The Pharma Innovation Journal 2023; 12(4): 1844-1845

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 1844-1845

www.thepharmajournal.com
Received: 10-02-2023
Accepted: 13-03-2023

Akanksha Gupta
Ph.D. Scholar, Department of Animal Physiology, National Dairy Research Institute Karnal, Haryana, India

AK Dang
P.S and Head of Department of Animal Physiology, National Dairy Research Institute Karnal, Haryana, India

Corresponding Author:
Akanksha Gupta
Ph.D. Scholar, Department of Animal Physiology, National Dairy Research Institute Karnal, Haryana, India

Studies of IFNG levels on LPS-treated PBMCS in Karan fries heifers

Akanksha Gupta and AK Dang

Abstract
To study the *in vitro* effect of LPS on PBMCs, apparently, healthy KF heifers were selected. To achieve this objective, the duration of exposure and different concentration of, LPS in PBMCs culture was used and IFNG levels were estimated. It was found that at 200 ng/ml dose rate of LPS IFNG levels were elevated with the increase in incubation hours. At 2, 4 hr incubation, the level of IFNG was high. Therefore, we can say with the increase in the duration of exposure of PBMCs IFNG levels increased.

Keywords: LPS, PBMCs, IFNG, *in vitro*

1. Introduction
Peripheral blood mononuclear cells (PBMCs) are the sentinels of the innate immune system (Asehnoune *et al.,* 2004) [2] and include monocytes, natural killer (NK) cells, and T and B lymphocytes. Lipopolysaccharide (LPS) is the most abundant component within the cell wall of Gram-negative bacteria. It can stimulate the release of IFNG and other inflammatory cytokines in various cell types, leading to an acute inflammatory response towards pathogens. We evaluated plasma inflammatory cytokine levels as well as the production of inflammatory cytokines produced by the peripheral blood mononuclear cells (PBMCs) upon lipopolysaccharide (LPS) stimulation.

2. Material and Methods
To study the *in vitro* effect of LPS on PBMCs, apparently healthy KF heifers were selected. To achieve this objective, the duration of exposure and different concentration of, LPS in PBMCs culture was used and IFNG levels were estimated.

2.1 Ethical permission
The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of ICAR-National Dairy Research Institute (Application no 41-IAEC-18-32) constituted as per the article 13 of the CPCSEA rules, laid on by the Government of India (Reg.No.1705/GO/AC/13/CPCSEA dated. 3/7/2013). All the ethical guidelines were followed throughout the experiment.

2.2 Processing of blood samples
The isolation of PBMCs from whole blood was performed by the gradient density centrifugation method using histopaque solutions 1119 (Catalog No. 11191, Sigma Aldrich, St Louis, MO, USA) in 15 ml polypropylene Falcon tubes, reported by (Panda *et al.,* 2020) [4] with little modifications. Briefly about 3 ml of histopaque 1077 was carefully layered on top of 3ml of histopaque 1119 in a 15 ml polypropylene falcon tube. Subsequently, 6ml of the fresh blood was decanted on this discontinuous density gradient. The tube was centrifuged at 900 g for 40 minutes at 27 °C. Once the centrifugation was complete, the PBMCs phase containing lymphocytes and monocytes was removed carefully and completelypette. Separate polypropylene Falcon tubes containing PBMCs fractions were diluted with double the volume of Dulbecco's phosphate buffer saline (DPBS) (Catalog No. TS1006, Himedia, India Pvt. Ltd) without Ca2+ and Mg2+. The cellular suspensions were then simultaneously centrifuged at 750 g for 12 minutes at 4 °C. The supernatant was decanted and RBC lysis buffer (Himedia, India Pvt. Ltd) was added to the pellet and re-suspended. The suspension was then centrifuged at 750 g for 10 minutes at 4 °C. The PBMCs were cultured and treated with LPS at different dose rates and duration. IFNG in the supernatant of cultured PBMCs treated with LPS at

200 ng/ml in different time intervals was determined by "Bovine interferon α ELISA Kit" (Catalog Number. CSB-E0005Bo) supplied by Bioassay technology laboratory. This assay employed the competitive enzyme immunoassay technique. The sensitivity of the assay was less than 2.35 pg/ml with a detection range of 5-2000 pg/ml. Inter assay CV was 10% and intra assay CV was 8%.

Table 1: Incubation time and dose of LPS in PBMC culture

Incubation time (hours)	1	2	4
Concentration of LPS (ng/ml)	200	200	200

2.3 Statistical analyses
Statistical analyses were performed using the Graph Pad Prism software. All the data were expressed as mean ± standard error of the mean (S.E.M.) and were analyzed by one-way analysis of variance (ANOVA).

3. Result
To evaluate the effect of 200 ng/ml LPS on IFNG in PBMCs culture incubated at 1, 2, 4 hr interval. Fig 2 graphically represents the results of *in vitro* culture of LPS in blood PBMCs culture. IFNG levels did not show any change at 1hr incubation. The levels of IFNG were highly significant ($p \leq 0.01$) at 2, 4 hr interval with respect to control.

Table 2: Effect of 200 ng/ml LPS on PBMCs at 0, 1, 2, 4 hr interval

Time	IFNG (pg/ml)
0	5.16±2.4
1	5.99±2.64
2	22.5±5.24**
4	32.3±3.04**

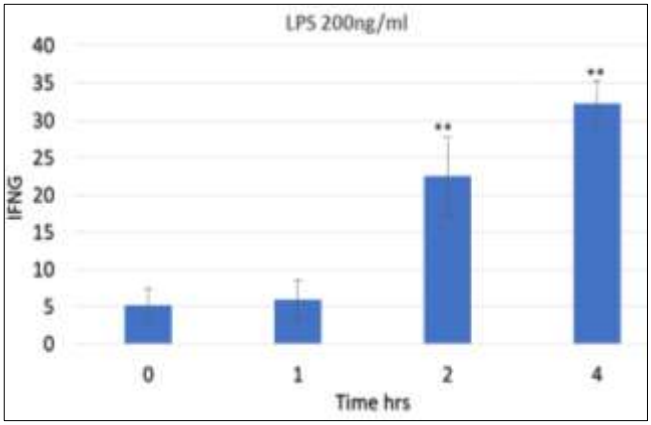

Fig 1: Effect of 200 ng/ml LPS on PBMCs at 0, 1, 2, 4 hr interval

4. Discussion
IFNG is mainly secreted by activated T cells and natural killer (NK) cells, and can promote macrophage activation, mediate antiviral and antibacterial immunity, enhance antigen presentation, activation of the innate immune system, regulate Th1/Th2 balance, and control cellular proliferation and apoptosis (BIlliau *et al*., 1996) [3]. IFNG potentiate the effects of LPS, but the mechanism is not thoroughly understood. Previous reports emphasized the ability of IFN-γ to up-regulate CD14 expression (the receptor for LPS), and nearly all studies have utilized sequential stimulation with IFN-γ followed by LPS to exploit this phenomenon. This study demonstrates that IFN-γ can up-regulate the effect of LPS at the level of transcription (Lee *et al*., 2001) [1]. In our study we evaluated the effect of 200 ng/ml LPS on IFNG in PBMCs culture incubated at 1, 2, 4 hr interval. Fig 2 graphically represents the results of *in vitro* culture of LPS in blood PBMCs culture. IFNG levels did not show any change at 1hr incubation. The levels of IFNG were highly significant ($p \leq 0.01$) at 2, 4 hr intervals with respect to control.

5. Conclusions
It was found that at 200 ng/ml dose rate of LPS IFNG levels were elevated with the increase in incubation hours. At 2, 4 hr incubation, the level of IFNG was high. Therefore we can say with the increase in the duration of exposure of PBMCs IFNG levels are increased.

6. Funding sources
This study was supported by the Department of Biotechnology (Grant No. BT/ PR23570/ AAQ/ 1/ 691/ 2017; Dated: 10/05/2018), Ministry of Science and Technology, Government of India.

7. Declaration of Competing Interest
The authors declare no conflict of interest, financial or otherwise.

8. Acknowledgment
The authors thank the Director of the Institute for providing all the facilities to carry out this research work.

9. References
1. Lee JY, Sullivan KE. Gamma interferon and lipopolysaccharide interact at the level of transcription to induce tumor necrosis factor alpha expression. Infection and immunity. 2001;69(5):2847-2852. https://doi.org/10.1128/IAI.69.5.2847-2852.2001.
2. Asehnoune K, Strassheim D, Mitra S, Kim JY, Abraham E. Involvement of reactive oxygen species in Toll-like receptor 4-dependent activation of NF-κB. The Journal of Immunology. 2004;172(4):2522-2529. https://doi.org/10.4049/jimmunol.172.4.2522.
3. Billiau A. Interferon-γ: biology and role in pathogenesis. Advances in immunology. 1996;62:61-130. https://doi.org/10.1016/S0065-2776(08)60428-9.
4. Panda PK, Arul MN, Patel P, Verma SK, Luo W, Rubahn HG, Mishra YK, Suar M, Ahuja R. Structure-based drug designing and immunoinformatics approach for SARS-CoV-2. Science advances. 2020 Jul 10;6(28):eabb8097.