Int.J.Curr.Microbiol.App.Sci (2019) *8*(9): 2585-2589

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 09 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.809.299

Expression of pcDNA-Interferon Lambda-3 (pcDNA-IFNλ3)

S. Barathiraja*, Lijo John, N. Vijay, P. A. V. Gangadhara and G. R. Reddy

Department of Veterinary Biochemistry, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Kurumbapet, Puducherry- 605 009, India

**Corresponding author*

Keywords

Bovine Interferon Lambda3; pcDNA-IFNλ3, Mice; RT-PCR

Article Info

Accepted:
24 August 2019
Available Online:
10 September 2019

A B S T R A C T

Type III Interferons also known as Interferon lambdas (IFNλs/IFNLs) constitute a recently described IFN with antiviral activities by signaling through a unique receptor complex composed of IFNλs receptor 1 (IFNλR1) and interleukin-10 receptor 2 (IL10R2). It also found to have more of immune modulatory activities in addition to antiviral activities. In the present study, the construct pcDNA-bovine IFNλ3 was administered intramuscularly to mice. The mice were sacrificed on 7^{th} and 14^{th} day to collect the different organs/tissues. The tissues were used to isolate total RNA and following cDNA conversion. Then, the tissues were analyzed for the expression of IFNλ gene by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). The results had shown that there was expression of given IFNλ3 in organs: leg muscle, liver and spleen and also in blood.

Introduction

Type III Interferons (IFNs), also known as IFN Lambdas (IFNλs/IFNLs) were described as members of a new cytokine family (Kotenko *et al.*, 2003). IFNλs signals through a heterodimeric receptor, IFNλR1 and IL-10R2 and plays significant role in antiviral activity and also involve in initiation of immune response. Unlike the ubiquitously expressed type I and II IFN receptor complexes, type III IFNλR1 which is expressed predominantly in epithelial cells has more specialized role in the immediate immune response at the sites of virus entry (Hamming *et al.*, 2010). This has added advantageous in comparison to Type I cytokines in terms of specific site of action. IFNλ action is mediated by binding of receptor complex by the cytokine (IFNλ) which indices signal transduction by initiation of pre-associated Janus tyrosine kinases (JAK1 and TYK2), which phosphorylate receptor chain enabling recruitment and

phosphorylation of STAT (Signal Transducer and Activator of Transcription**)**. STAT heterodimers associate with Interferon regulatory factor 9 (IRF9) forming the ISGF3 (Interferon Stimulated Gene Factor 3). These complexes translocate to the nucleus to induce IFN-stimulated genes (ISGs) from ISRE or promoter elements (Sadler and Willams, 2008).

Human IFNλ acts as an immunomodulator of host immune system by modulating Th1/Th2 responses by altering the concentrations of IL-6, IL-8 and IL-10 in human PBMCs (Jordan *et al.,* 2007). Dellgren *et al.,* (2009) reported that IFNλ3 is 16 times more active than IFNλ2 and 2 times more active than IFNλ1.

So, IFNλ3 may better inhibit the initial replication of a virus due to a longer lasting anti-viral effect of induced ISGs which positively affect spontaneous clearance of virus (Egli *et al.,* 2014). There are many reports on expression and antiviral activities of human IFNλ3 but only few regarding bovine IFNλ3. So, the study was planned to study the in vivo expression of pcDNA-IFNλ3 in mice after 1st and 2nd weeks of administration. This positive expression studies will further help to study its antiviral effects and immune modulatory effects in further studies.

Materials and Methods

Mice

Mice used in our study were approved by Institutional Animal Ethics Committee, Bangalore, India. Male Balb/c mice (6 weeks old) were obtained from experimental animal facility in Indian Institute of Science, Bangalore and provided with *ad libitum* food and water. All mice were kept under observation for 7 days before treatment. Mice were observed daily during the treatment and there was no adverse events occurred.

Experimental conditions

Cationic Poly (lactic-co-glycolic acid) (PLG) particles coated with DNA (pcDNA-IFNλ3) were prepared by solvent extraction process using methylene chloride (Singh M *et al.,* 2000). Mice were divided into two groups where each group had six animals viz. control group (I), PLG/pcDNA-bovine IFNλ3 group (II). Mice in group I were given only PLG suspended in 1X Phosphate Buffered Saline (PBS) and group II was given 10µg PLG/pcDNA-bovine IFNλ3 suspended in 1X PBS intra-muscularly on left leg. The animals were sacrificed on 2nd, 7th and 14th day for the analysis of IFNλ3 gene expression in blood and tissues.

IFNλ3 gene expression in tissues

Blood and Tissue collection

Mice were anesthetized with pentobarbital sodium and blood was collected from retro-orbital route. Collected blood sample was used for separation of White blood cells (WBC) and stored in TRIzol (Invitrogen, USA). After that, mice were sacrificed by exsanguination through cutting cervical artery under anaesthesia. The abdomen of the anaesthetised mice was opened and spleen was located and separated from rest of the viscera.

Other organs namely muscle injected (left), muscle opposite (right), heart and kidney were also separated. The collected organs were washed three times in sterile 1XPBS. The collected organs were homogenized in ice cold conditions and stored in TRIzol for RNA isolation.

RNA extraction and RT-PCR analysis

Total RNA was extracted from the collected tissue and blood samples and cDNA was synthesized using oligodT primer and Reverse

transcriptase enzyme. Polymerase Chain Reaction (PCR) was run with the following conditions: Initial denaturation 94ºC for 5min and 35 cycles denaturation 94ºC for 30sec, annealing 58ºC for 30sec and extension 72ºC for 40sec with the final extension 72ºC for 10 min. The primers used in the study include IFNλ3-F-Mat: TATCTCGAGGACA CACT GGTCTCCGCTG and IFNλ3-R (NotI): GGCGGCGGCCGCTCAGACACACTGGT CTCC which will amplify 585bp of gene. Then, the amplified product was analyzed by 1% agarose gel electrophoresis for the expression of bovine IFNλ3 gene.

Results and Discussion

A biodegradable microparticle with a cationic surface has added advantage that it improves the delivery of adsorbed DNA into antigen-presenting cells especially after intramuscular injection (Singh *et al.,* 2000). So, in the present study is done using PLG coated with DNA for analysis.

During the present study, pcDNA-bovine IFNλ3 construct was analyzed for its gene expression following intramuscular injection into mice. The organs or tissues namely muscle injected (left), muscle opposite (right), heart and kidney were homogenized separately in ice cold conditions and stored in TRIzol.

The tissues stored in Trizol were used for RNA isolation. Then, cDNA was prepared from the total RNA and further subjected for PCR to analyze expression of bovine IFNλ3 gene.

The PCR samples were run on 1% agarose gel electrophoresis for analysis. Expression of gene was observed in blood on 7th and 14th day post administration of pcDNA-bovine IFNλ3 construct. There was expression of gene on 2nd day onwards in leg muscle administered (leg) and other (right) (Fig. 1).

Fig. 1IFNλ3 gene expression in blood and muscle: WBC (Blood): *Lane1*:control. *Lane2*: day7. *Lane3*:day14. Muscle: *Lane4:* control (left muscle). *Lane5*: muscle right (day2). *Lane6*: Muscle left (day2). *Lane7*: muscle right (day7). *Lane9*: muscle left (day7). *Lane10*: muscle right (day14). *Lane11*: muscle left (day14). *Lane12:* 1Kbp DNA ladder.

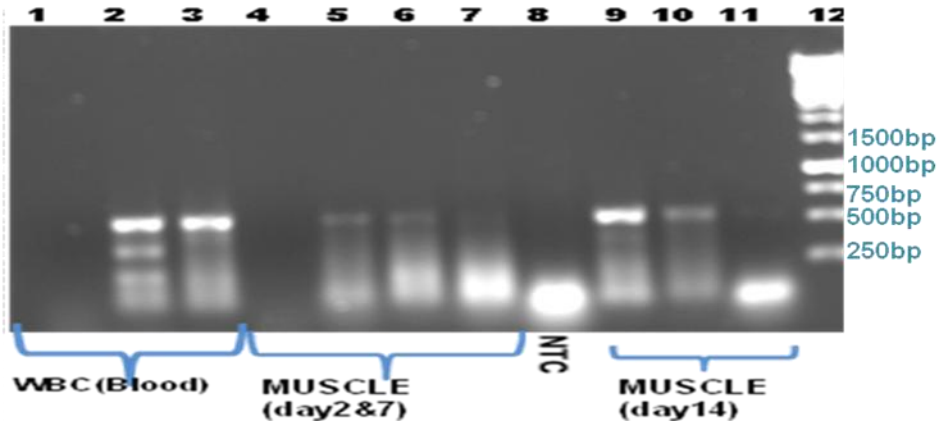
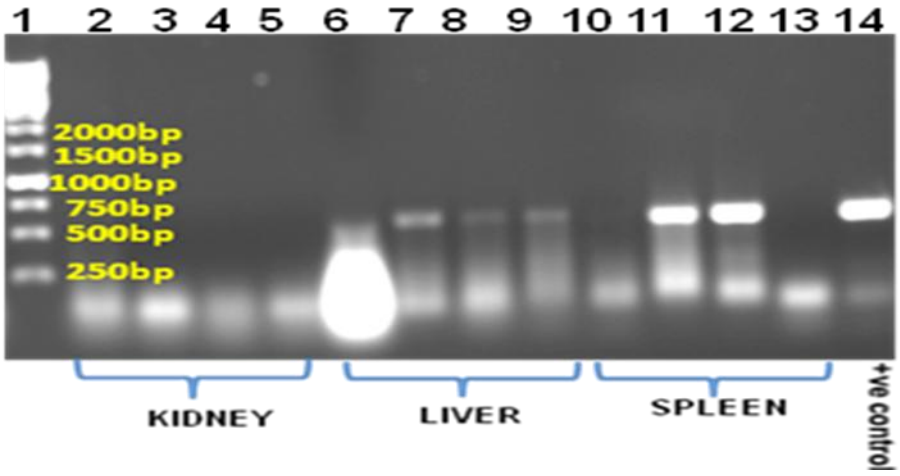


Fig. 2 IFNλ3 gene expression in Kidney, liver and Spleen: *Lane1:* 1Kbp DNA ladder. Kidney: *Lane2*: control. *Lane3*:day2. *Lane4*:day7. *Lane5*:day14. Liver: *Lane6*: control. *Lane7*:day2. *Lane8*:day7. *Lane9*:day14. Spleen: *Lane10*: control. *Lane11*:day7. *Lane12*:day14. *Lane 13:* non-template control (NTC). *Lane14*: positive control.



Expression of IFNλ3 gene was also analyzed in kidney, liver and spleen. Expression of gene was more prominent in spleen as spleenocytes have shown higher expression at mRNA level in comparison to liver. During our study, we could not found expression of bovine IFNλ3 gene in kidney (Fig.2).

Plasmids coated on PLG microparticle were used for intramuscular delivery to mice and the expression of construct gene was analyzed in blood (WBC), muscle, kidney, liver and spleen. Tissue collected at 2^{nd}, 7^{th} and 14^{th} day post inoculation was analyzed for bovine IFNλ3 gene expression.

RT-PCR results shown that there was mRNA expression of given pcDNA-IFNλ3 construct in organs: leg muscle, liver and spleen and also in blood. Reddy and coworkers (2011) reported the expression of pcDNA construct in guinea pig muscle seventh day onwards by qPCR.

Present results had shown the successful expression of the bovine IFNλ3 gene in blood and tissues especially after one week of administration. This will further help to use the DNA construct as antiviral agent or immune modulatory agent during animal studies.

Acknowledgement

The authors acknowledge the Director, IVRI (Indian Veterinary Research Institute) Izatnagar and Joint Director, IVRI, Bangalore for providing facilities. The first author also acknowledges ICAR (Indian Council of Agricultural Research) India for the ICAR-Senior Research Fellowship.

References

Dellgren, C., Gad, H.H., Hammin O.J., Melchjorsen, J. and Hartmann, R. 2009. Human interferon- lambda 3 is a potent member of type III interferon family, *Genes and Immunity.*, 10: 125-31.

Egli, A., Santer, D.M., Shea, D.O., Tyrrell, D.L., Houghton, M. 2014. The impact of the interferon λ family on the innate and adaptive immune response to viral

infections. *Emerging Microbes and Infections*., 3:-12.

Hamming, O.J., Gad, H.H., Paluda, S, Hartmann, R. 2010. λ Interferons: New Cytokines with Old Functions. *Pharmaceuticals*., 3(4):795-809.

Jordan, W.J., Eskdale, J., Srinivas, S. 2007. Human interferon λ1 (IFN-λ 1/IL- 29) modulates the Th1/Th2 response. *Genes Immun*. 8:254–261.

Kotenko, S.V., Gallagher, G., Baurin, V.V., Lewis-Antes, A., Shen, M.L. 2003. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nature Immunology*., 4: 69–77.

Reddy, S.K., Rashmi, B.R., Dechamma, H.J., Gopalakrishna, S., Banumathi, N., Ganesh K., Suryanarayana V.V.S., and Reddy, G.R. 2011. Improved immune response by ID-pVAC: A secretory DNA vaccine construct delivered by PLG micro particles against foot and mouth disease in guinea pigs. *Research in Veterinary Science*., 1: 86–89.

Sadler, A.J., and Williams, B.R. 2008. Interferon-inducible antiviral effectors. *Nature Reviews Immunology*., 8(7):559-68.

Singh, M., Briones, M., Ott G., O'hagan, D. 2000. Cationic micro particle: A potent delivery system for DNA vaccines. Proc. Nat. Acad. Sci., 97(2): 811:816.

How to cite this article:

Barathiraja, S., Lijo John, N. Vijay, P. A. V. Gangadhara and Reddy, G. R. 2019. Expression of pcDNA-Interferon LAMBDA-3 (pcDNA-IFN 3) *Int.J.Curr.Microbiol.App.Sci.* 8(09): 2585-2589. doi: https://doi.org/10.20546/ijcmas.2019.809.299