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โครงการ

ผลของฮอร์โมนเพศหญิงและการตอบสนองของเซลล์โมโนไซต์

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สนับสนุนโดย

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ชื่อโครงการ: ผลของฮอร์โมนเพศหญิงและการตอบสนองของเซลล์โมโนไซต์จากมนุษย์ต่อแอลพีเอสของเชื้อ*พอร์ไฟโรโมแนส จิงจิวาลิส*

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บทคัดย่อ:

ฮอร์โมนเพศหญิงมีระดับสูงขึ้นในระหว่างตั้งครรภ์และพบว่ามีบทบาทสำคัญต่อการตอบสนองของระบบภูมิคุ้มกันของมารดาได้ การเปลี่ยนแปลงของระบบภูมิคุ้มกันของมารดาอาจสัมพันธ์กับการอักเสบของเหงือกที่เกิดขึ้นตามมาขณะตั้งครรภ์ ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาบทบาทของ ฮอร์โมนบีต้า-เอสตราไดออลและโปรเจสเตอโรนต่อการตอบสนองของเซลล์โมโนไซต์ (monocytes) เพื่ออธิบายกลไกทางชีวภูมิคุ้มกันที่สัมพันธ์กันระหว่างการเกิดโรคปริทันต์ของมารดาขณะตั้งครรภ์ เซลล์โมโนไซต์ของอาสาสมัครที่มีสุขภาพดีจะถูกเพาะเลี้ยงโดยแยกมาจากบัฟฟี่โค๊ต (buffy coat) ด้วยชุดปั่นแยกเซลล์ Lymphoprep ตามกระบวนการมาตรฐาน แยกเซลล์ให้บริสุทธิ์โดยวิธี Adherent method วิเคราะห์การแสดงระดับของ TLR2, TLR4 และ CD14 ด้วยการทดสอบด้วย Flow cytometry วัดการแสดงออกของCox2 mRNA ด้วยเครื่องเพิ่มปริมาณสารพันธุกรรมชนิด Realtime (realtime PCR) และวัดปริมาณการหลั่งของพรอสตาแกลนดินอีทู (prostaglandin E_2; PGE_2) ด้วยการทำ ELISA ผลการศึกษาครั้งนี้พบว่าบีต้า-เอสตราไดออลและโปรเจสเตอโรนลดระดับของ TLR2 และ CD14 บนผิวเซลล์โมโนไซต์ แต่ไม่มีผลต่อระดับของ TLR4 นอกจากนี้บีต้า-เอสตราไดออลและโปรเจสเตอโรนยังลดการแสดงออกของ Cox2 mRNA โดยแปรพันไปตามความเข้มข้นของบีต้า-เอสตราไดออลและโปรเจสเตอโรน ในแง่ของการตอบสนองของโมโนไซต์ต่อ LPS ของแบคทีเรีย การบ่มเซลล์ด้วยบีต้า-เอสตราไดออล (1 นาโนกรัม/มล.) และโปรเจสเตอโรน (10 นาโนกรัม/มล.) ก่อนการกระตุ้นด้วยแอลพีเอสของเชื้อ*พอร์ไฟโรโมแนส จิงจิวาลิส* จะลดฤทธิ์ของแอลพีเอสใน การกระตุ้นระดับการแสดงออกของCox2 mRNA และการหลั่งของพรอสตาแกลนดินอีทูจากเซลล์โมโซต์ได้อย่างมีนัยสำคัญ ผลการทดลองที่กล่าวมาจึงอาจสรุปได้ว่าบีต้า-เอสตราไดออลและโปรเจสเตอโรนมีคุณสมบัติในการปรับเปลี่ยนการตอบสนองของเซลล์โมโนไซต์จากมนุษย์ ซึ่งกระบวนการนี้น่าจะมีบทบาทสำคัญในพยาธิกำเนิดของโรคปริทันต์ที่เกิดขึ้นขณะตั้งครรภ์

คำสำคัญ: เอสโตรเจน, โปรเจสเตอโรน, ทีแอลอาร์, *พอร์ไฟโรโมแนส จิงจิวาลิส*, โมโนไซต์

Abstract:

Female sex hormones are elevated and are potential host response modifiers during pregnancy. Modulation of immune responses by estrogen and progesterone may be responsible for periodontal inflammation. Therefore, we aimed to investigate the role of β-estradiol and progesterone in monocyte immune responses and to identify their role as a possible immunological link between pregnancy and periodontal disease. Human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of healthy donors by LymphoprepTM gradient according to standard procedures. Monocytes were purified from PBMCs by adherent method. Expression of TLR2, 4 and CD14 was analyzed by flow cytometry. Cox2 mRNA expression was measured using real-time RT-PCR and PGE_2 secretion was assayed by ELISA. We report herein that β-estradiol and progesterone reduced cell-surface protein expression of TLR2 and CD14 but had no effect on TLR4 expression in primary human monocytes. Significantly, β-estradiol and progesterone dose-dependently down-regulated monocyte expression of Cox2 mRNA. Additionally, pre-treatment monocytes with β-estradiol (1 ng/ml) or progesterone (10 ng/ml) reduced effects of *P.gingivalis* LPS on Cox2 mRNA expression and decreased PGE_2 secretion by human monocytes. Together, these results indicate the potential of β-estradiol and progesterone to alter monocyte immune response to periodontal pathogen and this process may have a role in the pathogenesis of periodontal disease associated with pregnancy.

Keywords: Progesterone; estrogen; TLRs; *P.gingivalis*; monocytes

1. Introduction

Pregnancy gingivitis is a common form of periodontal disease that affects more than 30% of pregnant women (Jensen et al., 1981; Carrillo-de-Albornoz et al., 2010). A number of studies suggest a bi-directional relationship between maternal periodontal diseases and adverse pregnancy outcomes (Goldenberg et al., 2008; Gursoy et al., 2008; Ruma et al., 2008; Figuero et al., 2010; Boggess et al., 2011). On the one hand, the risk for periodontal diseases is increased when pregnancy is present (Mealey and Moritz, 2003a; Gursoy et al., 2008). On the other hand, pregnant women with periodontal diseases or poor dental health had increased risk of having preterm birth (PTB) or low birthweight (LBW) baby (Offenbacher et al., 1996; Goldenberg et al., 2008; Baskaradoss et al., 2011). PTB has dramatic impact on the health care system not only due to high mortality and morbidity but also due to significant medical costs. The prevalence rate of preterm birth is significantly increasing annually in many countries (Goldenberg et al., 2008), including in Thailand (Ip et al., 2010). As periodontal disease and PTB are considered as a global public health problem the knowledge regarding the immunobiology between these conditions could be a key to establish rational diagnostic and therapeutic strategies for periodontal disease associated with pregnancy.

Although the clinical and histological characteristics of exacerbated gingival inflammation in pregnant individuals are well documented, its aetiology has not yet been clearly established and it is unknown why only some pregnant women develop frank signs of gingival inflammation. The function of female sex hormones could potentially link the causal relationship between pregnancy and pregnancy induced-gingival diseases. Indeed, the finding that salivary levels of estrogens and progesterone are elevated during pregnancy suggests a role for these hormones in the pathogenesis of periodontal inflammation (Figuero et al., 2010). The unbalanced/excessive production of pro-inflammatory cytokines such as IL-1β, TNF-α and PGE_2 has been detected in periodontal diseases (Graves and Cochran, 2003; Liu et al., 2010; Taylor, 2010). *In vitro*, it has been shown that the activation of immune and non-immune cells by female sex hormones can lead to the production of inflammatory cytokines such as IL-1β, TNF-α and PGE_2 (Nilsson, 2007; Gilliver, 2010). Recently, study from our group found that progesterone enhances VEGF-A mRNA expression in *P. gingivalis* LPS-treated THP-1 monocytes (Jitprasertwong et al., 2014). Because *Porphyromonas gingivalis* is a major bacterial species implicated in many form of gingival diseases, including pregnancy gingivitis (Slots and Ting, 1999; Carrillo-de-Albornoz et al., 2010; Jain and Darveau, 2010; Pathirana et al., 2010), the interaction between female sex hormones and *P. gingivalis* may be fundamentally important in the inter-relationship between periodontal diseases and pregnancy. However, the molecular mechanism regarding the interaction between sex

hormones and periodontal bacteria such as *P. gingivalis* is still lacking. The purpose of the present study is therefore to investigate the immunological role of sex hormones in term of TLRs and cell-surface activation in human monocytes stimulated with LPS *P.gingivalis*. The present study will also expand our knowledge of the molecular interaction between sex hormones and PGE_2 regulation in primary human monocytes.

2. Literature review

Pregnancy gingivitis is an important form of periodontal diseases which usually develops between the second and eighth month of pregnancy and affects 36–100% of pregnant women (Mealey and Moritz, 2003a; Ruma et al., 2008; Boggess et al., 2011). Many cross-sectional and longitudinal clinical studies have demonstrated that the prevalence and severity of gingival inflammation increase during pregnancy (Tilakaratne et al., 2000; Yalcin et al., 2002b; Gursoy et al., 2008). In an experimental gingivitis study, a more severe development of gingival inflammation was observed during pregnancy than after delivery (Raber-Durlacher et al., 1994).

The clinical signs of pregnancy gingivitis include red swollen gingiva, increased gingival probing depths, increased bleeding upon probing or mechanical stimulation, increased gingival crevicular fluid flow and increased tooth mobility. The most affected area appears to be in interproximal sites of anterior teeth. Preexisting gingivitis or periodontitis has been noted to worsen dramatically in pregnant women (Jensen et al., 1981). However, there is no evidence confirmed that steroid sex hormone-induced gingivitis could proceed to periodontitis (Mealey and Moritz, 2003a). In addition to the gingival changes seen due to an enhanced inflammatory response during pregnancy, 0.5–9.6% of women who are pregnant also experience localized gingival enlargement consistent with pyogenic granulomas (Arafat, 1974). The pregnancy-associated pyogenic granuloma, or "pregnancy tumor" is a painless, exophytic mass that has either a sessile or pedunculated base extending from the gingival margin or from the interproximal tissues of the teeth. These conditions could adversely affect the functions of the dentitions.

Gingivitis was previously believed inevitable following the formation of microbial plaque on teeth. It is now accepted that certain individuals will be more susceptible than others to gingivitis and indeed periodontitis (Kinane and Bartold, 2007). The development of periodontal disease is associated with a specific group of predominantly Gram-negative, anaerobic or microaerophilic bacteria. They colonize on the root surface in the subgingival area (Page and Kornman, 1997). The key periodontal pathogens in periodontal diseases are *Porphyromonas gingivalis, Tannerella forsythia*, *Treponema denticola Aggregatibacter* (formerly termed *Actinobacillus*) *actinomycetemcomitans* and *Prevotella intermedia* (Van Dyke and Serhan, 2003; Jain and Darveau, 2010).

Although bacteria are essential, the bacteria alone are not sufficient for the disease to occur. Periodontal disease results from the complex interaction between host response and periodontal pathogens (Gardy et al., 2009). After colonization on the gingival sulcus by

periodontal bacteria, the bacteria release their products, for example, lipopolysaccharide (LPS) which is a well known virulence factor of gram-negative bacteria. LPS has the potential to interact with nearly all cell types in periodontal tissues including immune cells and resident cells and thereby initiating a defense mechanism. The initial immune response in periodontal disease is characterized by the action of the innate immune system which, in this context, consists of the gingival epithelium, fibroblasts, neutrophils, dendritic cells, and monocytes/macrophages (Teng, 2006). In fact, innate host recognition of LPS is a key initiating event for the subsequent clearance of gram-negative bacteria from infected host tissues (Jain and Darveau, 2010). A group of receptors called pattern recognition receptors (PRRs) which include cell surface Toll-like receptors (TLRs) and intracellular NOD-like receptors (NLRs) are responsible for the detection of microbe-associated molecular patterns (MAMPs), i.e. LPS, and thereby leading to cellular activation. To date, a number of studies have demonstrated how periodontal pathogens, in particular *P. gingivalis* signal via TLRs (Pathirana et al., 2010; Taylor, 2010). However, little knowledge is yet known how NLRs sense oral bacteria. A recent study (Bostanci et al., 2009) revealed that the NLRP3 and NLRP2 mRNA expression are significantly increased in gingival tissues affected by periodontal disease compared to healthy ones. The same study also found that *P. gingivalis* culture supernatant up-regulates the NLRP3 mRNA expression in Mono-Mac-6 monocytic cells.

In the gingival epithelium, the binding of LPS to TLR on nearby cells induces the production of cytokines and chemokines resulting in the expression of adhesion molecules, increased permeability of gingival capillaries and chemotaxis of polymorphonuclear neutrophils (PMNs) through the junctional epithelium and into the gingival sulcus to phagocytose bacteria. One important component of innate immunity that plays a vital role in periodontal disease is monocytes. In response to inflammatory signals, monocytes can migrate quickly to sites of infection in the tissues and differentiate into macrophages which can effectively capture invading pathogens. The phagocytosis of bacteria by macrophages results in cytokine secretion and antigen-presentation to induce a more effective adaptive immunity (Teng, 2006; Liu et al., 2010).

Clearly, the primary cause of periodontal disease is plaque bacteria but the disease progression is modified by an individual's susceptibility (Kinane et al., 2007; Preshaw, 2008). A wide variety of determinants and factors, either environmental or acquired, e.g. smoking, diabetes, hormonal changes, systemic diseases, genetic factors, microbial composition of dental plaque are known to influence the host response (Nunn, 2003; Kinane and Bartold, 2007; Kinane et al., 2007). Therefore, these factors could subsequently have certain effects on the disease initiation and

progression (Gaspard, 1995; Grossi, 2000; Chavarry et al., 2009; Anner et al., 2010). Therefore, a complete understanding how sex hormones contribute periodontal disease progression could be a key to establish rational diagnostic and therapeutic strategies for periodontal disease associated with pregnancy.

There is increasing evidence suggests a bi-directional relationship between maternal periodontal diseases and adverse pregnancy outcomes (Mealey and Moritz, 2003a; Goldenberg et al., 2008; Carrillo-de-Albornoz et al., 2010; Baskaradoss et al., 2011; Corbella et al., 2011). The presence of pregnancy gingivitis in certain pregnant individuals may be recognized as indicator or predictive factor for higher risk of having preterm birth and may provide an indication for thorough health care and prevention in such conditions (Nabet et al., 2010; Chambrone et al., 2011; Corbella et al., 2011). There are some studies regarding preterm delivery caused by a premature increase in PGE_2 and a group of researchers suggest that periodontal infections, which serve as a reservoir of inflammatory mediators such as PGE_2, may pose a threat to the fetal placental unit (Offenbacher et al., 1996; Offenbacher et al., 2006; Gursoy et al., 2008). They have found that increasing ginigival crevicular fluid (GCF) levels of PGE_2 may help estimate the risk of preterm low birth weight as a maker of current periodontal disease activity (Offenbacher et al., 2006).

Exploring the underlying mechanism how pregnancy contributes to periodontal disease highlights the link between these two conditions. Several biological mechanisms have been proposed to explain the pathobiology of the interaction between pregnancy and periodontal disease (Mealey and Moritz, 2003a), including depression of the immune system, increased vascularity and vascular flow, cellular changes and changes in oral biofilms (Raber-Durlacher et al., 1993; Mariotti, 1994; Raber-Durlacher et al., 1994; Carrillo-de-Albornoz et al., 2010; Figuero et al., 2010). However, the precise mechanistic links between pregnancy and gingival changes have yet to be fully understood.

Alterations in immune-inflammatory process by hormonal imbalance have been proposed to explain the pathophysiolocal connections between periodontal disease and pregnancy. Female sex hormones have been shown to play a vital role on the function of immune cells including monocytes/macrophages (Whitacre, 2001; Fish, 2008; Gilliver, 2010). The sex hormone receptor is expressed in a number of immune cells such as neutrophils, mast cells, T and B lymphocytes and monocytes (Gilliver, 2010). In addition, it has been demonstrated that female sex hormones can activate the secretion of a myriad of cytokines and inflammatory mediators (Nilsson, 2007; Gilliver, 2010). For example, Polan et al. (1990) reported that IL-1 secretion by cultured peripheral monocytes isolated from pregnant women appears to be increased by luteal

levels of progesterone (Polan et al., 1990). Progesterone increases TNF-α secretion by U937 monocytic cell-treated with glucose or LPS whereas estrogen inhibits IL-6 secretion from these cells (Jain et al., 2004). Another study, however, demonstrated that progesterone enhanced the production of IL-1β and IL-8 but inhibited TNF-α production by monocytes stimulated by *E.coli* LPS or *U.urealyticum* (Peltier et al., 2008). This discrepancy may be explained by differences in cell types and experimental conditions. In addition, estrogen also has pleiotropic effect on cytokine production. Estrogen alters cytokine release through regulation of CD16 expression (Kramer et al., 2004). Moreover, estrogen was found to enhance TNF-α, IL-6 and TGF-β expression in macrophages derived from activated human monocytes (Montagna et al., 2010).

Hormonal imbalance is thought to be a major determinant that is responsible for the development of pregnancy gingivitis. Indeed, it has been postulated that the pathogenesis of pregnancy gingivitis is initiated by bacterial biofilm and exacerbated by endogenous sex steroid hormones (Mariotti 1999). The levels of serum sex hormones such as progesterone and estrogen are elevated throughout the 3 trimesters of pregnancy, reaching the highest levels during the third trimester (Edelstam et al., 2007). Interestingly, the levels of both hormones in saliva also significantly increased during pregnancy (Figuero et al., 2010). Studies suggested that alterations in hormone levels and host inflammatory response as a result of pregnancy may lead to further dysregulation of immune-inflammatory responses in the periodontium, causing periodontal inflammation. However, the information regarding the immunological role of sex hormones in periodontal diseases is still limited (Markou et al., 2009). For example, progesterone and estrogen have been shown to stimulate the production of PGE_2 (Miyagi et al., 1993) but inhibit the production of IL-1β from human peripheral monocytes (Morishita et al., 1999). A recent study showed that estrogen reduced the expression of chemokine CCL3 mRNA but increased the expression of CCL5 by periodontal ligament cells (Nebel et al., 2010).

Only a few clinical studies have examined the biological link between increased gingival inflammation during pregnancy and changes in the local immune system (Kinnby et al., 1996; Yalcin et al., 2002a; Figuero et al., 2010). Kinnby et al. (1996) reported that higher gingival inflammatory reaction in women during pregnancy may be associated with the negative effect of progesterone levels on GCF plasminogen activator inhibitor-2 (Kinnby et al., 1996). Another study demonstrated that PGE_2 levels in GCF during the second and third trimester in pregnant women decreased significantly following the non-surgical periodontal therapy suggesting that PGE_2 levels could be used as a marker of gingival inflammation during pregnancy (Yalcin et al., 2002a). Recently, Figuero et al. (2010) failed to show a correlation between exacerbated

gingival inflammation and GCF levels of PGE_2 and IL-1β during pregnancy due to limitation in sample collection (Figuero et al., 2010).

Periodontal disease is characterized by the interaction between bacterial challenge and the host response. The cellular processes that drive the immune responses are mediated by numerous cytokines functioning as a network. The bacterial products such as LPS are known to stimulate the expression and secretion of a number of cytokines and chemokines from periodontal resident and immune cells (Liu et al., 2010; Taylor, 2010; Preshaw and Taylor, 2011). Prostaglandins (PGs) belong to the class of prostanoid fatty acid derivatives of arachidonic acid. The production of prostaglandins starts with the release of arachidonic acid from membrane phospholipids by phosholipase A2. Arachidonic acid is converted into prostaglandin H2 (PGH2) by two isoenzymes, cyclo-oxygenase-1 (Cox-1) and Cox-2 and then converted into PGE_2 by prostaglandin E synthetase (PGES) (Helliwell et al., 2004). PGE_2 is considered to be one of key cytokines in the development of periodontal inflammation (Graves and Cochran, 2003; Serhan and Savill, 2005; Liu et al., 2010). This mediator is produced by activated monocytes, macrophages and fibroblasts within periodontal lesions. PGE_2 promote critical inflammatory responses during periodontal disease. In addition, PGE_2 stimulate the suppression of lymphocyte production, decrease collagen synthesis by fibroblasts and influencing osteoclastic bone resorption (Williams and Peck, 1977; Serhan and Savill, 2005). Several studies have demonstrated that PGE are involved in the pathogenesis of periodontal disease (Offenbacher et al., 1993). In particular, PGE_2 has been shown to be associated with attachment loss (Offenbacher et al., 1984). Studies have demonstrated that PGE_2 is elevated in patients with periodontal disease compared with healthy subjects and are associated with the clinical signs of periodontitis such as bone resorption, increased probing depth and attachment loss (Stashenko et al., 1991; Lee et al., 1995; Gamonal et al., 2000; Gorska et al., 2003). Moreover, a successful periodontal treatment often presents with a reduction in levels of PGE_2 (Gamonal et al., 2000; Al-Shammari et al., 2001). A summary of studies regarding the role of PGE_2 in pregnancy-related gingival diseases is shown in Table 1.

Table 1. The role of PGE_2 in pregnancy-related gingival inflammation

Reference	Study design	Conclusions
(Miyagi et al., 1993)	In vitro study PGE_2 response to sex hormones in monocytes	Low concentration of estrogen inhibits PGE_2 production whereas progesterone enhances the secretion of this mediator
(Offenbacher et al., 1998)	Case-control study Measurement of GCF PGE_2 using ELISA	Increasing GCF PGE_2 may be a marker of current periodontal disease activity and decreasing birth weight
(Morishita et al., 1999)	In vitro study PGE_2 response to sex hormones in monocytes	Estrogen and progesterone enhance PGE_2 production but inhibit IL-1β production
(Yalcin et al., 2002a)	Cohort study Measurement of GCF PGE_2 after periodontal treatment in pregnant women using ELISA	Periodontal therapy has resulted in statistically significant decrease in levels of GCF PGE_2
(Figuero et al., 2010)	Cohort study Measurement of GCF IL-1β and PGE_2 using ELISA	No significant association between gingival inflammation and an increase in progesterone or estradiol or with changes in GCF IL-1β and PGE_2

However, although the available data indicate that PGE_2 might play a role in gingival inflammation during pregnancy (Miyagi et al., 1993; Morishita et al., 1999; Figuero et al., 2010), the role of this mediator in pregnancy gingivitis is still not completely understood. In fact, there is no study has investigated the interaction between sex hormones and the periodontal

pathogens such as *P. gingivalis* in term of PGE_2 production by human monocytes. In addition, the mechanism that mediates this interaction is also unknown. Therefore, the aims of present study are to investigate the *in vitro* effect of female sex hormones (progesterone and estrogen) on the protein expression of TLR2, TLR4 and CD14 in primary human monocytes. In addition, the effect of progesterone and estrogen on Cox2 synthesis and PGE_2 production by human monocytes will be also determined.

3. Objectives

3.1 To investigate the effect of female sex hormones on Toll-like receptors (TLRs) expression and Cox2 in primary human monocytes

3.1.1 The effect of female sex hormones on Toll-like receptors expression in human monocytes using flow cytometry

3.1.2 Analysis of the effect of sex hormones on Cox2 synthesis using RT-PCR and realtime-PCR

3.2 To determine the effect of female sex hormones on PGE_2 production by human monocytes.

3.2.1 Investigation of the effect of estrogen and progesterone on PGE_2 production from human monocyte-stimulated with *P.gingivalis* LPS

4. Materials and Methods

Materials

Progesterone (P7556), estrogen (β-Estradiol, E4389) and cell culture media were purchased from Sigma-Aldrich (St. Louis, USA). All plasticware were purchased from (Nunc A/S, Denmark). Ultrapure LPS from *E. coli* 0111.B4 and ultrapure LPS from *P. gingivalis* were purchased from Invivogen (San Diego, USA).

Isolation and culture of primary human monocytes

Unless otherwise stated, all experiments were conducted in duplicate cultures from three independent healthy donors. The study was approved by the ethical research, Naresuan University Ethics Committee (HE54-Ex1-0042). Blood was obtained from Hospital Blood Service (Naresuan University Hospital, Phitsanulok). Human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of healthy donors by Lymphoprep™ gradient according to standard procedures. Briefly, blood was diluted 1:1 in PBS/2 mM EDTA and overlayed 1:1 on a Lymphoprep™ gradient. After a centrifuge step for 20 min at 800 g, 20 °C, the buffy coat layer was collected and diluted in 40 ml PBS/2 mM EDTA. Leukocytes were centrifuged for 10 min at 800 g, 20 °C, supernatant was discarded. 10 ml PBS was added and leukocytes were centrifuged for 5 min at 800 g, 20 °C. Monocytes were purified from PBMCs by adherent cell

method. Adherent cells (monocytes) were collected, resuspended in cell culture medium and centrifuged for 5 min at 2000 rpm, 20 °C. The cells were resuspended in cell culture medium, counted on a haemocytometer and were incubated over night at 37 °C, 5 % CO_2 and then used for stimulation experiments.

As shown in **Figure 1**, primary monocytes exhibit a round morphology and are single cells in suspension culture.

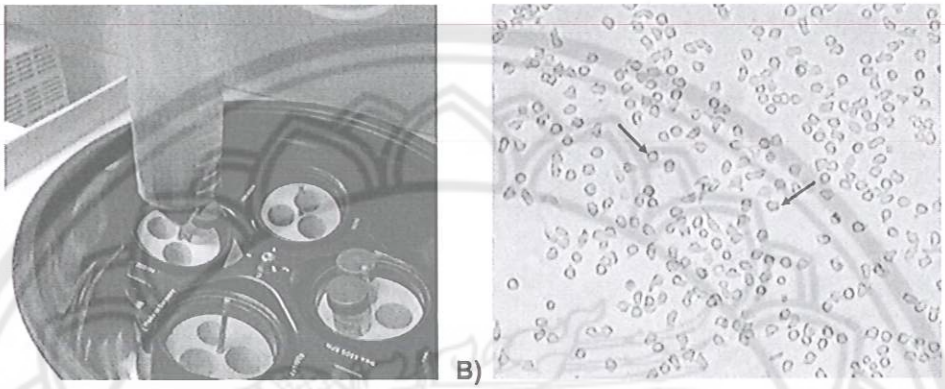

Figure 1 Morphology of primary human monocytes.

A) Human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of healthy donors by Lymphoprep™ gradient according to standard procedures. B) Monocytes typically appear as adherent round single cells (arrows).

Stimulation of monocytes with female sex hormones or LPS

Primary monocytes of 5 x 10^5 cells /well in 24-well plate were used for all stimulation experiments. For mRNA expression experiments, large scale experiments were set up with 2 x 10^6 cells/well in 6-well plate. Cells were stimulated with ultrapure LPS from *E. coli* 0111:B4, Ultrapure *P. gingivalis* LPS, progesterone or estrogen at different time points (3, 6 and 24 h). It has been shown earlier that 100 ng/ml *E.coli/P.gingivalis* LPS is an optimal concentration used in stimulation experiments (Foster *et al.*, 2005) and the progesterone and estrogen concentrations used in experiments are based on previous studies applying a comparable experimental setup (Morishita et al., 1999; Figuero et al., 2010). In addition, plasma progesterone levels during pregnancy may reach 100 ng/ml (90 to 120 ng/ml), approximately 10

times that seen during the luteal phase of the reproductive cycle whereas estradiol levels in plasma may similarly be increased up to 30 times (100 to 125 pg/ml) (Yuan et al., 2002; Mealey and Moritz, 2003b). Cells treated with LPS or hormones were compared with unstimulated cells cultured for the same time period.

Cell proliferation assay

The potential mitogenic or cytotoxic effects of hormones and LPS on primary human monocyte cultures were evaluated with the Cell Titer 96 Cell Proliferation Assay (Promega, USA). The assay was carried out according to the manufacturer's instructions. Briefly, human monocytes (1×10^5 cells/ well) were cultured in quadruplicate in a 96-well tissue culture plate in a total volume of 100 µl culture medium either in the absence or presence of the indicated concentration of hormones or LPS for 3 and 6 h. An 8-point standard curve of monocytes with 2×10^6 cells/ml as the highest standard was produced using a 2-fold dilution series in fresh cell culture medium. Twenty µl of Owen's reagent were added to each well and cells were incubated for a further 1 h. After incubation, absorption was measured at 460 nm on a spectrophotometer (Microplate Fluorescence Reader, Bio-Rad). Cell numbers of samples were calculated by linear standard curve fitting.

Flow cytometry

Primary human monocytes were isolated from PBMCs of five independent healthy donors. Expression of TLR2, TLR4, and CD14 on the cell surface of human monocytes was analyzed with flow cytometry. Briefly, 2×10^6 primary human monocytes per stimulation were collected, centrifuged for 5 min at 300 g, 20 °C and resuspended to a concentration of 1×10^6 cells/ml in FACS buffer (1 % BSA/PBS with 2 mM EDTA, w/v). 1 ml of cell suspension was transferred to a FACS tube (BD Falcon, Oxford, UK), 2 more ml of FACS buffer was added and cells were centrifuged for 5 min at 300 g, 20 °C. Cells were stained with IgG1 mouse antibodies (BioLegend, San Diego, CA, USA): CD14-PerCP/Cy5.5 (clone HCD14), isotype control-PerCP/Cy5.5 (clone MOPC-21) and the following IgG2a mouse antibodies (BioLegend, San Diego, CA, USA): TLR2-FITC (clone TL2.1), TLR4-PE (clone HTA125), isotype control-FITC (clone MOPC-173) and isotype control-PE (clone MOPC-173). 10,000 events were acquired on a FACSCalibur flow cytometer (Becton Dickinson). Data were analyzed with CellQuest software (Becton Dickinson). TLR2-FITC, TLR4-PE and CD14-PerCP were analyzed in a doublestaining whereas analysis of CD14 expression was performed in histograms of CD14 fluorescence and expressed as mean fluorescence intensity (MFI).

RNA extraction and cDNA synthesis by Reverse Transcription

Total RNA was isolated from culture cells using a commercial RNA extraction kit (RNeasy® Mini Kit, Qiagen, Germany) according to the manufacturer's instruction. The total RNA concentration of each sample was measured with a spectrophotometer (Nanodrop, Thermo Fisher, USA) and then stored at -80 °C. An aliquot containing 1.0 µg of total RNA was used for the reverse transcription reaction, which was conducted using iScript™ cDNA Synthesis kit (Bio-Rad, USA). cDNA samples were then stored at 2-8 °C.

Conventional RT-PCR

The mRNA expression of Cox2, TLR2 and TLR4 genes was detected using a conventional PCR method. All primers used for PCR analysis were purchased from Intron Bio (Korea). The primer sets specific for each gene and the PCR conditions are summarized in **Table 2.** The PCR was performed using i-Taq™ PCR Master mix (Intron Bio, Korea) with 2.0 µl of cDNA sample. PCR amplification was conducted for 35 cycles and the products of the PCR were then separated by 2% agarose gel electrophoresis, visualized with ethidium bromide staining, and viewed under UV light.

Table 2 Primers used in the polymerase chain reaction expression analyses

Gene	Primer sequence (5'-3')	Annealing Temp. (C)	Product size (bp)
Cox2	*F— TCC TTG CTG TTC CCA CCC ATG*	65	802
	R— CAT CAT CAG ACC AGG CAC CAG		
TLR2	*F—GCC AAA GTC TTG ATT GAT TG*	60	400
	R—TTG AAG TTC TCC AGC TCC TG		
TLR4	*F— TCC CTC CAG GTT CTT GAT TA*	60	495
	R— GTA GTG AAG GCA GAG CTG AA		
β2M	*F—ACC CCC ACT GAA AAA GAT GA*	60	120
	R--CTT ATG CAC GCT TAA CTA TC		

Quantitative analyses for Cox2 mRNA using real-time RT-PCR

The quantification of VEGF-A mRNA levels was performed using a LightCycler® 480II detection system (Roche). Reaction mixtures for PCR (20 µl) were prepared by mixing 1.0 µl of cDNA solution, LightCycler 480 SYBRGreen I Master (Roche), and VEGF-A primers. The relative fold changes between stimulations were calculated with the comparative Ct method ($2^{-\delta\delta}$Ct), using β2M mRNA as the reference gene.

PGE_2 ELISA

PGE_2 concentrations in cell culture supernatants were determined with commercial ELISA kits (R&D Systems). The assays were carried out according to the manufacturer's instructions. All assays were performed at room temperature. Absorbance was read at 450 nm on a spectrophotometer (Biorad Microplate Fluorescence Reader). A reading at 550 nm was subtracted to correct for plate background. Protein concentrations of samples were calculated from the standards with the supplied software for the spectrophotometer using a 4-parameter curve fit.

Statistical analysis

Results were expressed as means ± SD from 3 independent experiments. Statistical analysis of real-time RT-PCR data was performed on ΔCt values(Yuan et al., 2006). Shapiro-Wilk testing for normal distribution and Levene testing for homogeneity of variance were performed prior to ANOVA. *P*-values were corrected for multiple comparisons with the Bonferroni-Holm method. A *p*-value of < 0.05 was considered significant.

5. Results

5.1 Mitogenic and cytotoxic effect of sex hormones on primary human monocytes.

To test mitogenic or cytotoxic effect of hormones and LPS, primary monocytes (5×10^5) were stimulated with β-estradiol; Es (0.1-10 ng/ml), progesterone; Pr (1-100 ng/ml) or LPS from *P.gingivalis* (100 ng/ml) for 3 h and 6 h in quadruplicate cultures. Un-stimulated cells served as controls. Cells were collected and processed in the Cell Titer 96 cell proliferation assay. After 3 and 6 h of β-estradiol (0.1-10 ng/ml) and progesterone (1-100 ng/ml) stimulation, no significant change in number of monocytes was detected in comparison to controls ($p > 0.05$, **Figure 2**). These data show that after 3 and 6 h, neither β-estradiol nor progesterone has a mitogenic nor a cytotoxic effect on primary human monocytes. Any hormone-induced changes in monocyte responses are therefore not due to changes in cell numbers. This is in agreement with studies reported earlier that estrogen and progesterone had no effect on viability of U937 monocytes (Jain et al., 2004) and human monocytes (Bouman et al., 2004).

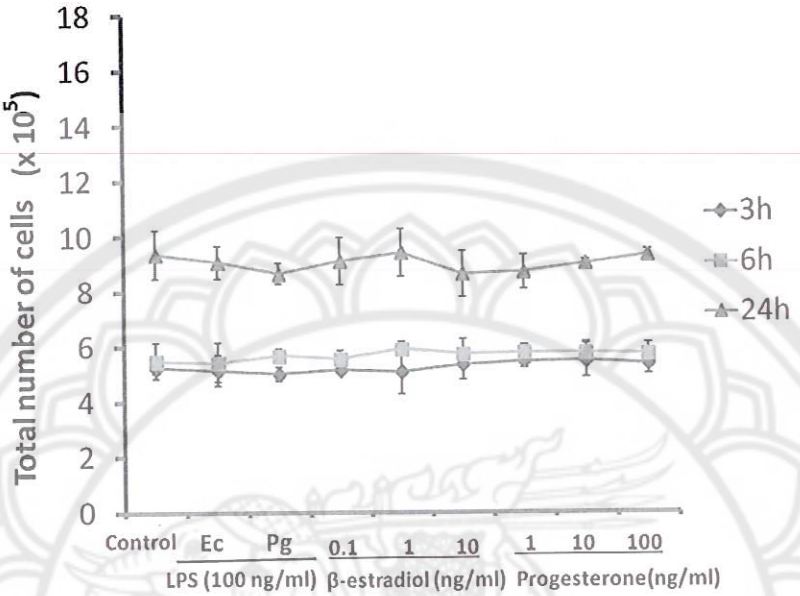

Figure 2 The effect of β-estradiol and progesterone on cell proliferation in primary human monocytes

Effects of β-estradiol and progesterone on cell proliferation in primary human monocytes. Monocytes were stimulated with the indicated concentration of hormones or LPS for 3, 6 and 24 h. Cell proliferation was analysed with the Cell Titer96 cell proliferation assay. The graph represents mean ± SD from one experiment with four independent cultures. Not significant compared with control.

5.2 β-estradiol and progesterone down-regulate monocyte TLR2 expression

The stimulation of TLR4 and CD14 expression by LPS is a key mechanism to activate the production of proinflammatory cytokines (Jiang et al., 2005; Lu et al., 2008). We therefore investigated the effect of female sex hormones on the expression of TLR in monocytes. Primary human monocytes were stimulated with β-estradiol, progesterone, *P.gingivalis* or *E. coli* LPS. As shown in Figure 3, flow cytometry analysis showed a significant increase in TLR2 expression on the cell surface of primary human monocytes after *P.gingivalis* LPS (TLR2 agonist) stimulation. However, *E. coli* LPS appeared to have no effect on TLR2 expression. Interestingly, there was a significant decrease in TLR2 expression in β-estradiol and progesterone-stimulated primary human monocytes in comparison with unstimulated controls.

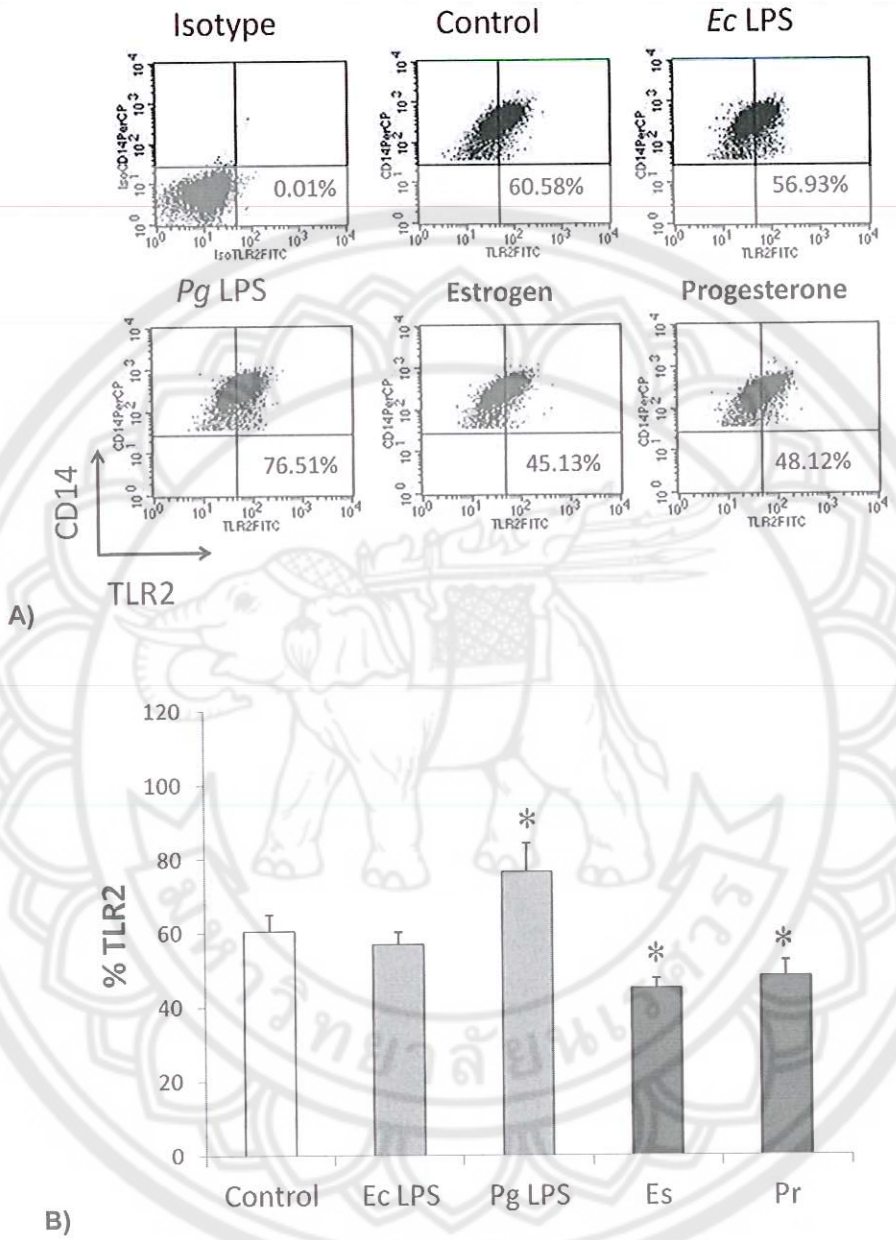

Figure 3 Effect of β-estradiol and progesterone on TLR2 expression in primary human monocytes.

Primary human monocytes (2 x 10^6 cells) were stimulated with *P.gingivalis* LPS; *Pg* LPS) or *E. coli* LPS; Ec LPS (100 ng/ml) or β-estradiol; Es (1 ng/ml) or progesterone; Pr (10 ng/ml) for 24 h. TLR2 protein expression on the cell surface of primary human monocytes was analyzed with flow cytometry. (A) Dot plots show one representative experiment. (B) Bar charts are derived from the analysis of the upper-right quadrant of five independent experiments compared with an isotype control (mean±SD; ANOVA; Student's *t*-test; $*P \leq 0.01$ compared with control).

5.3 β-estradiol and progesterone had no effect on monocyte TLR4 expression

Primary human monocytes were stimulated with β-estradiol, progesterone, *P.gingivalis* or *E. coli* LPS. *E. coli* LPS, which is a TLR4 agonist, induced significant up-regulation of TLR4 expression. To a lesser extent, *P.gingivalis* LPS also affects TLR4 expression although the effect was minimal when compared to *E. coli* LPS. Unlike TLR2 expression, incubation of primary human monocytes with either β-estradiol or progesterone did not affect expression of TLR4 (**Fig. 4A, B**).

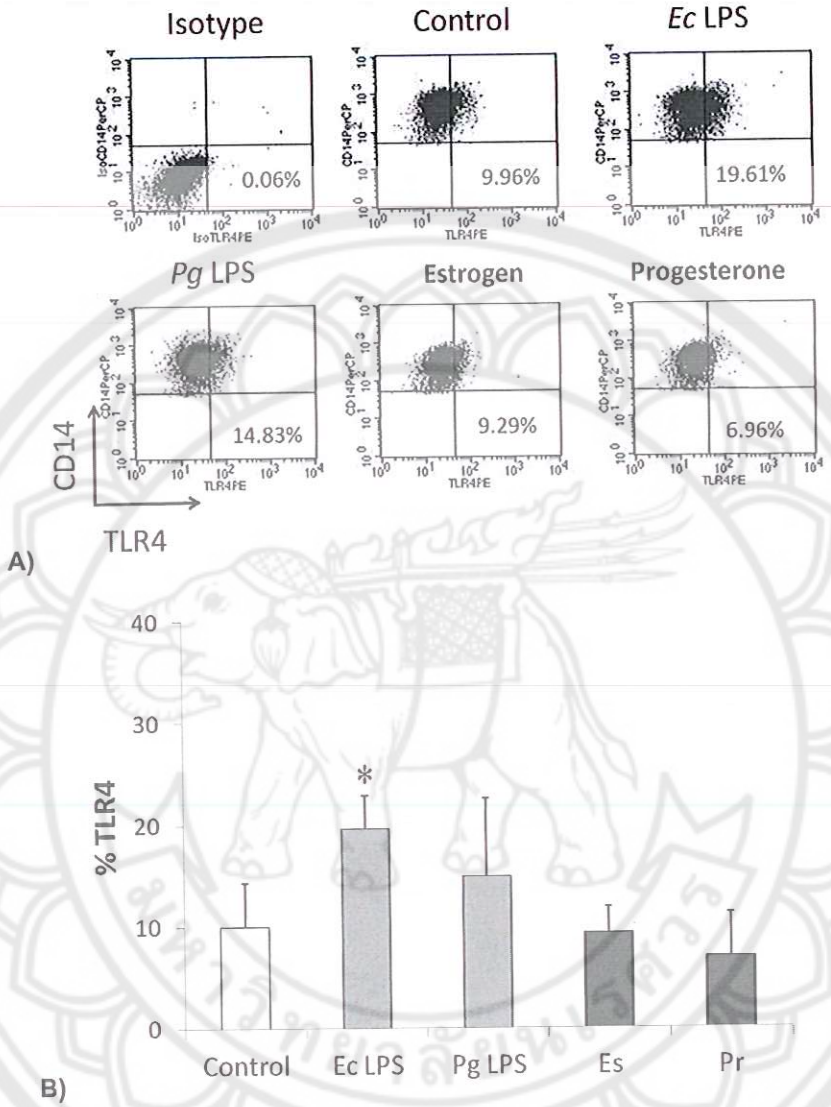

Figure 4 Effect of β-estradiol and progesterone on TLR4 expression in primary human monocytes.

Primary human monocytes (2 x 10^6 cells) were stimulated with *P.gingivalis* LPS; *Pg* LPS) or *E. coli* LPS; Ec LPS (100 ng/ml) or β-estradiol; Es (1 ng/ml) or progesterone; Pr (10 ng/ml) for 24 h. TLR4 protein expression on the cell surface of primary human monocytes was analyzed with flow cytometry. (A) Dot plots show one representative experiment. (B) Bar charts are derived from the analysis of the upper-right quadrant of five independent experiments compared with an isotype control (mean±SD; ANOVA; Student's *t*-test; $*P \leq 0.01$ compared with control).

5.4 Progesterone or β-estradiol down-regulate monocyte CD14 expression

CD14 is essential for LPS signaling in TLR2 and TLR4 (Miyake, 2007) and therefore, changes in CD14 expression impact on both TLRs' signaling capacity. To assess the impact of hormones or LPS on CD14 expression, primary human monocytes were stimulated with β-estradiol, progesterone or *P.gingivalis* LPS, and CD14 cell-surface expression was analyzed with flow cytometry. Compared with control, *P.gingivalis* LPS had no effect on cell-surface CD14 expression in primary human monocytes. Significantly, we found that β-estradiol and progesterone decreased cell-surface CD14 expression after 24 h stimulation in primary human monocytes (Fig. 5, middle and bottom panels).

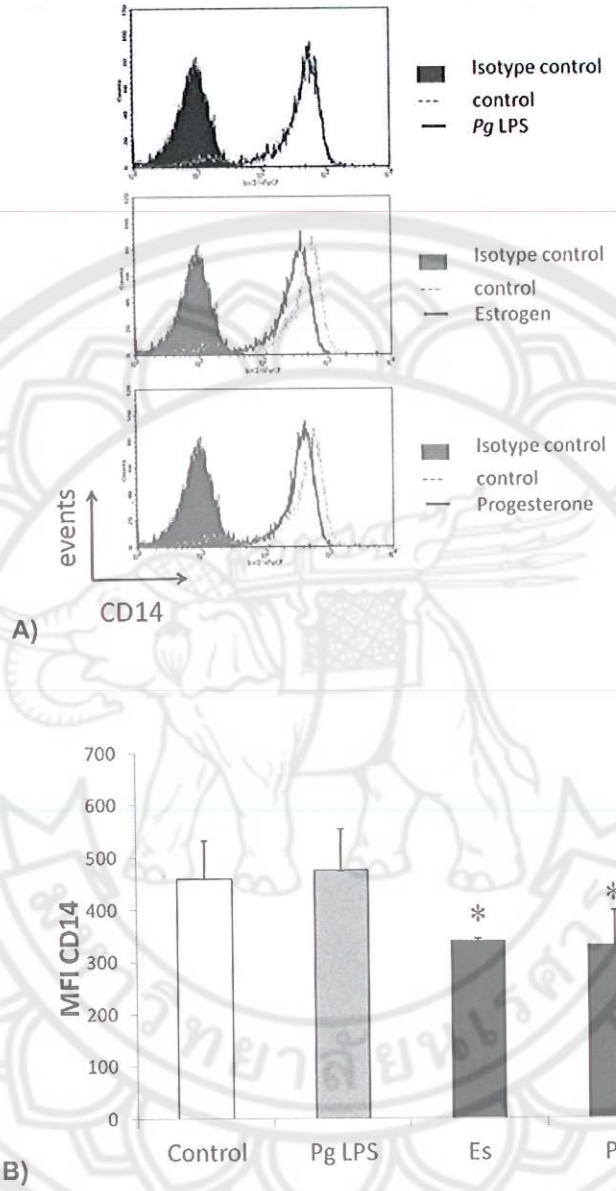

Figure 5 Effect of β-estradiol and progesterone on CD14 expression in primary human monocytes.

Primary human monocytes (2 x 10^6 cells) were stimulated with *P.gingivalis* LPS; *Pg* LPS) or *E. coli* LPS; Ec LPS (100 ng/ml) or β-estradiol; Es (1 ng/ml) or progesterone; Pr (10 ng/ml) for 24 h. CD14 protein expression on the cell surface of primary human monocytes was analyzed with flow cytometry compared with an isotype control. A) Histograms show one representative experiment. B) Bar charts are derived from analysis of MFI (mean±SD; ANOVA; Student's *t*-test; $^*P \leq 0.05$ compared with control) from five independent experiments.

5.5 Effect of progesterone and β-estradiol on mRNA expression of Cox2, TLR2 and TLR4 in primary human monocytes.

To investigate the effect of progesterone or β-estradiol on Cox2, TLR2 and TLR4 expression at mRNA level, monocytes (2×10^6) were stimulated with *P.gingivalis* LPS (100 ng/ml) or β-estradiol (0.1-10 ng/ml) or progesterone (1-100 ng/ml) for 3 h. Unstimulated cells were served as control. Total cellular RNA was extracted and reverse transcribed to produce cDNA. The cDNAs were analysed for the mRNA expression of Cox2, TLR2 and TLR4 by conventional RT-PCR.

As shown in **Figure 6**, primary monocytes constitutively expressed Cox2, TLR2 and TLR4 mRNA. Compared to control, a distinct up-regulation of Cox2 mRNA expression was detected after treatment with *P.gingivalis* LPS. In contrast, β-estradiol (0.1-10 ng/ml) and progesterone (1-100 ng/ml) dose-dependently down-regulated Cox2 mRNA expression after 3 h stimulation. However, there was no clear change of TLR2 and TLR4 mRNA expression in primary monocyte treated with hormones or LPS as compared with controls.

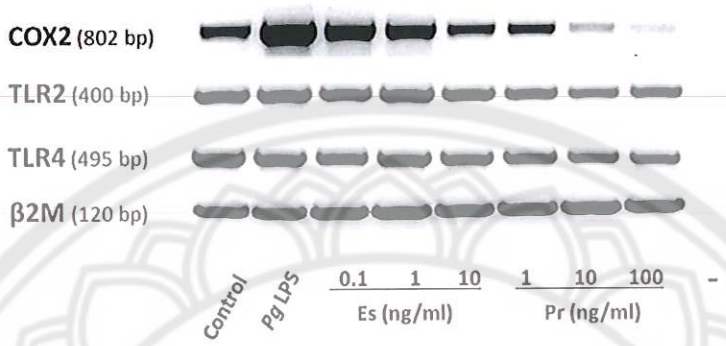

Figure 6 Effect of progesterone and β-estradiol on the mRNA expression of Cox2, TLR2 and TLR4 in primary human monocytes.

Primary human monocytes (2 x 10^6 cells) were stimulated with *P.gingivalis* LPS; *Pg* LPS (100 ng/ml) or β-estradiol; Es (0.1-10 ng/ml) or progesterone; Pr (1-100 ng/ml) for 3 h. Total RNA was isolated and the mRNA expression of Cox2, TLR2, TLR4 and β2M were analyzed by RT-PCR. Negative (-) control was H_2O. Shown are representative results of 3 independent experiments.

Because traditional PCR is only semi-quantitative at best, the quantification of Cox2 mRNA expression was then performed using realtime PCR. **Figure 7** shows the results from realtime PCR. Compared to control, both β-estradiol and progesterone significantly down-regulated Cox2 mRNA expression at 3 h ($p < 0.05$). The expressions of Cox2 mRNA by monocyte-simulated with β-estradiol at 0.1, 1 and 10 ng/ml were 0.49-fold, 0.40-fold and 0.09-fold, respectively. In addition, the expressions of Cox2 mRNA by monocyte-simulated with progesterone at 1, 10 and 100 ng/ml were 0.75-fold, 0.24-fold and 0.10-fold, respectively. However, *P.gingivalis* LPS significantly up-regulated Cox2 mRNA expression (11.74- fold; $p < 0.05$) at 3 h. These data suggested that female sex hormones regulate Cox2 production in human monocytes at mRNA level and play a role in immune response.

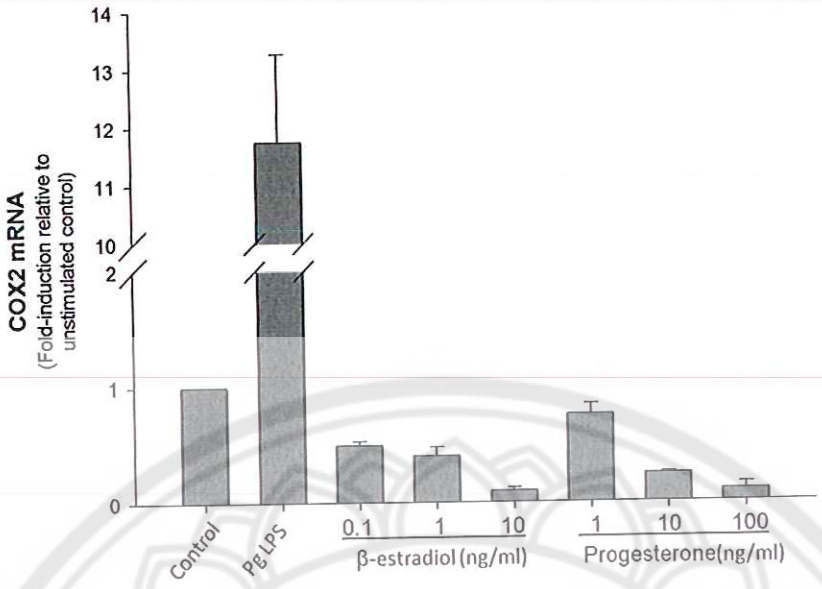

Figure 7 Cox2 mRNA expression by primary human monocytes after stimulation with *P. gingivalis* LPS and female sex hormones.

Primary monocytes (2 x 10^6 cells) were stimulated with *P.gingivalis* LPS; *Pg* LPS (100 ng/ml) or β-estradiol; Es (0.1-10 ng/ml) or progesterone; Pr (1-100 ng/ml) for 3 h. The amount of Cox2 mRNA was quantified by realtime PCR. The data are expressed as mean of duplicate cultures of 3 independent experiments (n=6). Statistics: ANOVA. *: $p < 0.05$ compared with controls.

5.6 Effect of progesterone and β-estradiol on Cox2 mRNA expression by primary monocytes in response to *P.gingivalis* LPS

To study effect of estrogen and progesterone on Cox2 mRNA expression by monocytes in response to *P.gingivalis* LPS, primary monocytes (2×10^6) were pre-incubated with β-estradiol (1 ng/ml) or progesterone (10 ng/ml) for 3 h. LPS from *P.gingivalis* (100 ng/ml) were then added for 3 h. Un-stimulated cells and cells stimulated with hormones or LPS alone served as control. Cox2 mRNA expression was quantified by realtime PCR.

As shown in **Figure 8**, *P.gingivalis* LPS alone enhanced Cox2 mRNA expression in monocytes whereas both β-estradiol (1 ng/ml) and progesterone (10 ng/ml) reduced it. Interestingly, pre-treatment monocytes with β-estradiol (1 ng/ml) and progesterone (10 ng/ml) significantly inhibited the effect of *P.gingivalis* LPS on Cox2 mRNA expression.

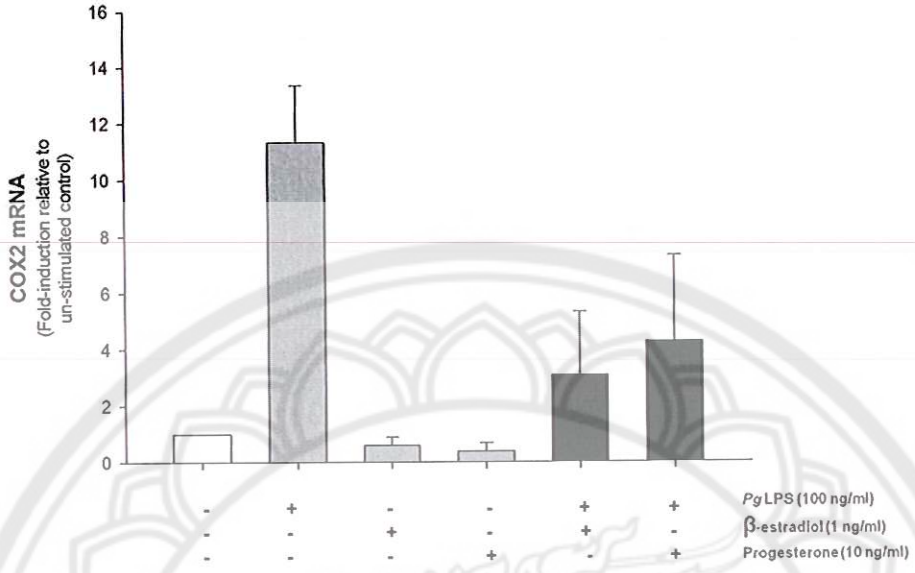

Figure 8 Cox2 expressions by primary monocytes in response to LPS and female sex hormones.

Primary monocytes (2 x 10^6 cells) were stimulated with β-estradiol (1 ng/ml) or progesterone (10 ng/ml) for 3 h, followed by stimulation with *P.gingivalis* LPS for 3 h. The amount of Cox2 mRNA was quantified by realtime PCR. The data are expressed as mean of duplicate cultures of 3 independent experiments (n=6). Statistics: ANOVA. *: $p < 0.05$ compared with controls.

5.7 The effect of estrogen and progesterone on PGE_2 production from primary human monocyte-stimulated with *P.gingivalis* LPS

To study the effect of estrogen and progesterone on PGE_2 release by primary human monocytes, human monocytes (0.5×10^6) were stimulated with β-estradiol (1 ng/ml) or progesterone (10 ng/ml) for 3 h, followed by stimulation with *P.gingivalis* LPS for 3 h. Un-stimulated cells were served as control. Supernatants were collected and assayed for PGE_2 concentrations with ELISA. As shown in Figure 9, both progesterone and β-estradiol inhibited PGE_2 secretion by primary human monocytes. In response to LPS, human macrophages were significantly up-regulated PGE_2 secretion after stimulation with *P.gingivalis* LPS. Interestingly, the effect of *P.gingivalis* LPS on PGE_2 secretion was reduced when cells were co-cultured with both progesterone (10 ng/ml) and β-estradiol (1 ng/ml). These data suggest an inhibitory effect of female sex hormones on monocyte-response to LPS.

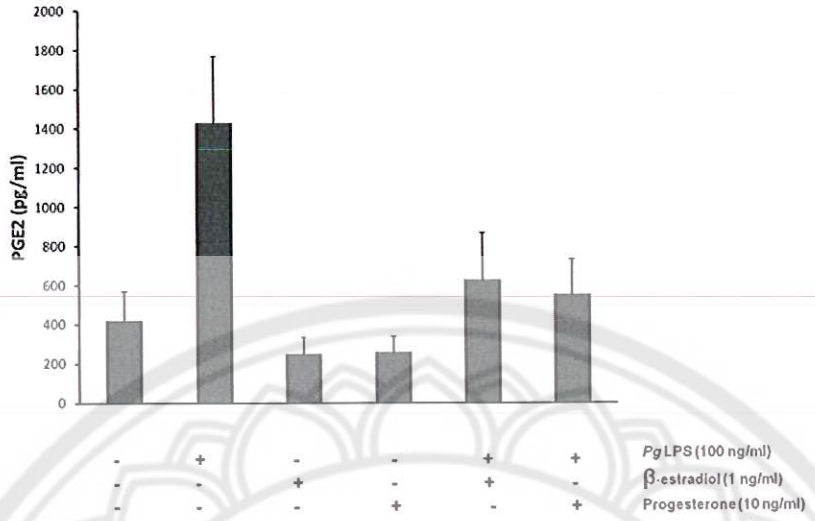

Figure 9 PGE_2 secretions by primary human monocytes in response to LPS and female sex hormones.

Primary monocytes (5 x 10^5 cells) were stimulated with β-estradiol (1 ng/ml) or progesterone (10 ng/ml) for 3 h, followed by stimulation with *P.gingivalis* LPS for 3 h. PGE_2 levels in supernatants was measured by ELISA and compared with controls. Each value is a mean (± SD) of duplicate cultures from 3 experiments.

6. Discussions

Alteration of maternal immune responses during pregnancy may increase the susceptibility of pregnant women to a number of infections, including periodontal disease (Armitage, 2013). As female sex hormones play a role in the pathogenesis of pregnancy-associated gingival diseases (Mealey and Moritz, 2003a; Kamal et al., 2012), understanding the effect of estrogen and progesterone on cellular immune responses may provide an insight in the mechanistic link between pregnancy and periodontal diseases. Previous studies have identified the role of estradiol and progesterone as important immune modulators (Gilliver, 2010; Garcia-Gomez et al., 2013). Interestingly, estradiol and progesterone can have either immunostimulant or immunosuppressive effects (Calippe et al., 2010; Giannoni et al., 2011). The present study supported that estradiol and progesterone can diminish the immune response by primary human monocytes. We demonstrated that estradiol and progesterone decreased TLR2 and CD14 expression but did not affect TLR4 expression in primary human monocytes. In addition, estradiol and progesterone significantly inhibited Cox2 mRNA expression and modulated monocyte responses to *P. gingivalis* LPS, a TLR2 ligand and key pathogen in periodontal disease. Pre-treatment monocytes with estradiol or progesterone reduced the effect of *P. gingivalis* LPS on the expression of Cox2 mRNA and the production of PGE_2 by primary human monocytes.

The activation of TLRs initiates and controls innate and acquired immune responses in host defense. Previous studies have reported an effect of female sex hormones on the expression of cell-surface activation markers, such as CD14, TLR2 or TLR4, in different cell types (Su et al., 2009; Giannoni et al., 2011; Fukuyama et al., 2012). For example, pre-treatment cells with progesterone can significantly inhibit TLR4 and TLR9-triggered IL-6 and nitric oxide (NO) production in macrophages (Su et al., 2009). Additionally, neither the number of TLR4-expressing macrophages nor the expression level of TLR4 was affected by E2 treatment (Calippe et al., 2010). Although Giannoni et al. (2011) failed to show that β-estradiol and progesterone can decrease expression of TLR4 and TLR2 in cord blood mononuclear cells (CBMCs), progesterone was found to be a potent inhibitor for LPS-stimulated TLR4 induction in the cultured fibroblasts from human uterine cervix (Fukuyama et al., 2012). Possible explanations for the observed differences may include discrepancies in concentration, timing and duration of hormone treatment, and expression of specific receptor subtypes and receptor coactivators in distinct cell types of different species. However, to the best of my knowledge, there is no study aimed to determine the direct effect of female sex hormones on the cell-

surface protein expression of these makers on human monocytes. In the present report, both β-estradiol and progesterone induced a down-regulation of TLR2 and CD14 cell-surface expression in primary human monocytes. However, we did not observe changes of TLR2 and TLR4 expressions at mRNA level although analysis of TLR2 and TLR4 mRNA expression was measured only by conventional RT-PCR. Therefore, it could be speculated that altered female sex hormone concentrations (Robinson and Klein, 2012) may play a role in the immunological shift that occurs during pregnancy by down-regulation of cell-surface activation markers in monocytes.

Several lines of evidence support that elevated levels of steroids have profound effects on the function of immune system. The results from the present study also demonstrated that female sex hormones regulate Cox2 production in human monocytes at mRNA level. In agreement with previous studies (Beyan et al., 2006; Rosignoli et al., 2013), the present study demonstrated that Cox2 mRNA is expressed in human monocytes and Cox2 expression can be induced by inflammatory stimuli such as LPS. Interestingly, both estradiol and progesterone down-regulate Cox2 mRNA expression in a dose-dependent manner.

Previous studies reported that effects of estradiol and progesterone on inflammatory response by monocytes/macrophages are dependent on their concentrations (Miyagi et al., 1992; Bouman et al., 2005; Armitage, 2013). For example, Bouman et al. (2005) reported that low estradiol concentrations enhanced IL-1, IL-6 and TNF-α production, whereas high estradiol concentrations reduced production of these cytokines. In addition, estradiol and progesterone inhibited the production of IL-1 from human peripheral monocytes (Morishita et al., 1999) and estrogen inhibited IL-6 in monocytes activated by LPS (Jain et al., 2004). These findings likely explain the fact that elevated levels of female sex hormones may suppress innate immune responses (Robinson and Klein, 2012).

Hormonal and immunological changes affect the outcome of immune-related diseases by altering functions of immune cells. For example, elevated concentrations of progesterone are hypothesized to alter not only local immune responses, but also genital tract mucosa, to increase the risk of HIV infection during pregnancy (Gray et al., 2005). The severity of many infectious diseases, which require inflammatory responses for the initial control and clearance of pathogens, is increased during pregnancy (Krishnan et al., 1996; Jamieson et al., 2009). In periodontal disease, it is postulated that pregnancy gingivitis is initiated by bacterial biofilm and exacerbated by endogenous sex steroid hormones (Mealey and Moritz, 2003). Therefore, the interaction between female sex hormones and periodontal bacteria such as *P.gingivalis* may exist. We demonstrated, for the first time, an inhibitory effect of female sex hormones for

P.gingivalis LPS, a TLR2 ligand and key pathogen in periodontal disease. Clearly, treatment with either estradiol or progesterone led to a significant decrease in the *P. gingivalis* LPS-stimulated expression of Cox2 mRNA expression and PGE_2 production in primary human monocytes. In agreement with the present results, previous studies have demonstrated the anti-inflammatory roles of female sex hormones. In newborn mononuclear cells, estradiol and progesterone strongly inhibited TNF and IL-6 production induced by purified microbial products as well as *E. coli* LPS (Giannoni et al., 2011). In addition, progesterone inhibited TNF-α production by monocytes stimulated with *E.coli* LPS (Peltier et al., 2008). Taken together, these data suggest the anti-inflammatory role of female sex hormones in cellular immune response to bacterial LPS. In conclusion, the present study supports that estradiol and progesterone alter innate immune responses of primary human monocytes by down-regulating of cell surface expression and inhibiting production of inflammatory mediators. The immunosuppressive effect of estradiol and progesterone may play a role in periodontal disease asscociated with pregnancy.

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ภาคผนวก

Abstract

IADR APR meeting
Type: Oral Session -O3
Slot: Wednesday, August 21, 2013: 10:30 a.m.-12:15 p.m.

SPEAKERS/PRESENTATIONS

Start Time: 10:30 a.m.

Female sex hormones modulate *P.gingivalis* LPS induced-PGE_2 release in monocytes/macrophages

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Abstract

Objective: Female sex hormones are elevated and are a potential host response modifier during pregnancy. Dysregulation of immune responses by progesterone and estrogen may be responsible for periodontal inflammation. Therefore, we aimed to investigate the effect of progesterone and estrogen on PGE_2 secretion in human monocytes and macrophages. Method: Human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of healthy donors by Lymphoprep™ gradient according to standard procedures. Monocytes were purified from PBMCs by adherent method and were differentiated into macrophage-like cells by the incubation with 50 units/ml of recombinant human granulocyte macrophage-colony stimulating factor (rhGM-CSF) for 6 days. Monocytes or macrophages were stimulated with progesterone (10-1000 ng/ml), β-estradiol (1-100 ng/ml) or lipopolysaccharide (LPS) from E.coli and P.gingivalis (100 ng/ml) for 6 h. Supernatants were collected and assayed for PGE_2 levels by ELISA. Result: In primary monocytes, progesterone dose-dependently increased PGE_2 secretion whereas β-estradiol (1 and 10 ng/ml) decreased PGE_2 secretion and β-estradiol (100 ng/ml) only had minimal effect on PGE_2 secretion compared to control. In monocyte-derived macrophages, differentiated cells expressed higher background levels of PGE_2 than of those primary monocytes. In contrast to primary monocytes, both progesterone and β -estradiol inhibited PGE_2 secretion by macrophages. These data suggest that monocytes and macrophages may exhibit a differential magnitude of inflammatory responses to female sex hormones. In response to LPS, human monocytes/macrophages significantly up-regulated PGE_2 secretion after stimulation with P.gingivalis and E.coli LPS. Interestingly, the effect of P.gingivalis and E.coli LPS on PGE_2 secretion was reduced when cells were co-cultured with either progesterone (10 ng/ml) or β -estradiol (1 ng/ml). Conclusion: Progesterone and β -estradiol inhibited PGE_2 secretion in monocytes/macrophages treated with P.gingivalis LPS and this process may have a role in the pathogenesis of periodontal disease associated with pregnancy.

Start Time: 10:45 a.m.

Oral commensals and pathogens enhance hBD-2 expression in HOKs