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

Qi-Jian Su1 | Xu Wang2 | Run-Hong Zhou3 | Le Guo3 | Hang Liu3 | Jie-Liang Li2 | Wen-Zhe Ho2,3

1Ruikang Hospital, Guangxi University of Chinese Medicine, Nanning, Guangxi, China
2Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, Pennsylvania
3School of Basic Medical Sciences/State Key Laboratory of Virology, Wuhan University, Wuhan, Hubei, China

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. 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. But there was also evidence supported that IFNL4 genotype is not associated with the antiviral interferon (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-λ R1 and IL-10R2 antibodies and sheep IgG were purchased from R & D Systems.

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. 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. To determine the role of IFN-λ1/IFN-λ1 receptor complex in IFN-λ4 mediated anti-HIV activity, the binding of IFN-λ4 to its receptor was blocked using antibodies to IFN-λ1 and IL-10R2. IFN-λ1 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.

2.4 | HIV RT assay

HIV RT activity analysis was performed as described previously. 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 (32P)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

TABLE 1 Primers used in the real-time PCR

| Target gene | Primer | Nucleotide sequence |
|-------------|--------|---------------------|
| GAPDH       | Forward| 5’-GGTGGTCTCTCTGACTTCAACA-3’ |
|             | Reverse| 5’-GGTCTGTCAGCCCAATTCTGCTGT-3’ |
| ISG56       | Forward| 5’-TTGGTCTCTGACTTCAACAATCTGCTGT-3’ |
|             | Reverse| 5’-TTGGTCTCTGACTTCAACAATCTGCTGT-3’ |
| Viperin     | Forward| 5’-TTGAGTCTCTGACTTCAACAATCTGCTGT-3’ |
|             | Reverse| 5’-TTGAGTCTCTGACTTCAACAATCTGCTGT-3’ |
| GBP5        | Forward| 5’-TCATGTTATTAACAGTCCTCTGG-3’ |
|             | Reverse| 5’-TCATGTTATTAACAGTCCTCTGG-3’ |
| APOBEC3G    | Forward| 5’-AGCAGGACCCAGGTATTCAGGACTTAC-3’ |
|             | Reverse| 5’-AGCAGGACCCAGGTATTCAGGACTTAC-3’ |
| APOBEC3F    | Forward| 5’-TTGGTCTCTGACTTCAACAATCTGCTGT-3’ |
|             | Reverse| 5’-TTGGTCTCTGACTTCAACAATCTGCTGT-3’ |
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

Statistical 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...
inhibit HIV replication,\textsuperscript{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 findings 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{18} in IFN-\(\lambda\)-treated macrophages. The middle concentration of IFN-\(\lambda\), 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-\(\lambda\) treatment. The highest levels of p-STAT1 were observed at 30 minutes post-treatment (Figure 2A). This effect of IFN-\(\lambda\) 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-\(\lambda\) treatment (Figure 2D-F).

In conclusion, this is the first study to reveal that IFN-\(\lambda\) 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-\(\lambda\)-mediated HIV inhibition. Further studies are necessary in order to determine the effect of IFN-\(\lambda\) on HIV in ex vivo and in vivo systems.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (grant numbers 81360258, 81571962), and in part by grants from the National Institutes of Health (grant numbers DA022177, DA041302, DA 040329, MH109385).

CONFLICT OF INTERESTS

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

ORCID

Qi-Jian Su \(\text{http://orcid.org/0000-0002-6194-0317}\)

REFERENCES

1. Fensterl V, Sen GC. Interferons and viral infections. BioFactors. 2009;35:14-20.
2. Egli A, Santen DM, O’Shea D, et al. The impact of the interferon-lambda family on the innate and adaptive immune response to viral infections. Emerg Microbes Infect. 2014;3:e51.
3. Sheppard P, Kindsvogel W, Xu W, et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol. 2003;4:63-68.
4. Prokunina-Olsson L, Muchmore B, Tang W, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet. 2013;45:164-171.
5. Real LM, Herrera R, Rivero-Juárez A, et al. IFNL4 rs368234815 polymorphism is associated with innate resistance to HIV-1 infection. AIDS. 2015;29:1895-1897.
6. Machmach K, Abad-Molina C, Romero-Sa’nchez MC, et al. IFNL4 ss469415590 polymorphism is associated with unfavourable clinical and immunological status in HIV-infected individuals. Clin Microbiol Infect. 2015;21:289, e1-4.
7. Monteleone K, Scheri GC, Statzu M, et al. IFN-stimulated gene expression is independent of the IFNL4 genotype in chronic HIV-1 infection. Arch Virol. 2016;161:3263-3268.
8. Wang YZ, Li JL, Wang X, et al. Comparison of antiviral activity of lambda-interferons against HIV replication in macrophages. J Interferon Cytokine Res. 2015;35:213-221.
9. Hou W, Wang X, Ye L, et al. Lambda interferon inhibits human immunodeficiency virus type 1 infection of macrophages. J Virol. 2009;83:3834-3842.
10. Liu MQ, Zhou DJ, Wang X, et al. IFN-\(\lambda\) inhibits HIV infection of macrophages through the JAK-STAT pathway. PLoS One. 2012;7:e35902.
11. Hassan NF, Campbell DE, Douglas SD. Purification of human monocytes on gelatin-coated surfaces. J Immunol Methods. 1986;95:273-276.
12. Wang X, Ye L, Hou W, et al. Cellular microRNA expression correlates with susceptibility of monocytes/macrophages to HIV-1 infection. Blood. 2009;113:671-674.
13. Li J, Hu S, Zhou L, et al. Interferon lambda inhibits herpes simplex virus type I infection of human astrocytes and neurons. GLia. 2011;59:58-67.
14. Willey RL, Smith DH, Lasky LA, et al. In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. J Virol. 1988;62:139-147.
15. Hammering OJ, Terczyńska-Dyla E, Vieyres G, et al. Interferon lambda 4 signals via the IFN\(\alpha\)/IFN\(\beta\) receptor to regulate antiviral activity against HCV and coronaviruses. EMBO J. 2013;32:3055-3065.
16. Hong M, Schwerk J, Lim C, et al. Interferon lambda 4 expression is suppressed by the host during viral infection. J Exp Med. 2016;213:2539-2552.
17. Kotenko SV, Gallagher G, Baurin VV, et al. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol. 2003;4:69-77.
18. Ma TC, Zhou RH, Wang X, et al. Soybean-derived Bowman-Birk inhibitor (BBI) inhibits HIV replication in macrophages. Sci Rep. 2016:6:34752.

How to cite this article: Su Q-J, Wang X, Zhou R-H, et al. IFN-\(\lambda\) inhibits HIV infection of macrophages through signalling of IFN-\(\lambda\)R1/IL-10R2 receptor complex. Scand J Immunol. 2018;88:e12717. https://doi.org/10.1111/sji.12717