Investigating the mRNA expression of IL-8 in Response to Lipopolysaccharide (LPS) and Lipoteichoic Acid (LTA) Stimulation in Sheep Alveolar Macrophages,

A. Q. Naji[1, 2], Md M. Hasan Sohel[3], M. Ulaş Çınar[4]

[1]*Iraqi Ministry of Agriculture, State Company for Agricultural Supplies, Babylon Branch, Iraq*
[2]*Department of Agricultural Science and Technologies, Graduate School of Natural and Applied Sciences, Erciyes University, Kayseri,* Turkey
[3] *Department of Genetics, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey*
[4] *Department of Animal Science, Faculty of Agriculture, Erciyes University, Kayseri, Turkey*
Corresponding Author: e-mail: qasimnaji73@gmail.com

Introduction

The immune system of an organism is the most important defense system against harmful pathogens and this system is tightly regulated by the timely expression of several genes. Interleukin-8 (*IL-8*) is a chemoattractant cytokine produced by a variety of tissue and blood cells. It has a distinct target specificity for the neutrophil cells. *IL-8* attracts and activates neutrophils in inflammatory regions. The aim of present study was to investigate the dynamics of expression of *IL-8* in response to LPS and LTA in different time points which could later be used to select sheep against respiratory diseases effectively (Janette and Etheresia, 2016).

Material and Methods

In the current study, we employed both *in vitro* and *in vivo* experimental setup. In the case of *in vitro* study two different time points (4 and 24 h) have been used. After the collection and purification of alveolar macrophages cells (AMs), they were stimulated with LPS 10 ug/ml, LTA 10 ug/ml and with the combination of LPS 10 ug/ml + LTA 10 ug/ml and left for 4 h. Then, total RNA extraction, cDNA synthesis and quantitative Real-Time PCR were performed to find out the dynamics of mRNA expression of *IL-8*.

For *in vivo* study, a total of twenty-four healthy lambs from two different breeds, 12 Akkaraman and 12 Romanov lambs, were used in this study. The animals in the treatment groups were exposed to LPS (20 µg/kg), LTA (500 µg/kg) and with the combination of LPS + LTA (20+ 500 µg/kg), while the control group was given 500 µg/kg of PBS (1X). After 24 h the lamb was euthanized, then, from the collected lungs the BAL fluid was isolated and the alveolar macrophages cells were purified, then total RNA extraction, cDNA synthesis and quantitative Real-Time PCR were performed to find out the level of mRNA expression of *IL-8* (Islam *et al.* 2013).

Results

In vitro study: 4 h exposure of AMs cells to LPS, LTA and a combination of LPS+LTA treatments resulted in a significant increase in the expression of mRNA level of *IL-8* compared to control. In addition, there were significant differences in the expression levels of *IL-8* between 4 h and 24 h trials in response to LPS and a combination of LPS + LTA, were 4 h treatment showed higher levels of *IL-8* expression than 24 h treatment, but there were no significant differences mRNA expression of *IL-8* with LTA between 4 h and 24 h trials.

In vivo study: When the Romanov and Akkaraman lambs were exposed to LPS or LTA or a combination of LPS + LTA, the expression of *IL-8* in alveolar macrophages was significantly higher than that of control groups after 24 h of treatment. Furthermore, *IL-8* resulted in a significant difference in the mRNA expression levels in Akkaraman and Romanov lambs in response to LPS or LTA or a combination of LPS + LTA after 24 h of the treatment. were Romanov lambs showed higher levels of *IL-8* expression than Akkaraman lambs after 24 h treatment.

Discussion

In vitro study: In 4 h trial, the exposure of AMs to LPS, LTA and with the combination of LPS + LTA treatments resulted in a higher mRNA expression of *IL-8* compared to 24 h trial. This is in accordance with previous findings which indicate that the production of *IL-8* secreted at high levels between 4 h and 8 h after activation of the monocytes (Kang *et al*. 2002).

In vivo study: The results of our study indicated that the Romanov lambs present high and significant mRNA expression levels differences of *IL-8* compared with Akkaraman lambs in exposure to LPS or LTA or a combination of LPS + LTA after 24 h of the treatment. Previous study showed that macrophages and other cells such as endothelial cells, airway smooth muscle cells, and macrophages can produce *IL-8*. As well as, *IL-8* can be involved in most acute and chronic inflammatory diseases and several infections which is acute in nature (Konrad and Reutershan, 2012).

Conclusion

Our study clearly demonstrated that stimulation of alveolar macrophages with LPS, LTA and LPS + LTA in *in vitro* for 4 h treatment significantly increase the expression of *IL-8* compared to 24 h treatment. In addition, the in vivo experiments revealed that the Akkaraman lambs showed higher resistant to respiratory diseases than Romanov lambs, this was clear from the higher and significant mRNA expression levels of *IL-8* presented in Romanov lambs in response to all treatments compared with Akkaraman lambs after 24 h of treatments.

Acknowledgements

Authors are indebted to staff of Betül-Ziya Eren Genome and Stem Cell Research Center at Erciyes University for their helps during experiments. This study was supported by Erciyes University Scientific Research Projects Unit under the code of FDK-2017-7657.

References

Islam, M.A., Pröll, M., Hölker, M., Tholen, E., Tesfaye, D., Looft, C., Schellander, K., Cinar M.U. 2013. Alveolar macrophage phagocytic activity is enhanced with LPS priming, and combined stimulation of LPS and lipoteichoic acid synergistically induce pro-inflammatory cytokines in pigs. Innate Immunity, 19 (6): 631-643.

Janette. B. and Etheresia. P. 2016. Effects of IL-1β, IL-6 and IL-8 on erythrocytes, platelets and clot viscoelasticity. Scientific Reports, 6: 1–10.

Kang, T.J., Lee, S.B., and Chae, G.T. 2002. A polymorphism in the toll-like receptor 2 is associated with IL-12 production from monocyte in lepromatous leprosy. Cytokine, 20(2): 56-62.

Konrad, F.M. and Reutershan, J. 2012. CXCR2 in acute lung injury. Mediators of Inflammation, 2012: 1-8.