV-1 *in vitro* infection **(C)** were higher in HESN compared to HC. IL-6 mRNA levels in PBMCs at baseline were higher in HESN compared to HC **(D)** but at 7 days post infection there was no significant difference **(E)**. Perforin mRNA levels in PBMCs at baseline **(F)** and 7 days post HIV-1 *in vitro* infection **(G)** were higher in HESN compared to HC, but these differences did not reach statistical significance. Likewise, although not statistically significant, the Granzyme mRNA levels were also higher in HESN than HC at baseline (H) and 7 days post HIV-1 infection (I). The mRNA expression of all genes at baseline condition were analyzed by Kluscall–Wallis test with Dunn's multiple comparison correction, gene expression 7 days post HIV-1 infection and p24 levels were analyzed by Welch's t-test, except the Perforin and Granzyme mRNA expression that were analyzed by Mann–Whitney test. Mean values and S.E. are shown. * = $p < 0.05$, ** $p < 0.005$, **** $p < 0.0001$.

expression levels of target genes were calculated by 2-ΔΔCt equation as ratios between the target and B-actin (Fwd-5′-CTTTGCCGATCCGCCGC-3′ Rev-5′-ATCACGCCCTGGTGCCTGG-3′) considering one internal reference subject.

1.4. Statistical analysis

Depending on the normality distribution evaluated by Shapiro–Wilk normality test, the mRNA expression of all genes at baseline condition were analyzed by non-parametric Kluscall–Wallis test with Dunn's multiple comparison correction, gene expression 7 days post HIV-1 infection and p24 levels were analyzed by parametric t-test with Welch's correction because normal distribution of data except the mRNA expression of Perforin and Granzyme that were analyzed by non-parametric Mann–Whitney test. All statistical analyzes were performed using GraphPad PRISM version 8 (GraphPad Software), and p-values of 0.05 or less were considered to be significant.

2. Results

As expected, HESN individuals show a reduced susceptibility to HIV-infection compared with HC (267.4 ng/mL and 430.5 ng/mL; $p = 0.01$. Fig. 1A), as well as higher IL-21 mRNA levels at basal (13-fold, $p = 0.0049$. Fig. 1B), and seven days post-infection (2-fold, $p = 0.0154$. Fig. 1C). IL-6 expression by PBMCs at baseline was 136-fold higher in HESN than HC ($p < 0.0001$. Fig. 1D), this trend was maintained at 7 days post-infection (Fig. 1E).

We observed similar results with perforin and granzyme at basal (35-fold, $p = 0.0089$ and 10-fold, $p = 0.0021$) (Fig. 1F, H) and at 7 days post-infection (8-fold and 3-fold) (Fig. 1G, I). Nevertheless, the differences at 7 days post-infection were not statistically significant.

3. Discussion

IL-21 is a pleiotropic cytokine with an immunomodulatory influence over the innate and adaptive immune system, particularly limiting the HIV-1 infection [7–10]. In addition, IL-21 was inversely associated with disease progression [11] and higher viral suppression in response to antiretroviral therapy [12].

In our previous study, in an Italian cohort [3], we observed an increased expression of IL-21 in HESN versus HC. These results were confirmed in this Colombian HESN cohort, where the IL-21 increase was associated with a substantial reduced viral replication. Likewise, at 7 days post-infection this increment remained significant in HESN than HC. These data coincides with other studies performed on HIV Elite Controllers [11,12] suggesting that the maintenance of IL-21 levels could contribute to the natural control of the infection.

As for IL-6, its expression was significantly augmented in HESN PBMCs at baseline but not after *in vitro* infection. IL-6 is known to be a key inducer of IL-21 in CD4+ T cells [13]. Additionally, IL-6, along with IL-21, regulates CD4+ T-cell differentiation, mainly towards TH17 and T-follicular helper profile [14,15]. It is therefore plausible to speculate that IL-6 could exert a defensive role in HESN by regulating such cellular mechanisms.

IL-21 induces the expression of the cytotoxic molecules perforin and granzyme in CD8+ T cells and NK cells of mice chronically infected with lymphocytic choriomeningitis virus [7,16–18]. The statistical difference of perforin and granzyme expression gene at baseline conditions support these effects.

This evidence suggests that resistance or reduced susceptibility to HIV-1 infection may involve the increase of some cytokines categorized as "proinflammatory" counteracting the assumption that their role is exclusively deleterious during HIV-infection. It is plausible to hypothesize that the same cytokines which speed up the progression of HIV-infection could play a protective role during the early phases of exposure. Indeed, HESN phenotype has been associated with higher responsiveness to stimuli [19–21] supporting our findings.

Until now, the evidence found in our current investigations and by other groups suggests that this multifaceted cytokine has essential characteristics that could contribute to the innate control of HIV infection [22,23], pointing it as a useful tool in the control of HIV-1 infection.

The fact that both HESN cohorts, Italian and Colombian, have shown similar immunological characteristics, despite having different demographic origin and dissimilar genetic background suggests that IL-21 increase may be a distinctive quality in the cell-mediated immunity profile of HIV-1 resistance, at least during sexual exposure more likely resulting in a reduction of viral infection/replication. However, to deepen the role of IL-21 and its immunological network in this process, further studies with different HESN cohorts around the world are required, ideally including different exposure routes and higher sample sizes. It is also necessary to evaluate the effect of knockdowns of Il-21 on the susceptibility to infection to determine its direct role in the natural resistance phenomenon. The final objective is to delineate a profile of resistance against HIV or reduced susceptibility to this infection that could point towards the development of therapeutic strategies.

Declaration of Competing Interest

There are no conflicts of interest.

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