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PROSIDING TEMU ILMIAH FORUM DIES 54

Fakultas Kedokteran Gigi
Universitas Padjadjaran
6-7 September 2013

Prosiding Temu Ilmiah
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Arlette Suzy Puspa Pertiwi, Amalia, Alwin Kasim, Kosterman Usri, Elih, Gantini Subrata, Winny Yohana, Dudi Arifin, Sri Susilawati, Fitriana Sari, Ria Noerianingsih

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Kepada semua pihak yang terlibat langsung dalam penyusunan Prosiding Forum Dies 54 FKG Unpad ini kami sampaikan penghargaan dan ucapan terima kasih. Semoga pemaparan makalah-makalah yang tersaji dapat mendatangkan manfaat bagi pengembangan pendidikan dan profesi dokter gigi.

Panitia Forum Dies 54

Daftar Isi

PENGARUH PENYAKIT PERIODONTAL MATERNAL TERHADAP EKSPRESI TUMOR NECROSIS FACTOR-ALPHA PADA SEL PLASENTA TIKUS

(Effect of Maternal Periodontal Disease on Tumor Necrosis Factor-alpha Expression in the Rat Placental Cells)

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ABSTRACT

Background: Periodontal infection caused by Gram-negative anaerobic organisms can act as a potential threat in the feto-placental unit. The placenta forms a barrier against the maternal immune system. This study aimed to analyze the effect of periodontal disease on TNF-α expression in the rat placental cells. **Methods:** Female rats were infected with live-*Porphyromonas gingivalis* at concentration of $2x10^9$ cells/ml into subgingival sulcus area of the maxillary first molar before and/or during pregnancy. They were sacrificed on gestational day 14 and 20. The expression of TNF-α in trophoblast cells was detected by immunohistochemistry. **Results:** TNF-α expressions of the control, Pg-BD, Pg-B and Pg-D groups were significantly different ($P<0.05$) in syncytiotrophoblasts, trophoblastic giant cells and spongiotrophoblasts on GD14, while on GD 20 TNF-α expressions were significantly different only in syncytiotrophoblasts and trophoblastic giant cells on GD 20. **Conclusion:** The role of TNF-α in the control group is thought to be involved in the early growth of the fetus. A little expression of TNF-α can be beneficial for pregnancy, while increased expression of TNF-α is very adverse. Initial implantation and a successful pregnancy depend on the tight regulation of the TNF-α expression in placental tissue.

Key words: periodontal disease, pregnancy, tumor necrosis factor-alpha, placental tissue

ABSTRAK

Pendahuluan: Infeksi periodontal yang disebabkan oleh bakteri anaerob Gram-negatif dapat bertindak sebagai ancaman potensial di unit feto-plasenta. Plasenta membentuk suatu penghalang terhadap sistem imun maternal. Penelitian ini bertujuan untuk menganalisa pengaruh penyakit periodontal terhadap ekspresi TNF-α pada sel

plasenta tikus. **Metode:** Tikus betina diinfeksi dengan *live-Porphyromonas gingivalis* pada konsentrasi $2x10^9$ sel/ml di area sulkus subgingival molar pertama rahang atas sebelum dan/atau selama kehamilan. Tikus dikorbankan pada kehamilan hari ke-14 dan 20. Ekspresi TNF-α pada sel trofoblas dideteksi secara imunohistokimia. **Hasil:** Ekspresi TNF-α pada kelompok kontrol, Pg-BD, Pg-B dan Pg-D secara signifikan berbeda ($P<0,05$) pada sinsitiotrofoblas, *trophoblastic giant cell* dan spongiotrofoblas pada GD14, sedangkan pada GD 20 ekspresi TNF-α berbeda secara signifikan hanya pada sinsitiotrofoblas dan *trophoblastic giant cell*. **Kesimpulan:** Peran TNF-α pada kelompok kontrol diduga terlibat dalam pertumbuhan awal janin. Ekspresi TNF-α yang sedikit dapat bermanfaat untuk kehamilan, sementara peningkatan ekspresi TNF-α sangat merugikan. Implantasi awal dan kehamilan yang sukses tergantung pada regulasi ketat ekspresi TNF-α dalam jaringan plasenta.

Kata kunci: penyakit periodontal, kehamilan, *tumor necrosis factor-alpha*, jaringan plasenta

INTRODUCTION

Survival and growth of the fetus depends on the placenta that forms the interface between the maternal and fetal circulation, and facilitate metabolism and gas exchange as well as waste disposal fetal. In addition, the placenta produces hormones that alter maternal physiology during pregnancy and forms a barrier against maternal immune system.[1] Growth and development of the placenta depends on the trophoblast cells. The factors involved in the regulation of the placenta are associated with development and differentiation of trophoblast.[2] One of the factors that can lead to increased apoptosis of trophoblast during pregnancy is intrauterine infection.[3] The exact mechanism of this disorder is not clearly understood, but recent research suggests that bacterial products can have a direct effect on the trophoblast. Trophoblast can respond to infection through the expression of the innate receptor family, Toll-like receptor (TLR). TLR is able to recognize conserved-sequences on the surface of microorganisms.[4-6]

Our previous studies showed that Gram-negative bacteria as the primary aetology of periodontal disease can adversely affect on pregnancy.[7] The subgingival *Porphyromonas gingivalis* infection in pregnant rats can increase the activation of TLR-2 and TLR-4 on macrophages and trophoblast cells resulting in fetal growth retardation.[8] TLR ligation resulted in activation of nuclear factor-κB (NF-κB) and production of cytokines.[4] Several cytokines have been involved in the balance of the body's immune system and can affect the growth of the placenta and fetus. In the second trimester of human pregnancy, the level of TNF-α in amniotic fluid is associated with normal and abnormal fetal growth.[9] Based on the description, this study aimed to analyze the effect of periodontal disease on TNF-α expression in the rat placental cells.

MATERIAL AND METHODS

All procedures were approved by the Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta-Indonesia. This study had taken female *Sprague-Dawley* rats, adult, 2 months, 150-250 g and primiparous. The rats were maintained on the controlled and standardized conditions. The subjects of study were consisted of two blocks, they were sacrificed on gestational day (GD) 14 and GD 20. Each block was

subdivided into four groups, which consisted of the control group, no *Porphyromonas gingivalis* infection; the Pg-BD group, an infection of *Porphyromonas gingivalis* before and during pregnancy; the Pg-B group, an infection of *Porphyromonas gingivalis* before pregnancy; and the Pg-D group, an infection of *Porphyromonas gingivalis* during pregnancy. Each group consisted of five pregnant rats.

Before injecting *Porphyromonas gingivalis*, all female rats were given antibiotic to prevent infection from other bacterias. Induction of experimental periodontitis was performed by injection of 0.05 ml live-*Porphyromonas gingivalis* ATCC 33277 with a concentration of $2x10^9$ cells/ml that was dissolved in saline into the distopalatal and distobuccal gingival sulcus area of maxillary first molar. Injection was repeated every 3 days for 30 days. For infection after pregnancy, it was also performed by a repeated injection every 3 days for 19 days. Control group rats were injected saline 0.05 ml as the treatment schedule of the treated group rats. Then, the female rats were mated with the same strain of male rat overnight ratio 2:1. The next morning, female rats were removed from the cages and examined the vaginal plug. If the vaginal plug was found, the day was recorded as GD 1.

Furthermore, the placental immunohistochemically was undertaken to determine the expression of TNF-α. Samples were incubated overnight at 4°C with primary antibody, rabbit polyclonal anti-TNF-α antibody dilution 1:500 (Abbiotec, San Diego, CA, 1:100-1:500), whereas negative control was incubated with secondary antibody as a substitute for the primary antibody. TNF-α was expressed in the cytoplasm. Data were presented as mean number of cells expressing TNF-α in each type of cell. These specimens were evaluated in macrophages of labyrinth zone (LM), junctional zone (SM) and decidua zone (DM), as well as syncytiotrophoblast (LS), spongitrophoblast (ST) and trophoblastic giant cell (DG).

One-way analysis of variance (ANOVA) with post hoc test was performed to compare the TNF-α expression in trophoblast cells of maternal periodontal infection. Value of significance was determined as $P<0.05$. Numerical data were presented in mean ± standard deviation.

RESULT

Tumor necrosis factor-α was strongly expressed in the cytoplasm of the syncytiotrophoblast, spongiotrophoblast, macrophages and trophoblastic giant cell (Fig.1). TNF-α expression in the placenta of the control, Pg-BD, Pg-B and Pg-D groups were significantly different ($P<0.05$) in the syncytiotrophoblast, trophoblastic giant cell and spongiotrophoblast on GD14. However, the expression of TNF-α in the placenta of the control, Pg-BD, Pg-B and Pg-D groups were only significantly different ($P<0.05$) in the syncytiotrophoblast and trophoblastic giant cell on GD 20 (Table 1).

DISCUSSION

In this study, TNF-α is not only expressed in the infected group, but also in the control group. Its role in the control group is very speculative, but is thought to be involved in the early growth of the fetus. TNF-α is produced to regulate trophoblast proliferation and differentiation, cell adhesion, tissue remodeling, villous trophoblast apoptosis and trophoblast hormone production.[10] Therefore, the lower expression of TNF-α is beneficial to the pregnancy, while increased expression was very detrimental.

On GD 14 placental control group, TNF-α has been identified mainly in the syncytiotrophoblast with lower expression than in spongiotrophoblast and trophoblastic giant cell. TNF-α has also been found in macrophages junctional zone and the labyrinth zone. So the presence of TNF-α in the trophoblast showed that the trophoblast is also a source and target of cytokines. These data indicate the complexity of the regulation of TNF-α at the fetomaternal interface. In the aspect of maternal, TNF-α has been identified in the macrophages decidua zone.

In humans, extravillous differentiation program, such as the formation of anchoring villi through adhesion, and the proliferation and generation of extravillous trophoblast subtypes that invade the different compartments of the maternal decidua, is an important process of placental development. Invasive trophoblasts migrate into the decidua and spiral arteries stroma that replaces the maternal endothelial cells. This process is thought to be associated with enlarged diameter of the blood vessels resulting in increased blood supply and oxygen to the placenta and fetus. Mechanisms that initiate cell proliferation islands during early pregnancy and in different areas of attachment anchoring villi are largely unknown but is thought to TNF-α has the potential to be involved in this mechanism.[11]

Tumor necrosis factor-α can modulate the growth of rat blastocysts and trophoblast.[12] In general, TNF-α is considered a negative impact on blastocyst because of the increased levels of TNF-α shown to decrease proliferation and increase apoptosis primarily through TNFR1-dependent signal.[13] This is mainly due to the adverse effects of TNF-α in the blastocyst inner cell mass, but not in the trophoblast.[14] In the mouse blastocyst, TNF-α did not alter trophoblast proliferation but increase the number of multinucleated trophoblast cells.[14] However, TNF-α did not affect the growth of the first trimester human trophoblast cells.[15-17]

In cultured human first trimester villous explants, TNF-α has been shown to decrease migration primarily through upregulation of plasminogen activator inhibitor-1 (PAI-1) because the inhibitor PAI-1 antibody abolished the suppressive effect of TNF-α.[16,17] PAI-1 enzyme is known to specifically block the invasive pro-urokinase plasminogen activator-1 (uPA), which plays an important role in trophoblast invasion.[18] PAI-1 dependent induction of TNF-α would involve NF-κB dependent signaling.[17] Similarly, TNF-α secretion from activated macrophages shown to limit the invasion of trophoblast cells in vitro through the production of PAI-1-dependent TNF-α and inhibition of uPA activity.[19]

Although the negative impact on the migration and invasion of trophoblast, but TNF-α can stimulate the expression of MMP-9 in the first trimester trophoblast cells in vitro and human decidua.[16,20] Since MMP-9 is thought to be one of the major enzymes in trophoblast invasion, protease-dependent induction of TNF-α may be a mechanism to balance the adverse effects of cytokines. This may also apply to other MMPs are regulated by TNF-α in the trophoblast. Aberration TNF-α levels can interfere important stages of adhesion and trophoblast invasion by increasing the expression of MMP-9, and the degradation of the decidua or trophoblast.[21,22]

CONCLUSION

It can be concluded that the role of TNF-α in the control group is thought to be involved in the early growth of the fetus. A little expression of TNF-α can be beneficial for pregnancy, while increased expression of TNF-α is very adverse. Initial implantation and a successful pregnancy depend on the tight regulation of the TNF-α expression in placental tissue.

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Figure 1. TNF-α expression in the placental control (i), Pg-BD (ii), Pg-B (iii) and PG-D (iv) groups on GD 20. TNF-α was strongly expressed in the cytoplasm of the syncytiotrophoblast (a), spongiotrophoblast (b), macrophages (c) and trophoblastic giant cell (d). Magnification 400x.

Table 1. Effect of Maternal Periodontal Infection on TNF-α Expression in the Rat Placental Cells at GD 14 and GD 20

Variable	Maternal periodontal infection				*P*
	Control	Pg-BD	Pg-B	Pg-D	
GD 14:					
TNFα-LM	1,22±0,60	1,90±0,88	1,31±0,75	1,20±0,79	0,084
TNFα-LS	1,65±0,71*†§	2,70±0,95*†	2,23±1,01*§	2,20±0,63	0,010
TNFα-SM	1,48±0,67	2,30±1,25	1,62±0,65	1,80±1,14	0,111
TNFα-ST	2,00±0,95*†§	6,30±1,16*†§	3,54±1,66*†§	4,70±1,25*†§	0,000
TNFα-DM	1,83±0,72	2,60±1,51	1,92±1,32	1,80±1,14	0,290
TNFα-DG	1,83±0,98*†§	5,20±1,14*†§	3,85±1,04*†§	4,50±1,08*	0,000
GD 20:					
TNFα-LM	1,91±0,73	1,90±0,88	2,00±0,82	2,00±0,67	0,979
TNFα-LS	3,13±0,97*†	5,10±2,38*†	3,85±1,57§	5,50±1,35*§	0,000
TNFα-SM	2,96±0,77	3,40±2,07	2,54±1,13	3,00±1,25	0,440
TNFα-ST	3,48±1,44	3,90±1,59	3,62±1,61	3,40±0,97	0,857
TNFα-DM	2,39±1,23	3,40±1,65	2,69±1,23	3,10±1,37	0,209
TNFα-DG	2,61±0,99*†	3,80±1,14*†	3,15±1,28	3,50±1,08*	0,027

The control group, no *Porphyromonas gingivalis* infection; the Pg-BD group, an infection of *Porphyromonas gingivalis* before and during pregnancy; the Pg-B group, an infection of *Porphyromonas gingivalis* before pregnancy; and the Pg-D group, an infection of *Porphyromonas gingivalis* during pregnancy. *, †, § : mean difference was significant at the 0.05 level.