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IFN-λ inhibits HIV-1 integration and post-transcriptional events *in vitro*, but there is only limited *in vivo* repression of viral production

Ren-Rong Tian [a,1], Hong-Xiong Guo [b,1], Ji-Fu Wei [c], Chuan-Kun Yang [b], Shao-Heng He [c,*], Jian-Hua Wang [a,*]

[a] Key Laboratory of Molecular Virology and Immunology, Institute Pasteur of Shanghai, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China
[b] Jiangsu Center for Disease Control and Prevention, Nanjing, Jiangsu, China
[c] Clinical Experiment Center, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

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ABSTRACT

The lambda interferons (IL-28a, 28b, and IL-29) inhibit the replication of many viruses, but their role in the inhibition of HIV-1 infection remains unclear. During this study, we monitored IL-29 production in HIV-1 infected individuals and analyzed the *in vitro* and *in vivo* inhibition of HIV-1 production. Prior treatment with IL-28a or IL-29 induced an antiviral state in cultured primary T-cells, which suppressed HIV-1 integration and post-transcriptional events. The antiviral factors MxA, OAS, and PKR were up-regulated. In HIV-1 infected patients, IL-29 level was increased along with the depletion of $CD4^+$ T-cells in peripheral blood, while the elevated IL-29 did not show a significantly negative correlation with viral load. Further analysis of HIV-1 infected individuals showed that IL-29 was positively correlated with IFN-β and anti-inflammatory cytokine IL-10, and was negatively correlated with IFN-γ, which might suggest that IFN-λ participates in modulating antiviral immune responses during HIV-1 infection *in vivo*. Together, although IFN-λ impeded HIV-1 infection of T-cells *in vitro*, IFN-λ showed only limited *in vivo* repression of viral production. The modulation of IFN-λ on inflammatory factors might be worthy for further concentrating on for better understanding the host immune response during HIV-1 infection.

1. Introduction

Viral infection induces the expression of interferons (IFNs) (Diegelmann et al., 2010; Megjugorac et al., 2009; Wang et al., 2009; Yu et al., 2011) and these IFNs then modulate innate and adaptive immune responses against pathogen invaders (Ank et al., 2006b; Commins et al., 2008; Dumoutier et al., 2004; Kempuraj et al., 2004; Renauld, 2003; Sheppard et al., 2003; Uze and Monneron, 2007; Witte et al., 2010). Human IFNs are classified as type I, II, and III based on the type of receptors used for signaling. Type III IFNs (also known as IFN-λ) are composed of three structure-related cytokines, i.e., interleukins (IL)-28a, -28b, and -29. IFN-λ signals via a receptor complex containing IL-10Rβ and IL-28Rα. Peripheral blood mononuclear cells and dendritic cells are considered to be the major producers of IFN-λ (Lauterbach et al., 2010; Megjugorac et al., 2010; Wolk et al., 2008).

Like other types of IFNs, IFN-λ is known to inhibit replication in a range of viruses, including, hepatitis C and B virus (HCV and HBV) (Diegelmann et al., 2010; Doyle et al., 2006; Yu et al., 2011), influenza virus (Osterlund et al., 2005; Wang et al., 2009), rotavirus (Pott et al., 2011), herpes simplex virus-1 and -2, encephalomyocarditis virus, vesicular stomatitis virus, cytomegalovirus, and West Nile virus (Ank et al., 2008, 2006a; Doyle et al., 2006; Ma et al., 2009; Zhou et al., 2011). IL-28b has been drawn recently much attention by HCV researchers, because of the discovery of single nucleotide polymorphisms linked to IL-28b at the population level that are associated with spontaneous resolution and that determine anti-HCV treatment outcome (Ge et al., 2009; Thomas et al., 2009).

Limited studies have described the effects of IFN-λ on HIV-1 replication and disparate results were reported. IL-29 and IL-28a are reported to inhibit HIV-1 replication in macrophages. They promote the expression of CC-chemokine/MIP (macrophage inflammatory protein)-α/β and the host restrictive factor APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like)-3G/3F, which contribute to viral restriction (Hou et al., 2009). However, as reported, pretreatment of uninfected PBMCs or $CD4^+$ T-cell lines with IFN-λ improved the expression of HIV-1 receptor and co-receptors that increase viral binding and replication (Serra et al., 2008). The effect of IFN-λ on HIV-1 replication remains controversial.

We monitored IL-29 production in HIV-1 infected individuals and analyzed the potential role of IFN-λ in repressing viral production *in vivo* and *in vitro*. We confirmed that IFN-λ induced an

* Corresponding authors. Tel.: +86 21 63848688; fax: +86 21 63843571 (J. Wang), tel./fax: +86 25 83672085 (S. He).
E-mail addresses: Shaohenghe@live.cn (S.-H. He), Jh_wang@sibs.ac.cn (J.-H. Wang).
[1] These authors contributed equally to this work.

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antiviral state in cultured primary T-cells, which impeded HIV-1 integration and post-transcriptional events. In contrast, although plasma IL-29 level was increased along with the depletion of $CD4^+$ T-cells in HIV-1 infected patients, the elevated IL-29 showed limited repression of viral production. The modulatory effect of IFN-λ on inflammatory cytokines might contribute to immune hyper-activation and suppression during HIV-1 infection.

2. Material and methods

2.1. Subjects and ethics statement

This study was approved by the Ethics Committee of Jiangsu Provincial Center for Disease Prevention and Control (CDC). Written informed consent was provided by study participants or their legal guardians. Recruits were HIV-1 positive adults who were initially infected via heterosexual or homosexual intercourse, blood transfusion, or injected drug usage. All blood samples were confirmed as negative for HBV, HCV, and syphilis. HIV-1 load was tested using HIV-1 monitor version 1.5 (Roche Molecular Systems, Inc., Branchburg, New York, USA) with Roche Cobas Amplicor. The CD4 count was performed using BD Multitest CD3/CD8/CD45/CD4 reagent with a BD FACSCalibur™.

2.2. Cell culture and virus stock

Peripheral blood mononuclear cells (PBMCs) were isolated from the buffy coat units of healthy donors, provided by the Blood Center of Shanghai, Shanghai, China. PBLs (Peripheral blood lymphocytes) were recovered by the depletion of monocytes from PBMC using anti-CD14 antibody-coated magnetic beads (Miltenyi Biotec). $CD4^+$ T-cells were further purified from PBLs using anti-CD4 antibody-coated magnetic beads. PBLs and primary $CD4^+$ T-cells were activated using 5 μg/ml of phytohemagglutinin-P (PHA-P)

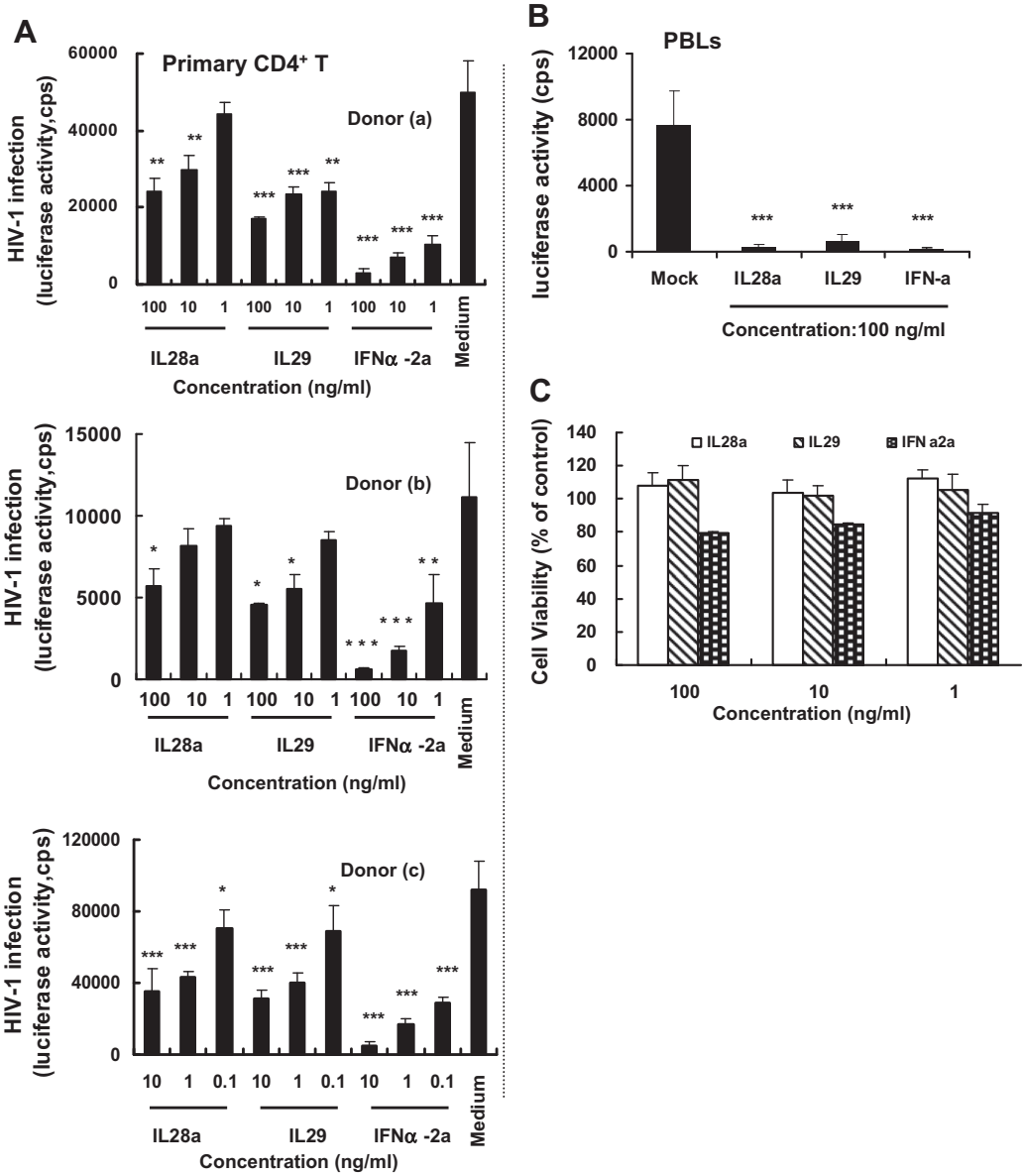

Fig. 1. Inhibitory effects of IFN-λ during HIV-1 infection. PHA-P-activated primary $CD4^+$ T-cells (A) or PBLs (B) were pretreated with IFNs and then pulsed with pseudotyped single-cycle infectious HIV-luc/NL4-3 (5 ng $p24^{gag}$). After 3 days of culture, HIV-1 infection was detected by measuring the luciferase activity in cell lysates. (C) Cell viability assay. PHA-activated primary $CD4^+$ T-cells were cultured for 3 days in the presence of different concentrations of cytokines. Cell viability was monitored using a MTT colorimetric assay. Data are mean values ± S.E.M. Cps, counts per second. The Mann–Whitney rank sum test was performed, $^{**}P < 0.01$ and $^{***}P < 0.001$ were considered to be significant.

(Sigma–Aldrich) and cultured in the presence of 20 IU/ml IL-2 (ProSpec, Cat# CYT-221) for 48 h. $CD14^+$ monocytes were purified from PBMCs using anti-CD14 antibody-coated magnetic beads (Miltenyi Biotec), and monocyte-derived macrophages (MDM) were generated by $CD14^+$ monocytes cultured in presence of macrophage colony-stimulating factor (M-CSF; 50 ng/ml) (PeproTech) for 9 days.

Single-cycle infectious HIV stocks were generated by calcium phosphate cotransfection of HEK293T cells with pLai-Δenv-Luc and the expression plasmid for HIV-1 envelope protein (Env) of NL4-3 (X4-tropic), as previously described (Wang et al., 2007). The plasmids were kind gifts from Dr. Li Wu (Ohio State University, USA). Virus stocks were quantified using $p24^{gag}$-capture enzyme-linked immunosorbent assay (ELISA).

2.3. Anti-HIV-1 assay

PHA-P-activated primary $CD4^+$ T-cells or PBLs were pretreated with cytokines for 24 h and then pulsed with pseudotyped luciferase reporter virus HIV-luc/NL4-3 (5 ng $p24^{gag}$) for 2 h at 37 °C. Cells were then washed and cultured for an additional 3 days. Viral infection was measured based on the detection of luciferase activity in cell lysates using a commercially available kit (Promega). The cell viability after cytokine treatments was measured by MTT colorimetric assay, as previously described (Wang et al., 2002). Recombinant IL-29 protein (Cat# 300-20L) and IL-28a (Cat# 96-300-02K) were purchased from Pepro Tech, and IFN-α2a (Cat# CYT-204) was purchased from ProSpec.

2.4. Real-time PCR

HIV-1 infection was also determined using real-time PCR to quantify the viral replication products. Cells were inoculated with HIV-luc/NL4-3 (5 ng $p24^{gag}$) for 2 h at 37 °C, then washed and cultured for the appropriate time. Total cellular DNA or RNA was extracted with a QIAamp DNA Mini Kit or QIAamp RNAeasy Mini Kit (Qiagen, Valencia, CA), respectively. HIV-1 entry was quantified by detecting the HIV-1 ssDNA level, while the integrated HIV-1

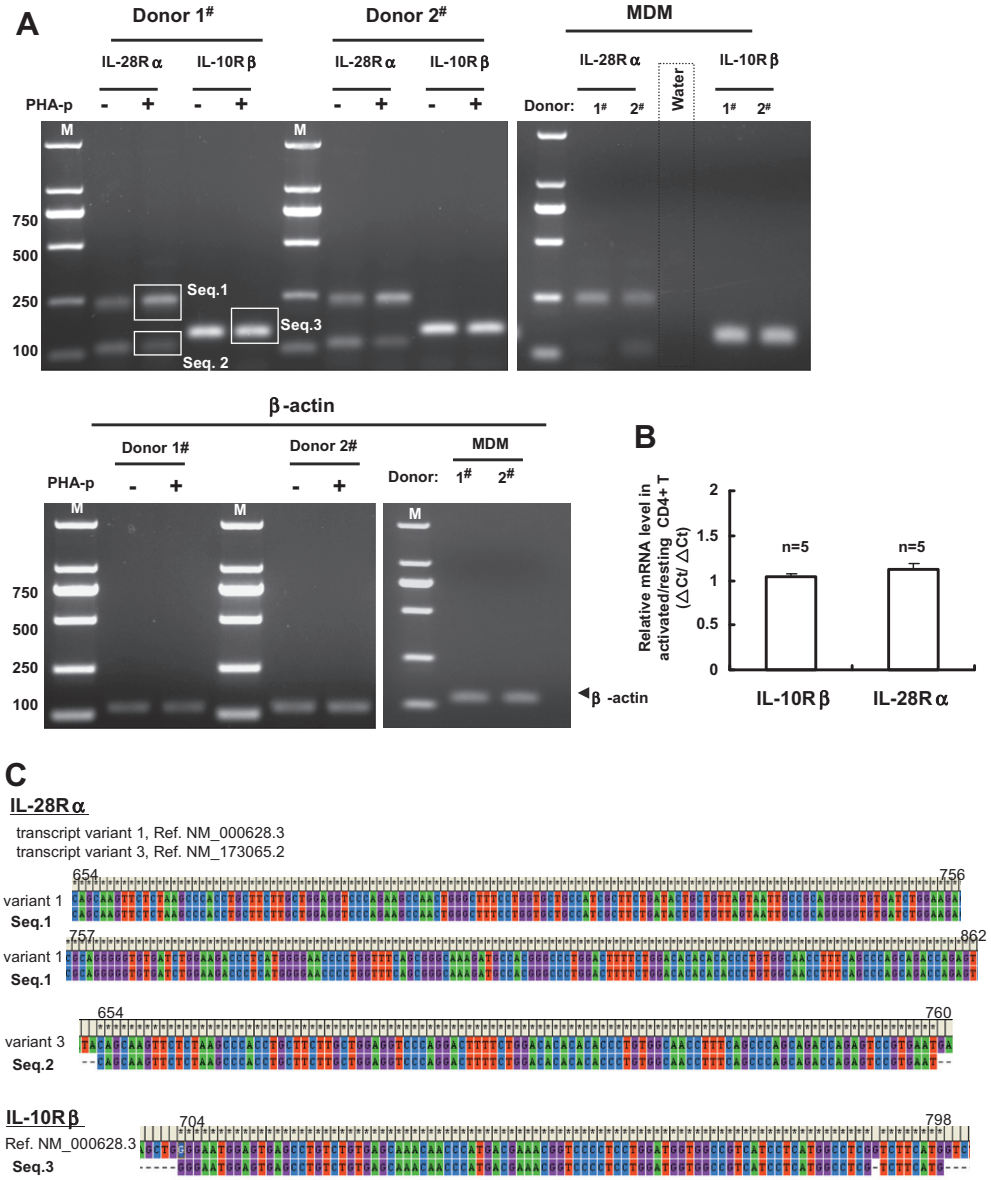

Fig. 2. The expression of IFN-λ receptors. (A) The expression of IL-10Rβ, IL-28Rα and β-actin in both PHA-p stimulated (+) and un-stimulated (−) $CD4^+$ T-cells was detected at mRNA level. Two donors results were shown. (B) Relative repression of IL-10Rβ and IL-28Rα by semi-quantification with SYBR green 1 and normalized with β-actin. (C) Sequencing of amplified IL-10Rβ and IL-28Rα by (RT-) PCR and alignments with transcript variants.

proviral DNA was quantified using a two-step Alu-PCR, as previously described (Dong et al., 2009). Viral transcription was quantified by detecting the products of unspliced, singly-spliced, and multiply-spliced HIV-1 mRNA. Expression of MxA, 2′,5′-OAS (oligoadenylate synthetase), PKR (protein kinase RNA regulated), Trim 22 (tripartite motif-containing 22), and APOBEC3G were monitored by detecting mRNA production. The primers and probes (synthesized from Invitrogen) used are listed in the Supplementary Table. Products were semi-quantified with SYBR green 1 (Toyobo) and normalized with β-actin. Real-time PCR was performed using an ABI 7900HT Real-Time PCR system.

2.5. Flow cytometry

Cells were stained with specific monoclonal antibodies (mAbs) or isotype-matched IgG controls. mAbs against the following human molecules were used (clone numbers and resources are given in parentheses), PE-CD4 (RPA-14; eBioscience), APC-CCR5 (2D7; BD Pharmingen), and PE-Cy™5-CXCR4 (12G5; BD Pharmingen). Stained cells were detected using a LSRII flow cytometer (BD Pharmingen) and analyzed with the FlowJo 7.6.1 program.

2.6. Cytokine assay

The plasma cytokine levels of IL-29 (Cat# 88-7296), IFN-α (Cat# BMS216), IFN-γ (Cat# 88-7316), IFN-ω (Cat# BMS233), and IL-10 (Cat# 88-7106) were measured using ELISA kits (eBioscience, San Diego, CA), according to the manufacturer's instructions. The IFN-beta was measured by using *VeriKine*™ Human IFN-Beta Serum ELISA Kit (Cat# 41415-1) from company of PBL Interferon Source. For the detection of cytokine production from *in vitro* culture, cells were pre-treated with IL-28a (100 ng/ml), IL-29 (100 ng/ml) or IFN-α (10 ng/ml) for indicated time, total cellular RNAs were isolated and the production IL-10 and IFN-β were quantified by real-time (RT)-PCR. The relative increased fold was calculated by comparing with un-treated controls.

2.7. Statistical analysis

The Spearman rank order correlation test and the Mann–Whitney rank sum test were performed using the SigmaStat 2.0 program.

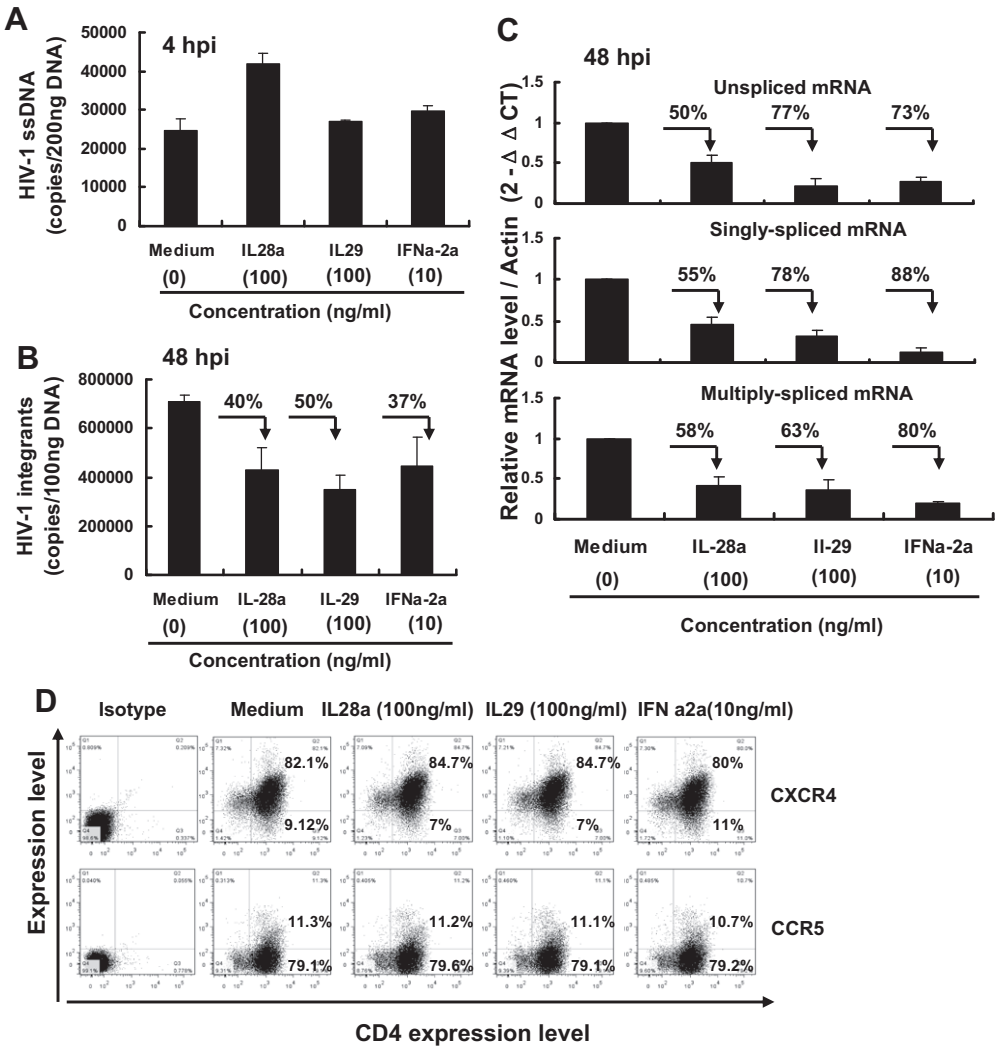

Fig. 3. IL-29 and IL-28a block HIV-1 integration and post-transcriptional events. PHA-activated primary CD4$^+$ T-cells were pretreated with IFNs and pulsed with HIV-luc/NL4-3 (5 ng p24^{gag}). Cellular RNAs or DNAs were isolated at the indicated times postinfection. (A, B) The production of HIV-1 ssDNA and integrants was quantified. Lower percentages relative to the medium-treated control are labeled. (C) HIV-1 post-transcriptional products of unspliced, singly-spliced, and multiply-spliced mRNA were quantified and normalized with β-actin. (D) Detection of HIV-1 receptor and co-receptor expression. PHA-activated primary CD4$^+$ T-cells were treated with cytokines for 24 h and the surface expression of CD4, CXCR4, and CCR5 were detected by flow cytometry. Positive percentages are shown. One representative experiment of three is shown.

3. Results

3.1. IL-28a and IL-29 inhibit HIV-1 infection of primary CD4+ T-cells and PBLs

To examine the anti-HIV-1 activity of IFN-λ, PHA-P-activated primary CD4+ T-cells and PBLs were pretreated with IL-29, IL-28a, or an IFN-α-2a control before infection with the HIV-luc/NL4-3 reporter virus. HIV-1 infection was measured by detecting the luciferase activity in cell lysates after 3 days of culture. The results showed that, like IFN-α-2a, IL-29 and IL-28a inhibited HIV-1 infection in activated primary CD4+ T-cells and PBLs, and the inhibition was shown to be dose-dependent in CD4+ T cells (Fig. 1A and B). The anti-HIV-1 activity of IFN-λ showed a little bit diversity in different donors, which might suggest the different T cells responsiveness to these cytokines. The cytokine treatments did not significantly reduce cell viability, as demonstrated by a MTT colorimetric assay that showed cells retained over 80% viability after treatments with different concentrations of IFNs for 3 days (Fig. 1C).

To support the interaction of IFN-λ with T cells, the primary CD4+ T-cells were monitored for the expression of IFN-λ receptors. Both resting and PHA-P-stimulated primary CD4+ T-cells expressed IL-10Rβ and IL-28Rα (Fig. 2A), The MDM, on which have been proved to possess the expression of IFN-λ receptors (Hou et al., 2009), was used as control. PHA-P stimulation did not alter much of either receptor expression (Fig. 2B). By sequencing confirmation, transcript variant 1 and 3 of IL-28Rα were expressed in both resting and activated CD4+ T-cells (Fig. 2C). Together, these results confirmed the inhibitory effects of IFN-λs on HIV-1 infection in cultured primary CD4+ T-cells or PBLs.

3.2. IL-28a and IL-29 block HIV-1 integration and post-transcriptional events

To better characterize the anti-HIV-1 activities of IFN-λ, we tracked the potential restriction steps during the HIV-1 life-cycle. Cytokine-treated, activated primary CD4+ T-cells were pulsed with HIV-luc/NL4-3. Cells were then harvested and lysed at the appropriate times, and total cellular DNA or RNA was isolated. HIV-1 fusion-based entry was assessed by the quantification of viral ssDNA after 4 h viral inoculation. Treatments with IL-28a (100 ng/ml), IL-29 (100 ng/ml) or IL-α2a (10 ng/ml) did not impair HIV-1 entry (Fig. 3A). HIV-1 integration was inhibited and the HIV-1integrants detected 48 h post-infection were decreased by 40% and 50% in IL-28a and IL-29 treated cells, respectively (Fig. 3B). When HIV-1 post-transcriptional products were examined 48 h after infection,

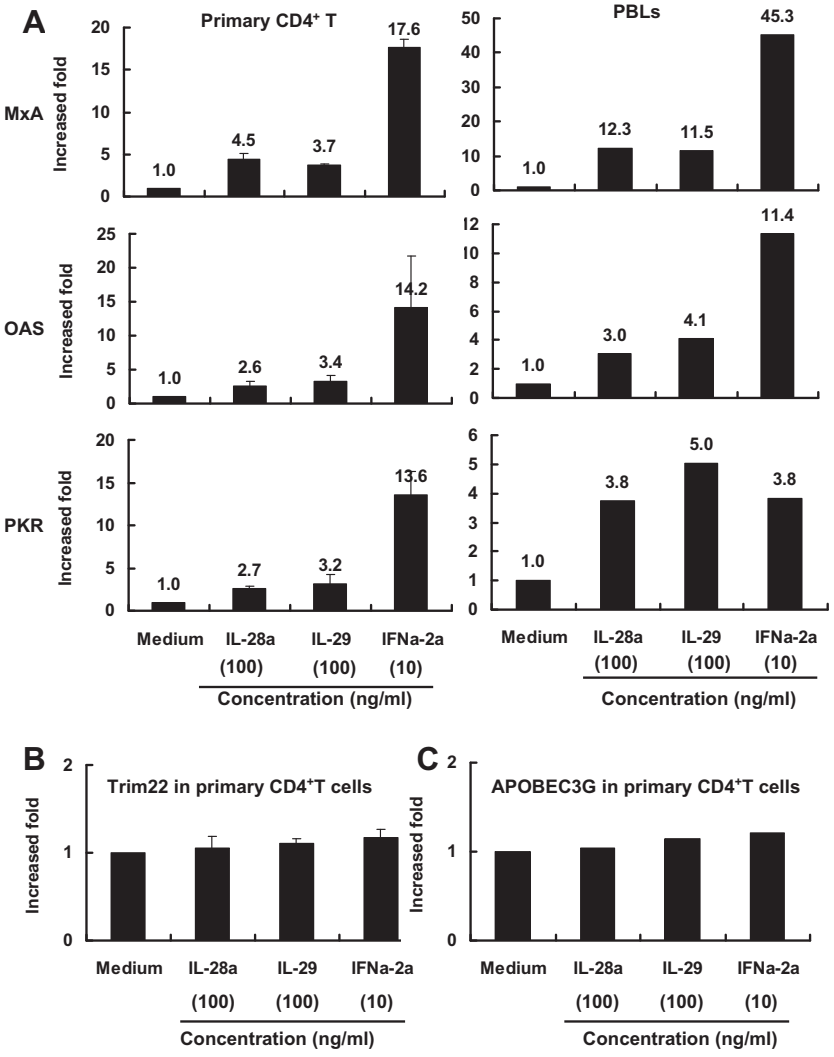

Fig. 4. Induction of antiviral factors. PHA-activated primary CD4+ T-cells or PBLs were treated with cytokines for 20 h, cells were lysed, and total RNAs were extracted. The production of MxA, OAS, PKR (A), Trim22 (B), and APOBEC3G (C) was quantified by real-time PCR and normalized with β-actin. The fold increases induced by cytokine relative to the medium-treated control are indicated. One representative experiment of three is shown.

the unspliced, singly-spliced, and multiply-spliced HIV-1 mRNA decreased over 50% in IFN-treated cells when compared with the medium-treated control (Fig. 3C). Unlike a previous report (Serra et al., 2008), we observed no alteration in the expression of CD4, CXCR4, and CCR5 by activated primary $CD4^+$ T-cells, even those treated with high concentrations (100 ng/ml) of IL-28a, IL-29, or IFN-α for 48 h (Fig. 3D). Overall, these data demonstrate that treatment with IL-28a, IL-29, or IFN-α efficiently blocked HIV-1 integration and post-transcriptional events in primary $CD4^+$ T cells, although the surface expression of HIV-1 receptors/co-receptors was not affected.

3.3. IFN-λ induces the expression of the antiviral factors MxA, OAS, and PKR in T-cells

IFN-stimulated genes (ISGs) for MxA, OAS, and PKR production have been reported to be induced by IL-29 in human primary liver cells or hepatoma cells, where they inhibit replication of HBV and HCV (Chai et al., 2011; Doyle et al., 2006). To investigate whether IFN-λ could induce expression of these antiviral factors in T-cells, PHA-P-activated primary $CD4^+$ T cells or PBLs were treated separately with IL-28a (100 ng/ml), IL-29 (100 ng/ml), or IFN-α(10 ng/ml) for 24 h and the expression of MxA, OAS and PKR were quantified at the mRNA level. Pretreatment with IL-28a, IL-29, or IFN-α induced 2.7- to 45-fold greater enhancement of MxA, OAS and PKR expression in $CD4^+$ T-cells or PBLs, respectively (Fig. 4A). However, the expression of Trim 22 and APOBEC3G, which are known anti-HIV-1 factors, did not increase in IFN-28a- or IL-29-treated, activated $CD4^+$ T-cells (Fig. 4B and C). Overall, these results demonstrate that IFN-λ, like type I IFNs (Foster et al., 2004), can induce the expression of the antiviral factors MxA, OAS and PKR in T cells, which probably accounts for the generation of an antiviral state in cells.

3.4. IL-29 production in HIV-1 infected individuals

It is accepted that viral infection can induce the production of IFNs (Diegelmann et al., 2010; Megjugorac et al., 2009; Wang et al., 2009; Yu et al., 2011). To monitor the *in vivo* production profile of IFN-λ during HIV-1 infection, HIV-1 infected, antiretroviral naïve-treated individuals were recruited and we further analyzed the relationship of IL-29 production with $CD4^+$ T cell frequency and viral load separately. HIV-1 infected individuals with $CD4^+$ T-cell numbers greater than 800/μL retained a similarly level of plasma IL-29 to the uninfected controls (Fig. 5A), while when CD4 dropped lower than 350 but higher than 200/μL (non-AIDS phase but permitting for antiretroviral medication), IL-29 production was significantly increased compared with uninfected group (Fig. 5A). IL-29 was significantly elevated along with the depletion of $CD4^+$ T-cells in HIV-1 infected individuals. While the IL-29 production was dropped when CD4 numbers were lower than 200/μL (known as the defined AIDS phase) (Fig. 5A), which may be attributable to defective cytokine secretion or the persistent depletion of blood myeloid cells, which are the main producers of IL-29.

When evaluating the correlation of IL-29 with viral load in the plasma, there was no significantly negative association of IL-29 with viral load was observed in any divided groups based on $CD4^+$ T-cell number (Fig. 5B), this may indicate the limited activity of IL-29 in inhibiting HIV-1 replication *in vivo*. Viral infection and/or engagement of the pattern recognition receptors such as the Toll-like receptors (TLRs) could induce both IFN-λ and type I IFNs production, the simultaneous induction of IFN-β and IFN-λ by poly I:C triggered TLR-3 signaling have been reported (Coccia et al., 2004), indicating the shared induced mechanisms by IFN-β and IFN-λ. So when analyzing the correlation of IFN-β with viral load, similarly, there was no significantly negative correlation was observed (Fig. 5C).

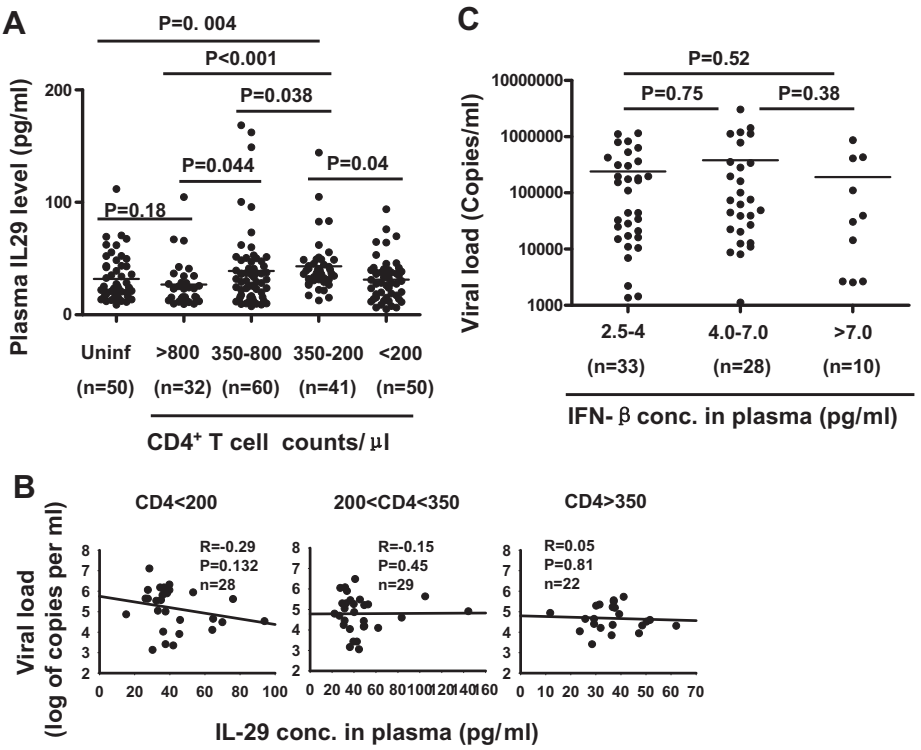

Fig. 5. Persistent HIV-1 infection induced *in vivo* IL-29 expression. (A, B) Antiretroviral naïve-treated HIV-1 infected individuals were recruited and we quantified their plasma IL-29 and IFN-β level, $CD3^+/CD4^+$ T cell frequency, and viral load. The relationship between IL-29 level with $CD4^+$ T cell count (A), IL-29 level with viral load (B), and IFN-β level with viral load (C), were analyzed. The Mann–Whitney rank sum test and Spearman rank order correlation test were performed, and a $P < 0.05$ was considered to be significant.

Together, these *in vivo* studies showed that, although IL-29 production increased along with depletion of CD4 $^{+}$ T-cell in HIV-1 infected individuals, the elevated IL-29 level in patients has limited *in vivo* repression of HIV-1 production. Instead, the production of IL-29 in HIV-1 infected individuals might reflect an accelerate disruption of the host immune system.

3.5. IL-29 was positively correlated with IFN-β and IL-10, but negatively correlated with IFN-γ in the plasma of HIV-1 infected individuals

IFN-λ modulates cytokine secretions and regulates host immunity (Jordan et al., 2007a,b). To better understand the immune regulation of IFN-λ during HIV-1 infection, we also quantified the production of type I IFNs (IFN-α, -β, and -ω), type II IFN (IFN-γ), and IL-10 in the plasma of HIV-1 infected individuals, before we analyzed their correlation with IL-29.

IL-29 was correlated with IFN-β production ($R = 0.4$, $P < 0.01$; Spearman rank order correlation test) (Fig. 6A–C). This indicated the *in vivo* modulation of IFN-λ and type I IFNs and it confirmed the regulatory effect of IFN-λ on immune activation during HIV-1 infection. The elevated IL-29 level in the plasma of HIV-1 infected individual was positively correlated with IL-10 ($R = 0.42$, $P < 0.01$) (Fig. 6D) and negatively correlated with IFN-γ production ($R = -0.456$, $P < 0.01$) (Fig. 6E). This might suggest that the elevated IFN-λ level was involved in immune suppression during the antiviral response of HIV-1 infected individuals. To prove the regulation on IL-10 and IFN-β by IFN-λ, cultured CD4^{+} T cells was treated with IL-28a (100 ng/ml), IL-29 (100 ng/ml) or IFN-α (10 ng/ml), for 2 or 6 h, as show in Fig. 5F, the treatment of CD4^{+} T cells by IFN-λ

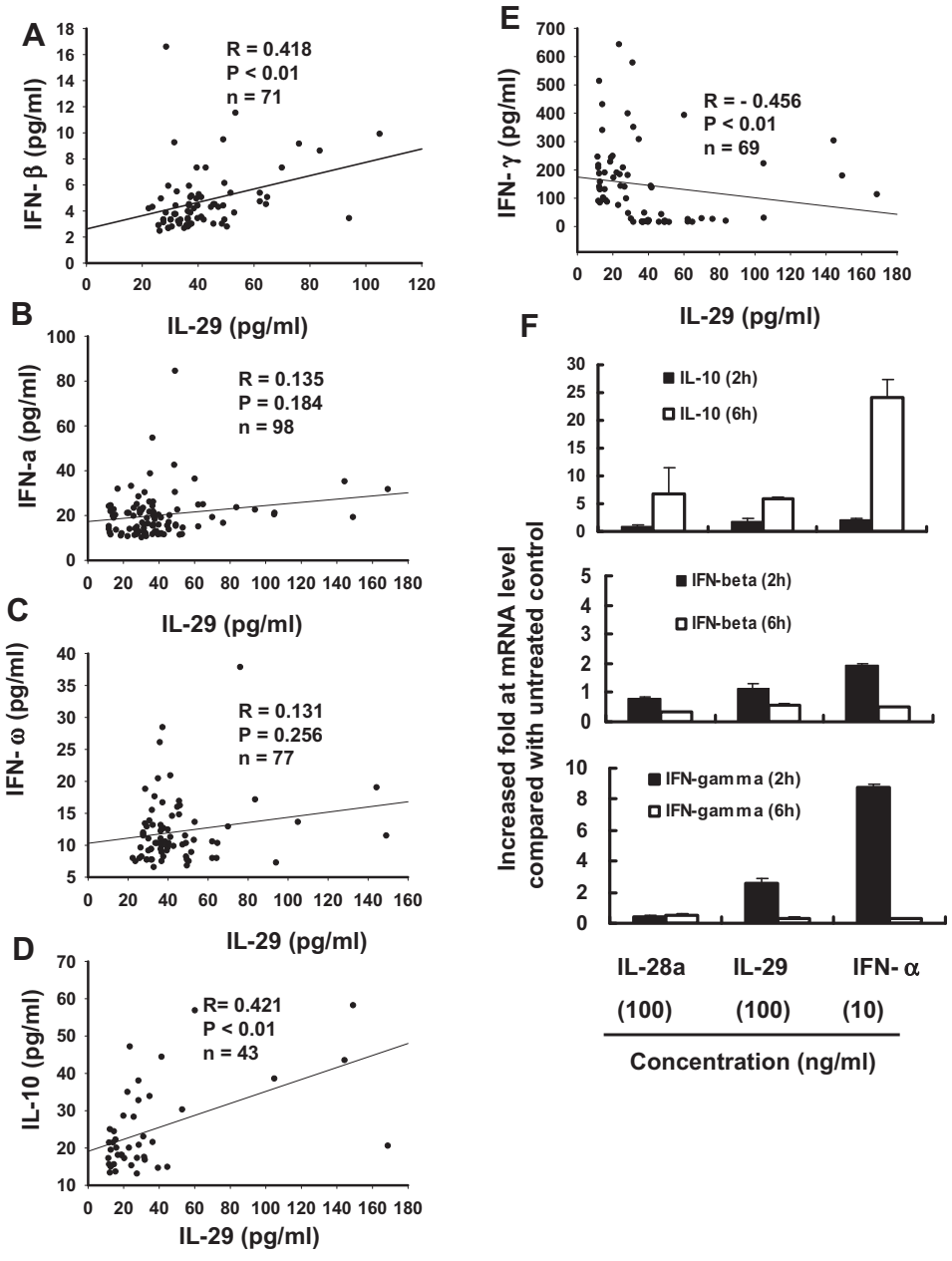

Fig. 6. The correlation of IFN-λ with other cytokines. (A–E) Antiretroviral naïve-treated HIV-1 infected individuals were recruited and we quantified the plasma production of the cytokines IFN-α, -β, -γ, -ω and IL-10 by ELISA. Correlations with IL-29 were analyzed using the Spearman rank order correlation test. The correlation (R) and *P*-values are labeled. (F) The IL-10, IFN-β and IFN-γ expression was detected at mRNA level. Cultured primary CD4^{+} T cell were treated by IFN-λ for indicated time. The relative increased fold was calculated by comparing with un-treated controls. Supplementary Table. The primers and probes used for PCR and RT-PCR.

induced IL-10 expression as detected at mRNA level, while the IFN-β was not much up-regulated, which may be due to $CD4^+$ T cells are not the major producer of IFN-β; Both of IL-28a and IL-29 did not show much up-regulation on the production of IFN-γ compared with the about 8-fold enhancement by 2 h-stimuation with IFN-α. IL-29 and IFN-α have been shown to differ in their ability for modulating IL-12 production in TLR-activated human macrophage and for regulating the expression of IFN-γ receptor (Liu et al., 2011).

Taken together, the *in vivo* studies demonstrate the correlation of IFN-λ with type I IFNs and IL-10. The modulation of IFN-λ on inflammatory factors might be worthy for further concentrating on for better understanding the host immune response during HIV-1 infection.

4. Discussion

We found that the antiviral state was induced by IL-29 or IL-28a in primary $CD4^+$ T-cells, which restricted HIV-1 infection and HIV-1 integration and post-transcriptional events were partially blocked. Our results were consistent with a previous report showing that IFN-λ inhibited HIV-1 infection in monocyte-derived macrophages (Hou et al., 2009). However, Serra et al. reported that type I and III IFNs promoted HIV-1 binding and replication in a T-cell line and PBMCs, by enhancing the expression of viral receptors or co-receptors (Serra et al., 2008). This discrepancy may be attributable to the different target cells and/or the methods used for HIV-1 infection detection.

IFNs preferably induce the expression of antiviral genes in uninfected cells, which promotes an antiviral state that blocks infection by incoming viruses (Chai et al., 2011; Doyle et al., 2006; Osterlund et al., 2005). These ISGs can impair many cellular processes that are required for viral replication or they may lead to the degradation of viral life cycle products. The induction of PKR, OAS, and MxA by IFN-λ has been previously reported (Chai et al., 2011; Doyle et al., 2006). Other ISGs, such as ADAR (adenosine deaminases acting on RNA) (Kawakubo and Samuel, 2000), P56 (Geiss et al., 2001), p200 gene family (Liu et al., 1999), ISG-20 (Eckert et al., 2006) and ISG-15 (D'Cunha et al., 1996) are well-known for their antiviral activities. The host restrictive factors of Trim 5α, Trim-22, and APOBEC3G/3F can also be induced by IFNs to inhibit the replication of retroviruses (Barr et al., 2008; Carthagena et al., 2008; Chen et al., 2010; Hou et al., 2009; Singh et al., 2011). We did not find that the expression of APOBEC3G and Trim-22 was promoted by IL-29 in primary $CD4^+$ T-cells, which was probably due to the specific cell types and assay methods that were used.

The *in vivo* production of IFN-λ also was monitored in HIV-1 infected individuals. We found that, although plasma IL-29 level was increased along with the depletion of $CD4^+$ T-cells in HIV-1 infected patients, the elevated IL-29 showed limited repression of viral production. Coincidentally, the weak *in vivo* antiviral activity of IFN-λ has been reported, intravenous injection with murine IFN-28a resulted in only limited inhibition of HBV replication in the liver of transgenic mice (Pagliaccetti et al., 2010).

IFN-λ can modulate cytokine production and regulate the host immune response. The positive correlation of IL-29 with IFN-β in HIV-1 infected individuals, may suggest that a type I IFN-like program is adopted by IFN-λ when promoting an antiviral response, as reported before (Chai et al., 2011; Doyle et al., 2006). IL-29 was negatively correlated with IFN-γ and positively correlated with anti-inflammatory cytokine IL-10. These results together might indicate the participation of IFN-λ in regulation of antiviral immune during HIV-1 infection.

Overall, IFN-λ was found to inhibit HIV-1 infection in cultured T-cells, whereas IFN-λ showed limited repression in HIV-1 production *in vivo*. Instead, the production of IL-29 in HIV-1 infected individuals showed a modulation of inflammatory factors, so the mutual regulation relationship might be worthy for further studies to better understand the antiviral immune response during HIV-1 infection. Clinical studies to assess the efficacy and safety of HCV treatment using exogenous IL-28b are currently underway, but whether the exogenous IFN-λ could be adopted for treating HIV-1 patient needs to be further carefully evaluated.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2012.04.011.

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