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IFN-λ4 inhibits HIV infection of macrophages through signalling of IFN-λR1/IL-10R2 receptor complex

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Abstract

The recently discovered IFN-λ4 has been found to have antiviral activity against several viruses. However, it's unknown whether IFN-λ4 can inhibit HIV infection. Here, we show that IFN-λ4 could suppress HIV infection of macrophages. This IFN-λ4-mediated HIV inhibition was compromised by the antibodies against IFN-λ receptor complex, IFN-λR1/IL-10R2. IFN-λ4 enhanced the phosphorylation of STAT1, and induced antiviral interferon-stimulated genes. These findings indicated that IFN-λ4 can inhibit HIV via JAK/STAT signalling pathway.

1 | INTRODUCTION

The mammalian interferon (IFN) is a multifunctional family of cytokines that have a key role in the host immune response to viral infections.[1] IFN family members are grouped into three types, type I, II and III, each of which exerts their functions through the unique receptor complex. Type I IFNs are represented by IFN-α and -β, type II by IFN-γ, and type III by IFN-λ family.[2] IFN-λ family consists of four members, IFN-λ1, IFN-λ2, IFN-λ3 and the newly discovered IFN-λ4 that is coded by four functional IFN-λ (IFNL) genes, IFNL1, -2, -3 and -4, respectively. IFN-λ1, 2 and 3 are highly similar to each other in the amino-acid sequences. The amino-acid identity between IFNL1 and IFNL2/3 is ~81%, and the identity between IFNL2 and IFNL3 is ~96%.[3] IFNL4 is most similar to IFNL3 compared to IFNL1 and IFNL2, but even that similarity is still very low, IFNL4 has only 29.1% amino-acid identity with IFNL3.[4] They have similar residues in the area that is known to interact with the primary receptor of IFN-λs (IFN-λ R1) but differ in the region of IFNL3 that interacts with the second chain of the IFN-λ receptor complex, IL-10R2.[4]

IFNL4 genome contains a dinucleotide variant, IFNL4-ΔG/TT (rs368234815, originally designated as ss469415590) in exon 1 of IFNL4, upstream of IFN-λ3 on chromosome 19q13.13. The IFNL4-ΔG allele generates a functional IFN-λ4 protein p179 (179 aa) by introducing a frameshift mutation that enables transcription, and the homozygous TT genotype creates a premature stop codon

and thus knockouts this gene. IFN-λ4 expresses in a small fraction of Asian and about half of European populations, but in most of Africans.[4] Genetic studies have demonstrated that IFNL4-TT allele has a strong positive correlation with HCV clearance, treatment outcome of HCV infection, and innate resistance to HIV infection, on the contrary, IFNL4-ΔG allele is associated with the impairment of HCV clearance, and unfavourable clinical and immunological status in HIV/HCV co-infected subjects.[4-6] But there was also evidence supported that IFNL4 genotype is not associated with the antiviral interferon-stimulated genes (ISGs) expression and HIV load in chronic HIV infection.[7]

Studies from different laboratories have documented that IFN-λ1, 2 and 3 have the ability to inhibit HIV replication.[8-10] It is unknown, however, whether IFN-λ4 has anti-HIV activity. In the present study, we investigated the antiviral effect of IFN-λ4 on HIV infection of macrophages, a major target of HIV infection and potential long-term HIV reservoir. We also examined whether IFN-λ4 acts through signalling of IFN-λR1/IL-10R2, the key receptor complex for IFN-λ1, 2 and 3.

2 | MATERIAL AND METHODS

2.1 | Reagents

Recombinant human IFN-λ4 was purchased from R&D Systems (Minneapolis, MN, USA). Rabbit monoclonal antibodies against human phospho-STAT1 (p-STAT1), STAT1, guanylate binding protein 5 (GBP5), IFN-stimulated gene 56 (ISG56, official gene symbol IFIT1), Virus inhibitory protein (Viperin) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and anti-rabbit secondary antibody were purchased from Cell Signalling Technology (Danvers, MA, USA). Sheep anti-human IFN-λR1 and IL-10R2 antibodies and sheep IgG were purchased from R & D Systems.

TABLE 1 Primers used in the real-time PCR

Target gene	Primer	Nucleotide sequence
GAPDH	Forward	5′-GGTGGTCTCCTCTGACTTCAACA-3
	Reverse	5′-GTTGCTGTAGCCAAATTCGTTGT-3′
ISG56	Forward	5′-TTCGGAGAAAGGCATTAGA-3′
	Reverse	5′-TCCAGGGCTTCATTCATAT-3′
Viperin	Forward	5′-TGGGTGCTTACACCTGCTG-3′
	Reverse	5′-TGAAGTGATAGTTGACGCTGGT-3′
GBP5	Forward	5′-CAGGAACAACAGATGCAGGA-3′
	Reverse	5′-TCATCGTTATTAACAGTCCTCTGG-3′
APOBEC3G	Forward	5′-TCAGAGGACGGCATGAGACTTAC-3′
	Reverse	5′-AGCAGGACCCAGGTGTCATTG-3′
APOBEC3F	Forward	5′-TTCGAGGCCAGGTGTATTCC-3′
	Reverse	5′-GGCAGCTGGTTGCCACAGA-3′

2.2 | HIV infection of macrophages

Purified human monocytes obtained from Human Immunology Core at the University of Pennsylvania were plated in the Corning CellBIND surface 96-well plate (10^5 cells/well) in complete Dulbecco's modified Eagle medium (DMEM) with 10% foetal calf serum (FCS). Corning CellBIND surface enhances cell attachment, which is capable of promoting monocytes differentiating into macrophages after cultured for 5-7 days without the addition of stimulating factor M-CSF.[11,12] Thereafter, DMEM with 10% FCS were replaced with DMEM with 5% FCS.

HIV Bal strain was obtained from the NIH AIDS Research and Reference Reagent Program. Equal amount of HIV Bal stock (RT activity of 158, 242 cpm) were added to the macrophage cultures. Cells were washed 3 times with fresh DMEM after overnight (14 hours) culture with the virus.

2.3 | IFN-λ4 treatment

IFN-λ4 toxicity was measured using the MTS assay which showed that IFN-λ4 had no toxicity to macrophages at the concentration of 1000 ng/mL or less. Macrophages were treated with different doses of IFN-λ4 (0, 100, 250 or 500 ng/mL) prior to, during, or after incubation with HIV Bal strain. IFN-λ1 (100 ng/mL) was used as a positive control, which was demonstrated to have strong anti-HIV activity in macrophages at this concentration.[8,9] To determine the role of IFN-λR1/IL-10R2 receptor complex in IFN-λ4 mediated anti-HIV activity, the binding of IFN-λ4 to its receptor was blocked using antibodies to IFN-λR1 and IL-10R2. IFN-λR1 antibody concentration of 1 μg/mL was chosen based on the product instruction and our preliminary experiment, and 5 μg/mL of IL-10R2 antibody was used as described in our previous study.[13]

2.4 | HIV RT assay

HIV RT activity analysis was performed as described previously.[14] Briefly, 10 μL of supernatant collected from HIV-infected macrophage cultures was added to 50 μL of a cocktail containing poly(A), oligo(dT), MgCl2, Nonidet P-40, and (^{32}P)dTTP and incubated overnight at 37°C. Thirty microlitres of the reaction mixture were spotted on DE81 paper and air-dried. The filters were then washed

four times in 2× standard saline citrate (SSC) (0.3 mol/L NaCl, 0.03 mol/L sodium citrate, pH 7) and 100% ethanol, dried, cut into pieces, and placed in a liquid scintillation counter (PerkinElmer, Boston, MA) for measurement of radioactivity.

2.5 | Real-time PCR and western blotting

Total cellular RNA was extracted from macrophages using Tri-Reagent (Molecular Research Center, Cincinnati, OH, USA). Total RNA was subjected to the reverse transcription using the Reverse Transcription System (Promega, Madison, WI, USA). The cDNA product was then used as a template for real-time PCR assay with an iCycler iQ real-time detection system (Bio-Rad, Hercules, CA, USA). Quantification of mRNA for ISGs and GAPDH were performed with SYBR green Master Mix (Bio-Rad Laboratories, Hercules, CA, USA). The primers used in the experiments are listed in Table 1.

Macrophages plated in 48-well plates (2.5×10^5 cells/well) were lysed by the RIPA buffer that contains protease and phosphatase inhibitors (Sigma, St. Louis, MO, USA). Proteins were collected and quantified with BCA method. Equal amount (20 μg) of each sample was subjected to SDS PAGE using 4%-12% Bis-Tris gels (Invitrogen, Carlsbad, CA, USA), and then transferred electrophoretically to nitrocellulose membrane. Protein bands were visualized using enhanced chemiluminescence (Amersham, Bucks, England) in a FujiFilm LAS-4000 imaging analyzer (GE Life Sciences, Piscataway, NJ, USA).

2.6 | Statistical analyses

Statistic analyses were performed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as the mean ± standard deviation, and statistical significance is determined using one-way ANOVA followed by the least significant difference test where appropriate.

3 | RESULTS AND DISCUSSION

IFN-λ4 has been shown to have the antiviral activity against hepatitis C virus (HCV), coronaviruses and West Nile virus.[15,16] Although studies have shown that other members of IFN-λ family, IFN-λ1, 2 and 3, are able to

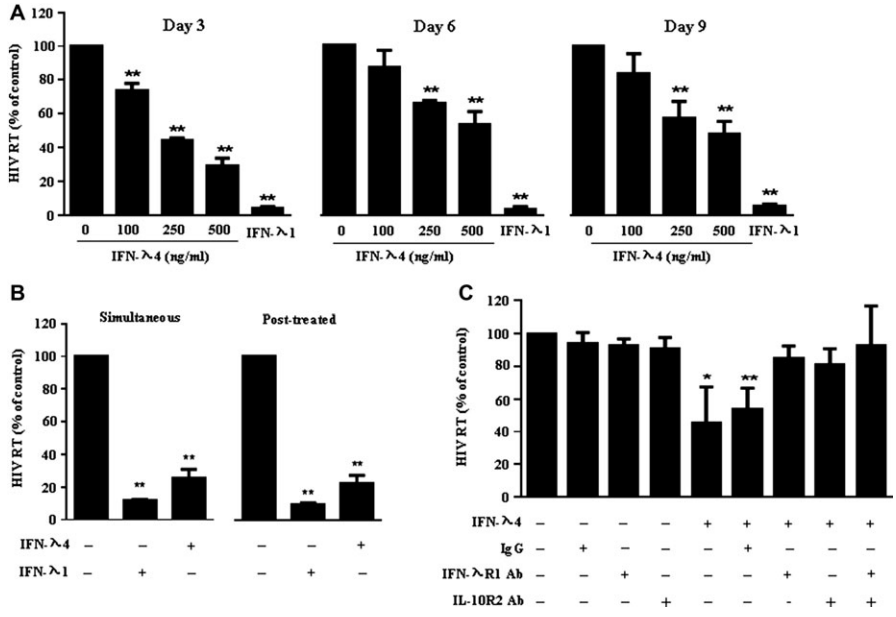

FIGURE 1 IFN-λ4 inhibits HIV replication in macrophages. A, IFN-λ4 pre-treatment of macrophages inhibits HIV infection. Macrophages were pre-treated with IFN-λ4 at the indicated concentrations for 24 h before HIV Bal strain infection overnight, with IFN-λ1 (100 ng/mL) as a positive control. Supernatant was collected for HIV RT activity analysis at day 3, 6 and 9 post-infection. Results are representative of three experiments with cells from three different donors. $^{**}P < 0.01$. B, IFN-λ4 simultaneous or post-treatment inhibits HIV infection of macrophages. Macrophages were treated with or without IFN-λ4 (500 ng/mL) during or after the incubation with HIV Bal strain. Cells were washed 3 times, and fresh DMEM with 5% FCS and IFN-λ4 (500 ng/mL) was added to the cell cultures. IFN-λ1 (100ng/mL) was used as a positive control. Supernatant was collected for HIV RT activity analysis at day 3 post-infection. Results are representative of three experiments with cells from three different donors. $^{**}P < 0.01$. C, Antibodies to IFN-λR1 and IL-10R2 block anti-HIV effect of IFN-λ4. Macrophages were incubated with HIV Bal strain overnight. Cells were washed 3 times and treated with antibody to IFN-λR1 (1 μg/mL) and/or IL-10R2 (5 μg/mL) for 1 h prior to the addition of IFN-λ4 (250 ng/mL). Sheep IgG (5 μg/mL) was used as a negative control. Supernatant was collected for HIV RT activity analysis at day 3 post-treatment. Results are representative of three experiments with cells from three donors. HIV RT activities were compared between the group treated with IFN-λ4 alone and the other groups. $^{*}P < 0.05$, $^{**}P < 0.01$

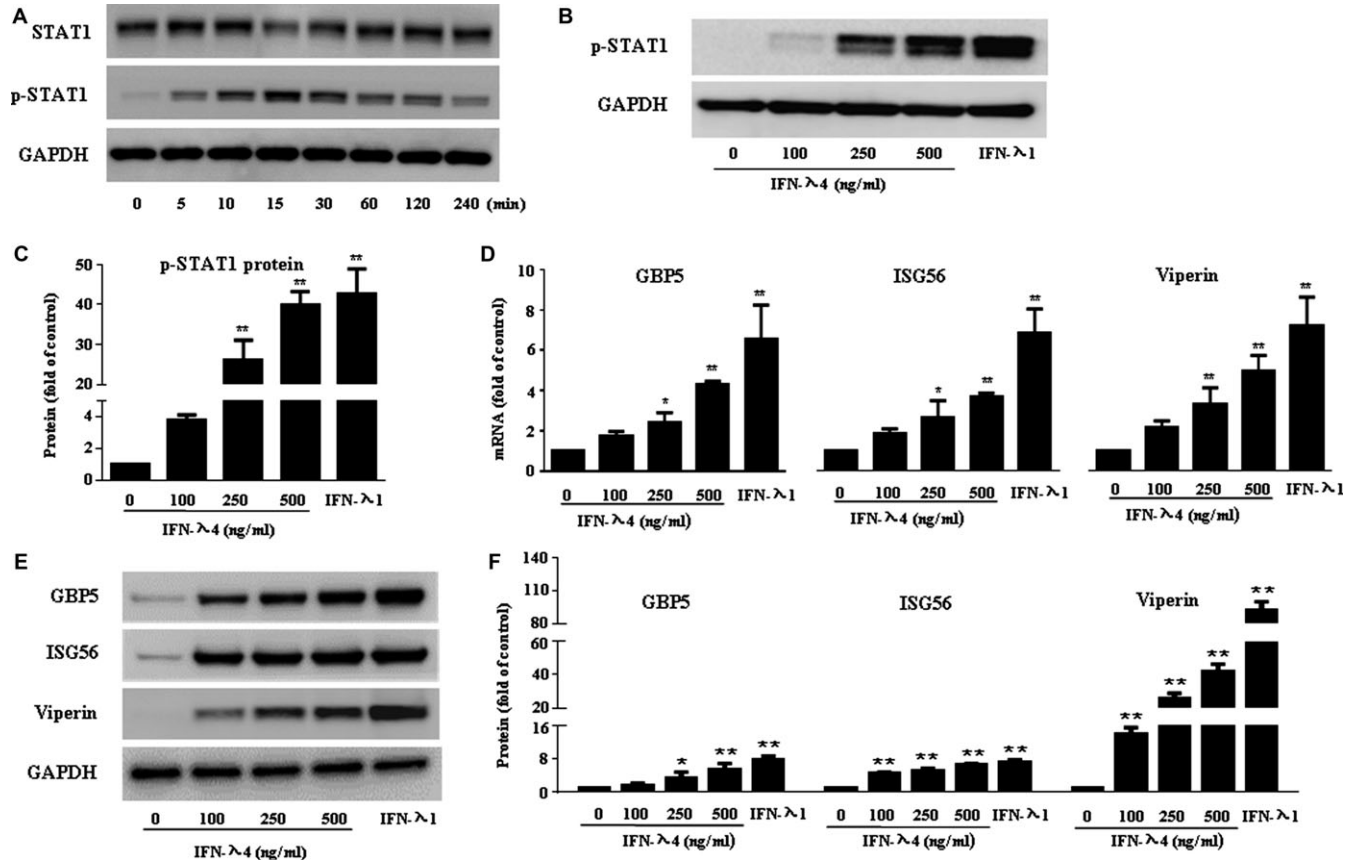

FIGURE 2 IFN-λ4 activates JAK/STAT signalling pathway and induces ISGs. A, Effect of IFN-λ4 on STAT1 phosphorylation. Macrophages treated with IFN-λ4 (250 ng/mL) were collected at 0, 5, 15, 30, 60, 120 and 240 min post-treatment. Proteins were extracted and subjected to Western blot analysis using antibodies against STAT1, p-STAT1 and GAPDH. (B, C) IFN-λ4 induces p-STAT1 expression. Macrophages were treated with IFN-λ4 at the indicated concentrations or IFN-λ1 (100 ng/mL) for 30 min. Protein were extracted for Western blot analysis using antibodies against p-STAT1 and GAPDH. Results are representative of three experiments with cells from three donors. $^{**}P < 0.01$. D, IFN-λ4 induces ISGs mRNA expression. Macrophages were treated with or without IFN-λ4 for 12 h. Total RNA was extracted and subjected to reverse transcription, followed by the real-time PCR for GBP5, ISG56 and Viperin mRNA quantification. Results are representative of three experiments with cells from three donors. $^{*}P < 0.05$; $^{**}P < 0.01$. (E, F) IFN-λ4 induces ISGs protein expression. Macrophages were treated with or without IFN-λ4 for 24 h. Proteins were extracted for Western blotting analysis using antibodies against GBP5, ISG56, Viperin and GAPDH. Results are representative of three experiments with cells from three different donors. $^{*}P < 0.05$; $^{**}P < 0.01$

inhibit HIV replication,[8-10] it remains to be determined whether IFN-λ4 possesses the anti-HIV function. In the present study, we treated macrophages with different doses of IFN-λ4 before, simultaneous or after HIV infection. HIV RT activities reduced in macrophage culture supernatant at days 3, 6 and 9 post-infection when treated with IFN-λ4 24 hours prior to the viral infection (Figure 1A). HIV inhibition was also observed in the macrophage cultures treated with IFN-λ4 during or after HIV infection (Figure 1B). These finding demonstrated that IFN-λ4 at non-cytotoxic concentrations could effectively and dose-dependently inhibit HIV replication in primary human macrophages.

It has been demonstrated that IFN-λ1, 2 or 3 acts through a cell-surface receptor complex composed of two chains, IFN-λR1 and IL-10R2.[17] Considering IFN-λ4 genetic sequence has low sequence similarity to other members of IFN-λ family in the region that interacts with IL-10R2, it has been speculated that IL-10R2 is not involved in IFN-λ4-mediated the JAK/STAT signalling pathway.[4] To determine whether IFN-λ4 functions through this receptor complex, we performed a blocking experiment with the antibodies against IFN-λR1 and IL-10R2. As shown in Figure 1C, the preincubation of the cells with either IFN-λR1 or IL-10R2 antibody largely blocked the anti-HIV activity of IFN-λ4. Blocking both IFN-λR1 and IL-10R2 receptors by the antibodies almost completely reversed the inhibitory effect of IFN-λ4 on HIV (Figure 1C).

It is known that IFN-λs bind to the IFN-λ receptor complex and activate the JAK-STAT signalling pathway, inducing a number of antiviral ISGs.[1] To confirm the effect of IFN-λ4 on the JAK-STAT signalling pathway, we measured the levels of phosphorylated STAT1 (p-SAT1) and several anti-HIV ISGs (GBP5, ISG56 and Viperin), which are the key elements in host cell innate immunity against

HIV,[18] in IFN-λ4-treated macrophages. The middle concentration of IFN-λ4, 250 ng/mL, was used as a representative dose for reducing the costs. As shown in Figure 2A, there was a rapid increase in p-STAT1 protein during the course of IFN-λ4 treatment. The highest levels of p-STAT1 were observed at 30 minutes post-treatment (Figure 2A). This effect of IFN-λ4 on p-STAT1 expression was dose-dependent (Figure 2B,C). Subsequently, macrophages expressed higher GBP5, ISG56 and Viperin at both mRNA and protein levels after IFN-λ4 treatment (Figure 2D-F).

In conclusion, this is the first study to reveal that IFN-λ4 can inhibit HIV infection of macrophages. Although the precise cellular and molecular mechanisms remain to be studied, the induction of key anti-HIV ISGs via activated JAK/STAT signalling pathway should account for much of IFN-λ4-mediated HIV inhibition. Further studies are necessary in order to determine the effect of IFN-λ4 on HIV in ex vivo and in vivo systems.

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CONFLICT OF INTERESTS

The authors of this manuscript have no conflict of interests to disclose.

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