Ref: Ro J Infect Dis. 2023;26(4)
DOI: 10.37897/RJID.2023.4.4

Induction of lipopolysaccharide during pregnancy will suppress IL-6 production but not activin B

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ABSTRACT

During inflammation, proinflammatory cytokines are released by various immune cells including IL-6 and activin B. Production of proinflammatory cytokines can be suppressed by estradiol and an increase in estradiol can be found in pregnancy. The aim of this study was to determine the response of IL-6 and activin B in pregnant mice through LPS induction. There were two groups of mice consisting of 8 mice which were not pregnant and 13 pregnant mice in the 2nd week. Inflammation was induced by intraperitoneal injection of LPS *Escherichia coli* serotype O111:B4. After 4 hours of treatment, the mice serum was collected from their intracardiac blood to measure estradiol, IL-6 and activin B using ELISA. Pregnancy significantly reduced IL-6 levels (P=0.015) but there was no significant difference in estradiol (P=0.169) and activin B (P=0.583) levels. We also found that there was no association of E2 with IL-6 (P=0.637) and activin B (P=0.306). Pregnancy influences the inflammatory response, especially IL-6. This condition can occur because during pregnancy physiological changes occur in the body which involve various complex mechanisms in regulating the immune system.

Keywords: Activin B, Estradiol, Interleukin-6, lipopolysaccharide

INTRODUCTION

Inflammation is known as a complex series of response changes that are beneficial in wound healing and infection control or pathology [1]. During inflammation, pro-inflammatory cytokines are released by various immune cells, such as IL-1β (interleukin-1β), IL-6 (interleukin-6), TNF-α (Tumour Necrosis Factor-α), inflammatory proteins and enzymes [2,3].

It has been reported that the concentration of pro-inflammatory proteins in the blood increases after lipopolysaccharide (LPS) induction [4,5]. LPS is one of the bacterial virulent factors that can induce inflammation [6]. LPS is recognized in infected lesions by PRRs (pathogen-recognition receptors) of immune cells such as monocytes and macrophages [7,8]. The interaction of LPS with the receptor will stimulate various signaling pathways, including

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Article History:
Received: 20 December 2023
Accepted: 28 December 2023

NF-κB and increase inflammatory cytokine mRNA transcription including IL-6 [7].

Activin B is a protein that increases during inflammation and has a role as an anti-inflammatory protein, the mechanism of production is not yet understood, but there is evidence that inflammation can induce Activin B [9,10]. However, in activin A which is family with activin B, it has been reported that its production can be induced directly by LPS or through TNF-α and neutrophil intermediaries [11,12].

Various studies report that pregnancy can suppress the inflammatory response [13.14]. Hormonal changes, especially steroid hormones, underlie immunological changes during pregnancy, as they are necessary for reproductive success [13]. E2 is reported to be a hormone that plays an anti-inflammatory role through genomic and non-genomic pathways [15-17].

Inflammatory conditions are capable of activating various pathological signaling leading to cancer, multiple sclerosis, rheumatoid arthritis, anemia, inflammatory bowel disease, Crohn's disease, and Alzheimer's disease [18]. Inflammation that occurs in pregnant women can cause fetal development disorders and even miscarriage [19,20].

The response of IL-6 and Activin B in pregnant women who experience inflammation is still unknown. Uncovering these cytokines in pregnant women experiencing inflammation opens the door to new treatment and management strategies. Taking into account the above-mentioned information, we hypothesized that pregnant women experiencing inflammation have reduced levels of IL-6 and Activin B. To test this hypothesis, we used wild-type pregnant mice.

MATRIALS AND METHODS

Ethical Approval

This research used female mice as an experimental unit and has been approved by the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, with ethical certificate number 2.KE.111.11.2020.

Experimental Animal

This study used non-pregnant female mice (28.8 ± 3.2 grams) and pregnant mice (38.7 ± grams) obtained from the Farma Surabaya Center for Veterinary Medicine, Indonesia. The age of each mouse is ±14 weeks with the day strain. The mating process was carried out by mixing female and male mice with a ratio of 2 females and a male mouse inside the in a cage for 3 days. Pregnancy was checked on sacrificed mice after two weeks of mating by finding the mice fetuses through surgery at the same time as collected laboratory samples.

Female mice in this study were divided into two groups, the first group consisted of 13 female mice with the second week of pregnancy as the treatment group, and the second group consisted of 8 non-pregnant female mice. Each group was injected with LPS (Lipopolysaccharide) *Escherichia coli* serotype O111:B4 (Sigma-Aldrich, Merck, Singapore) in a single dose of 1 μg/g body weight intraperitoneally [10,21].

Sample Collection

Mice were sacrificed at the indicated times, 4 hours after LPS injection. Intracardiac injections were performed to collect the blood sample, which was put into a tube and allowed to freeze for at least 1 hour. The clotted blood was centrifuged at 3000 rpm for 20 minutes to obtain serum.

ELISA

Estradiol, IL-6 and activin B concentrations of mouse were measured by enzyme-linked immunosorbent assay (ELISA). Estradiol levels were measured using Mouse Estradiol ELISA Kit (Cat. No. E0072Mo, Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd., Shanghai, China). IL-6 levels were measured using IL-6 Mouse ELISA Kit (Cat. No. 431307, Legen MaxTM, BioLegend®, San Diego, USA). Activin B levels were measured using Activin B Mouse ELISA Kit (Cat. No. E1813Mo, Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd., Shanghai, China). The measurements were carried out according to the manufacturer's instructions and the analysis was carried out in the molecular biology laboratory, Biosains Institute, Brawijaya University, Malang, Indonesia.

Statistical Analysis

All analyses were performed in IBM SPSS statistics for Windows version 21.0 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro-Wilk test. The difference test was carried out using the independent sample t-test. Apart from that, a correlation test was also carried out using Pearson correlation. A p value of <0.05 was set to determine statistical significance.

RESULTS

Pregnancy suppresses IL-6 production but not activin B and E2 in LPS-induced mice

LPS induction in pregnant mice peritoneally after 4 hours showed a decrease in IL-6 levels (p-value = 0.015) compared to controls that did not experience pregnancy. Activin B, which is a protein that responds during inflammation, showed no difference in levels (p-value = 0.583) in the pregnant and

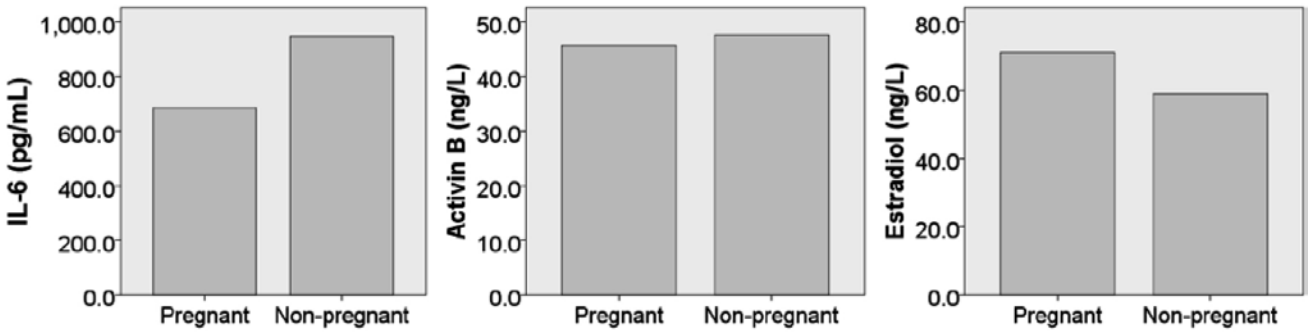

FIGURE 1. LPS-induced response of pregnant mice to IL-6, activin B and E2

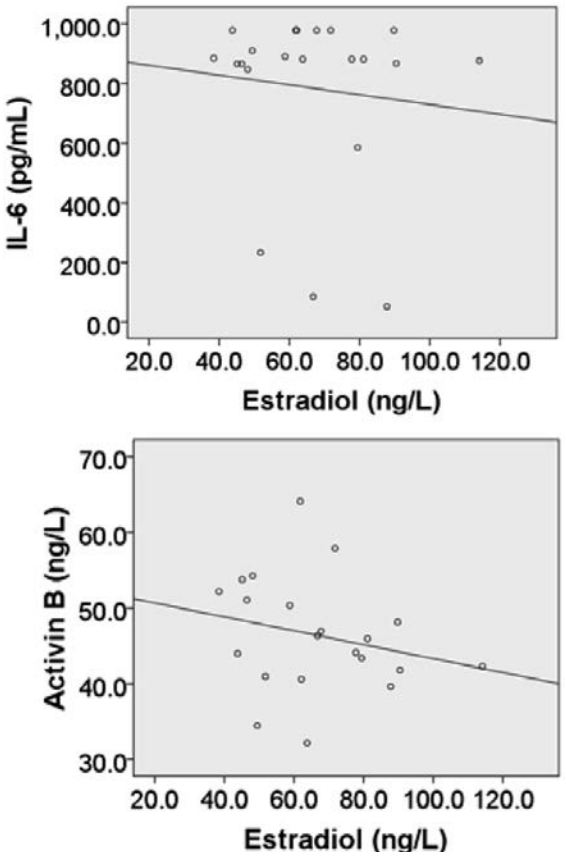

FIGURE 2. Relationship of E2 with IL-6 and activin B

non-pregnant groups. We also found no difference in E2 levels (p-value = 0.169) in the pregnant and non-pregnant groups.

E2 was not associated with IL-6 and activin B level

We performed an analysis of the association of E2 with IL-6 and activin B (Figure 2). E2 showed no significant relationship with IL-6 (p-value = 0.637; r = 0.109) and activin B (p-value = 0.306; r = 0.235). Thus, changes in IL-6 levels in pregnant mice are not caused by changes in E2, let alone activin B.

DISCUSSION

Many studies report that E2 is able to inhibit the inflammatory response. A study by Santos et al (2017) found that E2 in adipocytes showed an anti-inflammatory role, thereby reducing the transcription of TNF-α and IL-6 [16]. Song et al (2019) have proven that E2 is an anti-inflammatory protein [15]. Meanwhile, our research found that there was suppression of IL-6 production in pregnant mice that experienced inflammation, but there was no relationship between E2 and changes in IL-6 levels.

The condition of the absence of differences in E2 in the two groups may have caused E2 to have no significant relationship with IL-6. It is known that non-pregnant mice have different levels of E2 depending on the estrus cycle, while in proestrus, there is an increase in E2 and phase others are lower [22]. We did not control the phase in mice, and this was one of the factors that influenced this study.

There is a unique fact that activin B is able to control E2 because increased levels of activin B will induce E2 [23,24]. This factor may contribute and also be the reason for the E2 levels that were not significantly different in the LPS-induced group of pregnant and non-pregnant mice. Activin B in our study showed no significant difference, research conducted by Kanamori et al. (2016) on normal mice with inflammation showed an increase in activin [25].

CONCLUSION

This study shows that inflammation during pregnancy will suppress IL-6 production but not activin B. E2 does not directly control IL-6 suppression. This condition occurs because of the complex mechanisms involved as a form of homeostasis during pregnancy.

Acknowledgements:
This work was supported by a grant from Indonesia Endowment Fund for Education (LPDP) grant no. FR19112020237466.

Conflict of interest: none declared

Financial support: none declared

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