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

E2 tidak berhubungan dengan kadar IL-6 dan activin B

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].

In 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.

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