Effects of combined 17β-estradiol with TCDD on secretion of chemokine IL-8 and expression of its receptor CXCR1 in endometriotic focus-associated cells in co-culture

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BACKGROUND: Chemokines play an important role in the pathogenesis of endometriosis. In the present study, the transcription of 18 chemokine receptors in eutopic endometrium and ectopic tissue with endometriosis was first analysed by RT–PCR. Dioxin, an air pollutant, and estrogen are reported to be associated with endometriosis. The regulatory mechanisms of dioxin and estrogen in the expression of CXCR1/IL-8 in the corresponding cells will help in elucidating roles of the chemokine in the aetiology of endometriosis. METHODS AND RESULTS: CXCR1, a type of chemokine receptor, was over-expressed in endometriotic tissue. The high translation of the receptor and its ligand, interleukin (IL-8), in endometriotic tissue was then demonstrated by immunochemistry. Estradiol and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alone inhibited expression of CXCR1, whereas the combination of estradiol with TCDD up-regulated the expression. TCDD promoted IL-8 secretion by human pelvic mesothelial cells (HPMC), and 17β-estradiol magnified the stimulatory effect. Both 17β-estradiol and TCDD alone inhibited IL-8 secretion of U937 (a cell line of monocyte), but combination of 17β-estradiol and TCDD had no further inhibitory effect. The co-culture of endometrial stromal cells (ESC) with HPMC produced more IL-8 than respective or total production of either of the cells alone, and estradiol played a synergistic stimulatory role with TCDD in IL-8 secretion of the co-culture. Interaction of HPMC and the monocytes significantly stimulated IL-8 secretion, suggesting a main resource of IL-8 in peritoneal cavity with endometriosis. TCDD promoted IL-8 secretion by HPMC–U937 co-culture, but exerted a contrary effect for IL-8 secretion when combined with estradiol. CONCLUSION: Estradiol and TCDD in the peritoneal cavity can lead to a persistent and serious inflammation, which gives a new insight into the interactions of estrogen and TCDD in endometriosis.

Key words: endometriosis/17β-estradiol/interleukin-8/TCDD/co-culture

Introduction

Endometriosis is a common gynaecological disorder that causes pelvic pain and infertility in ∼10% of fertile women (Strathy et al., 1982). Although multiple theories of the histogenesis of endometriosis exist, no single paradigm can explain all cases of endometriosis (Witz et al., 1999). There are essentially four different forms of endometriosis: rectovaginal endometriosis, adenomyosis, ovarian cystic endometriosis and peritoneal endometriosis, each with its own pathogenesis (Dunselman et al., 2001). The present study focused on the pathogenesis of peritoneal endometriosis, the most accepted theory being Sampson’s transplantation theory. Since retrograde menstruation is a normal process occurring in fertile women, other elements must be involved in the disease progression. Chemokines, a superfamily of specific cytokines, consist of four families: CXC (α), CC (β), XC (γ) and CX3C (δ); the corresponding receptors are CXCR1-6, CCR1-10, XCR1 and CX3CR1 respectively (Zlotnik and Yoshie, 2000). These chemokines have the ability to chemotactically attract and activate inflammatory cells (Visser et al., 1998). It is also increasingly clear that chemokines, especially interleukin-8 (IL-8), monocyte chemotactic protein (MCP)-1, regulated-upon-activation normal-T-cell-expressed and -secreted (RANTES), and growth-regulated oncogene-α (GRO-α), are produced by endometrial, myometrial, and trophoblast cell types in a timed and co-ordinated manner, and play a relevant role in many physiological and pathological situations, such as ovulation, menstruation, implantation, cervical ripening, preterm labour, and endometriosis (Garcia-Velasco and Arici, 1999; Kayisli et al., 2002).

Current evidence suggests local peritoneal immune inflammation might be associated with the origin of endometriosis. The onset of endometriosis is accompanied by a massive leukocyte infiltration that is intensively orchestrated by a complex
network of cytokines and chemokines. At direction of the chemokine gradient, leukocytes migrate from blood vessels embedded in peritoneal interstitium across the monolayer of mesothelial cells into the peritoneal cavity (PC). These small polypeptide chemokines display remarkable specificity toward various leukocyte subpopulations (Rollins et al., 1997). Most CXC chemokines act as potent chemoattractants for neutrophils, whereas members of the CC chemokine subfamily mediate mainly chemotaxis of the mononuclear cells (Janusz et al., 2001). So far ~60 kinds of chemokines have been identified; it is difficult to investigate the role of each chemokine in pathogenesis of endometriosis. The biological function of chemokines depends on binding to the corresponding receptors, which show overlapping specificities. Therefore identifying chemokine receptors that are highly expressed in the ectopic tissues and exploring functions of these receptors as well as their ligands may provide some useful insights into the molecular mechanisms in the pathogenesis of endometriosis.

To elucidate the potential mechanisms for initiation events in the pathogenesis of endometriosis and the phenomenon of the increased chemokine receptors as well as their ligands in the PC with endometriosis, the elements should be recruited in regulating the expression of chemokines and their receptors. In humans, an increased incidence of endometriosis has been associated with exposure to the environmental chemicals (Cotroneo and Lamartiniere, 2000). TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), one of the family of chlorinated aromatic hydrocarbons known as dioxins, is ubiquitous and probably the most feared environmental contaminant worldwide. Dioxins are produced as unwanted contaminants of many industrial and combustion processes (Guo, 2004), are resistant to degradation and, because of their lipophilic nature, bioaccumulate and biomagnify at higher trophic levels of the food chain. The myriad biological effects of TCDD are believed to be mediated essentially via aryl hydrocarbon receptor (AhR) binding. The liganded AhR associates with its arylhydrocarbon receptor nuclear translocator (Arnt), and moves from the cytoplasm to the nucleus. The heteromeric complex acts as a signal transducer and transcription factor for target genes, including cytochromes P450 1A1, 1A2, 1B1 (CYP1A1, CYP1A2, CYP1B1), and growth regulatory genes are involved in cell proliferation, differentiation and inflammation (Zhao et al., 2002). In view of the dependence of endometriosis upon ovarian hormones, our aim was to investigate the potential links between endometriosis and TCDD exposure in the environment, which was reported to exert an estrogen-related effect. TCDD might give rise to increased levels of CYP1A1, 1A2, and 1B1 in endometrial tissues that reach pelvic peritoneal mesothelium by way of retrograde menstruation. These enzymes are 17β-estradiol hydroxylases, which catalyse C19 steroids to estrogen. Furthermore, transcriptional level of CYP1A1 was found to be strikingly higher in endometriotic tissues than in eutopic endometrium, but undetectable in normal endometrium, suggesting that increased P450 1A1 enzyme activity may promote the development and growth of endometriosis by inducing the formation of catechol estrogens (Noble et al., 1996; Bulun et al., 2000). Besides pro-inflammatory effects, TCDD have endocrine-disrupting properties as either estrogens or anti-estrogens in a tissue- and species-specific manners, depending on the level of estradiol in vivo (Ohtake et al., 2003).

We hypothesized that coordination of over-expressed chemokine receptors in endometriotic tissue with their corresponding ligands may be an important step in the beginning of endometriosis. In the present study, we first confirmed that CXCR1 among 18 chemokine receptors was expressed significantly more in ectopic tissue than in eutopic endometrium by using RT–PCR and immunohistochemistry. IL-8, a ligand of CXCR1, is also known to be elevated in peritoneal fluid of women with endometriosis, and plays an important role in the progress of endometriosis. Therefore we looked at CXCR1/IL-8 for further study. Both TCDD and estradiol are involved in signal transduction of some chemokines (Zhao et al., 2002). It is plausible to postulate that TCDD and estradiol may lead to different expression of CXCR1/IL-8 in endometriotic and eutopic endometrial tissues, and thus elucidate the regulatory effect in vitro of 17β-estradiol and TCDD on the expression of CXCR1/IL-8 by the corresponding ectopic cells.

**Materials and methods**

**Tissue collection and cell culture**

All the endometriotic and eutopic endometrial tissues were obtained from 15 patients with endometriosis (mean age 41.6 years; range 26–47) at the time of laparoscopy and by uterine curetage, respectively, in the Hospital of Obstetrics and Gynecology, Fudan University. The endometriotic tissues included peritoneal implants (n = 3) and ovarian endometriomas (n = 12). The patients were classified according to the revised American Fertility Society classification (AFS): four had stage 1, and 11 had stage 2, endometriosis. Patients had not received any GnRH analogue or other hormonal drug in the 6 months prior to the surgical procedure. Before surgery, informed consent was obtained from each patient using protocols approved by the Human Investigation Committee of Fudan University. All the samples were obtained in the proliferative phase of the cycle, which was confirmed histologically according to established criteria (Noyes et al., 1950). All of the endometriotic samples and a part of eutopic endometrium specimens were snap-frozen in liquid nitrogen, and the other eutopic tissues were immediately transferred to the laboratory.

The minced eutopic endometrium was digested with collagenase type α (0.1%; Sigma, USA) for 30 min at 37°C with constant agitation. The tissue pieces were filtrated through a 400 μmol/l wire sieve to remove debris. Following gentle centrifugation, the supernatant was discarded, and the cells were resuspended in 1:1 formula of DMEM (Dulbecco’s modified Eagle’s medium)/F-12 (Gibco, USA). The ESC were separated from epithelial cells by passing over a 400 μmol/l wire sieve. The filtrated suspension was layered over Ficoll, and centrifuged at 200 rpm for 20 min to further remove leukocytes and erythrocytes. The middle layer of cells was collected, and washed with D-Hanks. The ESC were placed in a culture flask, and allowed to adhere for 20 min. The adherent stromal cells were cultured as monolayer in flasks with DMEM/F-12 containing 10% fetal calf serum (FCS; Hyclone, Logan, UT, USA), 20 mmol/l HEPES, 100 IU/ml penicillin and 100 μg/ml streptomycin.

In 24 h of culture, the ESC were stained using the Vectastain Elite ABC kit (Vector Laboratories, Inc. Burlingame, CA, USA). An immunoperoxidase staining was performed using anti-human vimentin and anti-human cytokeratin monoclonal antibody (Maixin Co. Ltd, Fuzhou, China). An isotypic control was used at the same concentration.
The primer sequences of chemokine receptors and GAPDH were prepared by Prof. Jian Yao, the First People’s Hospital, Shanghai, China) were maintained in a humidified, CO₂-controlled (5%) incubator. The medium was changed every other day.

**RT-PCR**

The total RNA of the ESC, endometriotic and eutopic endometrial tissues was prepared by Trizol reagent (Sigma, USA). The RT reaction was performed using a nonamer primer and 1 μg of RNA in a volume of 20 μl. PCR primers of the chemokines and house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are indicated in Table I. The PCR cycles and conditions for denaturation, annealing, and elongation were 35 cycles, 1 min at 94°C, 30 s at 55°C, and 30 s at 72°C respectively. The amplified DNA was fractionated by electrophoresis, and ethidium bromide-stained bands were photographed.

**Immunohistochemistry**

The endometriotic and eutopic endometrial tissues were cut into serial sections of 5 μm, and stained using Vectastain Elite ABC kit (Vector Laboratories, Inc., Burlingame, CA, USA). An immunoperoxidase staining was performed using mouse monoclonal IgG antibodies against human CXCR1 (25 μg/ml; RD Systems, Wiesbaden, Germany). An isotypic control was used at the same concentration. The CXCR1 expression was analysed by IMS DNA colour image analysis system.

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Flow cytometry

The ESC (1×10^5/well) were incubated for 30 min at room temperature with 100 μl phosphate-buffered saline containing 0.2% bovine serum albumin (PBS–BSA) supplemented with 2 μl anti-human CXCR1 antibody (10 μg/ml; R&D Systems, Wiesbaden, Germany). After three washes with PBS–BSA, the bound primary antibody was revealed by addition of 100 μl of the FITC-conjugated secondary antibody (1:10 dilution according to the manufacturer’s instructions). After 30 min staining, the cells were washed with PBS–BSA, and the fluorescence intensity at 543 nm was analysed by a FACSCAN flow cytometer (Becton Dickinson, Mountain View, CA, USA).

Enzyme-linked immunosorbent assay (ELISA) for determination of IL-8

The culture supernatants were harvested, centrifuged to remove cellular debris, and stored at −170°C until assayed by ELISA. The IL-8 concentration in the culture supernatant was quantified by ELISA kits (R&D Systems, Wiesbaden, Germany) according to the manufacturer’s instructions. The limit of detection was <3.5 pg/ml.

Statistical analysis

RT–PCR and immunohistochemistry results were expressed as mean ± SD, and analysed by paired-sample t-test. ELISA and FCM data were expressed as mean ± SEM, and performed using analysis of variance (one-way analysis of variance), with application of the least significant difference test. P < 0.05 was considered statistically significant.

Results

Immunocytochemical characterization for the purity of ESC

We evaluated expression of cytokeratin and vimentin on the cultured ESC. As shown in Figure 1A, the isolated cells almost all stained for vimentin, whereas in Figure 1B, no cells were found stained by anti-cytokeratin antibody. The purity of isolated ESC was >95% (Figure 1).

Chemokine receptor transcription in endometriotic and eutopic endometrial tissues and ESC

Among all the 18 chemokine receptors, transcriptions of CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CXCR1, CXCR4, CXCR5, CXCR6 and XCR1 in ectopic tissues were higher than those in eutopic endometrium, whereas the transcription of CXCR1, CCR10, CXCR2, CXCR3 and CX3CR1 was lower in ectopic tissue. Among the higher transcribed chemokine receptors, only CCR5, CCR8 and CXCR1 showed significantly higher levels in ectopic tissue compared to that of eutopic endometrium (P < 0.05), and among the lower transcribed chemokine receptors, only CX3CR1 was expressed significantly lower in ectopic tissue compared to that of eutopic endometrium (P < 0.05) (Figure 2B).

The ESC transcribed almost all chemokine receptors except for CXCR5. The transcription pattern of the chemokine receptors in the primary eutopic endometrial stromal cells was in accordance with that of the eutopic endometrial tissue. In view of this, we chose ESC for further study in the pathogenesis of endometriosis (Figure 2B).

Immunohistochemistry of CXCR1 in the endometriotic and eutopic endometrium

Immunohistochemistry was used to localize CXCR1 protein in the frozen section. In eutopic endometrial samples, epithelial and stromal CXCR1 expression was observed in 13 of the 14 proliferative samples. CXCR1 immunoreactivity was localized in the surface epithelium and glands. The epithelial and stromal staining was mostly cytoplasmic and membranous. In all of the 15 endometriotic tissues, we observed an increased amount of CXCR1 immunoreactivity in the stromal compartment, but the epithelial cells were hardly observed.

The scoring of CXCR1 immunoreactivity in the stromal compartment of the endometriotic tissues, by blinded observers, showed significantly greater intensity than that of the eutopic endometrium (P < 0.05) (Figure 3).

CXCR1 expression on the ESC by FCM

CXCR1 expression was found on ESC (65.11 ± 1.2%). 17β-Estradiol or TCDD alone inhibited CXCR1 expression on the ESC in a dose-dependent manner (P < 0.05), but combination of estradiol and TCDD up-regulated the molecular expression on the cells, and the increased level was positively correlated with the concentration of estradiol in the combination (P < 0.05) (Figure 4).

Effect of estradiol and/or TCDD on IL-8 secretion by U937 cells

U937 cells also secreted naturally IL-8 in vitro. Either estradiol or TCDD alone resulted in a dose-dependent inhibition of IL-8 secretion by U937 cells (P < 0.05), but the combination of estradiol and TCDD had no further effect on the monocyte line (Figure 5).

Figure 1. Immunocytochemical characterization of vimentin (A), cytokeratin (B) and negative control (C) on ESC of primary culture.
Effect of estradiol and/or TCDD on IL-8 secretion by HPMC
HPMC secreted naturally IL-8 in vitro. TCDD significantly stimulated HPMC IL-8 secretion in a dose-dependent manner. Although estradiol alone had no obvious effect on IL-8 secretion, it facilitated the stimulatory effect of TCDD on IL-8 secretion of the HPMC (Figure 6).

Effect of the cell co-culture on IL-8 secretion
The respective culture of ESC, HPMC and U937 cells for 48 h had almost equivalent secretion of IL-8. The co-culture of ESC with U937 cells showed no effect on IL-8 secretion, whereas the co-culture of ESC with HPMC promoted IL-8 release. The IL-8 production in the non-contact co-culture and in the contact co-culture unit was 3.37- and 8.7-fold respectively more than the total production of the respective ESC and HPMC culture (P < 0.05). More strikingly, when HPMC were co-cultured with U937 cells for 48 h, the IL-8 production in the contact co-culture unit was 16.87-fold more than the total production of the respective HPMC and U937 culture (P < 0.05) although production in the non-contact co-culture unit increased only 1.93-fold. The results above showed that direct contact of the corresponding cells would promote the secretion of IL-8 (Figure 7).

Effect of estradiol and/or TCDD on IL-8 secretion by co-culture of ESC and HPMC
Compared to the control group, TCDD significantly stimulated IL-8 secretion of the co-culture unit of ESC and HPMC. The 17β-estradiol had no obvious effect on IL-8 production of the culture unit, but when combined with TCDD, showed a synergistic effect on the IL-8 secretion (Figure 8A).
Interactions of estrogen and TCDD in endometriosis

Effect of estradiol and/or TCDD on IL-8 secretion by co-culture of HPMC and U937 cells

Estradiol showed little effect on IL-8 secretion of the co-culture unit. TCDD significantly stimulated IL-8 release, and estradiol appeared antagonistic to TCDD on IL-8 secretion (Figure 8B).

Discussion

The involvement of chemokines in endometriosis is becoming more evident as research in this area progresses. Chemokines such as IL-8, MCP-1, RANTES, eotaxin and GRO-α are implicated directly or indirectly in the formation, maintenance and proliferation of endometriotic implants (Kayisli et al., 2002). They exert effects by binding to their cell membrane receptors, and the levels of the receptors modulate partly their actions (Ulukus et al., 2005), which led us to speculate that the over-expressed chemokine receptors in endometriotic foci might play a key role in the progress of endometriosis by cooperating with their corresponding chemokines. Our present data showed that CCR5, CCR8 and CXCR1 were transcribed significantly more in the ectopic tissue than in the eutopic endometrium. Immunohistochemistry analysis confirmed that CXCR1 was also translated more in the endometriotic tissue, which is in accord with the findings of a recent study (Ulukus et al., 2005). The observations suggest that the over-expression of CXCR1 in endometriotic tissue supports a potential role of IL-8 in the pathogenesis of endometriosis.

IL-8, a major ligand of CXCR1, is known to be elevated in peritoneal fluid of women with endometriosis, and the level correlates with the severity of the disease. It is a cytokine with chemotactic, activating, and surviving functions on neutrophils and T-cells (Kwak et al., 2002). Its other known actions in endometriosis include producing a local immuno-tolerant environment (Selam et al., 2002), directly affecting endometrial cell proliferation, taking part in neovascularization (Barcz et al., 2002), promoting the vicious circle of endometrial cell attachment (Arici, 2002), and increasing matrix metalloproteinase activity and invasive capability of ESC (Mulayim et al., 2004). The increased IL-8 enhances the adhesion and invasion of ESC to peritonium partly by binding to CXCR1 on the ESC surface.

Estrogen is believed to be essential for the maintenance and growth of ectopic implants, but little work has been done to investigate the biochemical mechanisms of estrogen in endometriosis (Akoum et al., 2001). In normal endometrium, the epithelial and stromal CXCR1 expression shows significant menstrual cycle-dependent variation, suggesting that the level of CXCR1 is modulated by sex hormone (Mulayim et al., 2003). In this study, 17β-estradiol or TCDD alone inhibited CXCR1 expression on the ESC of the primary culture. The
combined estradiol and TCDD up-regulated CXCR1 expression on ESC, suggesting a potential interaction between estradiol and TCDD in regulating the CXCR1 expression on ESC. In view of previous reports that TCDD exhibited both estrogen and anti-estrogen effect (Ohtake et al., 2003), we postulate that TCDD alone exerts an estrogen-related effect on CXCR1 surface expression on ESC, whereas when estradiol is present, TCDD appears to produce an anti-estrogen effect.

The potential sources of IL-8 in peritoneal fluid with endometriosis are macrophages, masothelial cells, endometrial cells and endometrial endothelial cells (HEEC) (Garcia-Velasco and Arici, 1999; Luk et al., 2005), but the precise mechanisms by which it increases are still not known. Recently, Luk et al. (2005) found that estradiol up-regulated IL-8 expression in HEEC with endometriosis, whereas it reduced it in HEEC without endometriosis. Furthermore, TCDD, as endocrine-disrupting and pro-inflammatory compounds, have the ability to up-regulate RANTES gene expression of ESC with endometriosis (Zhao et al., 2002). To test the hypothesis that estrogen and TCDD also have the potential link
Interactions of estrogen and TCDD in endometriosis

on IL-8 secretion, and thus may be a path for the genesis of endometriosis, we treated the main cells in endometriotic foci with a series of concentrations of 17β-estradiol and/or TCDD, and analysed IL-8 level in the cell culture supernatant. Our results suggest that TCDD significantly stimulate HPMC secretion of IL-8 in a dose-dependent manner. Estradiol facilitates the stimulatory effect of TCDD although it alone has no obvious effect on IL-8 secretion, suggesting that TCDD can induce the peritoneal inflammation via stimulating a pro-inflammatory cytokine such as IL-8.

The adhering and invading of the retrograded endometrial cells to the HPMC is a key step for the early stage of endometriosis. Because the retrograded ESC are responsible for the adherence and implantation of endometrium to peritoneum in the early stage of endometriosis (Julia et al., 2001; Witz et al., 2001), we constructed the co-culture unit of ESC and HPMC. This model was divided into two groups: one group had transwell chamber inserts such that ESC and HPMC could share common soluble molecule exchange, but not have contact with each other. The other group used a 24-well plate to ensure adequate contact between ESC and HPMC. We found that either indirect or direct cell interaction could induce IL-8 secretion, suggesting that ESC in the shed endometrium represent a foreign entity, initiating the acute inflammatory response by recruiting neutrophils. Since most women have retrograde flow of menstrual debris into the PC, but few suffer from endometriosis, we proposed that the retrograde ESC into the PC could induce a physiological rather than a pathological inflammatory response to eliminate the menstrual debris, which only leads to transitory and mild disturbance in the peritoneal milieu (Dunselman et al., 2001). Unfortunately, in the presence of 17β-estradiol and TCDD, IL-8 secretion abnormally increased.

Figure 6. Effect of 17β-estradiol or/and TCDD on IL-8 secretion by HPMC. HPMCs were treated with various concentration of 17β-estradiol (0.01–100nM) (A) or TCDD (0.01–10nM) (B) or 17β-estradiol (1nM) plus TCDD (1nM) (C), respectively, for 48 h. At the end of the culture, the supernatant was collected. IL-8 level was determined by ELISA. Data are expressed as mean ± SEM. * P < 0.05 compared to the control.

Figure 7. IL-8 secretion by ESC, HPMC and U937 cells alone or the co-culture. The cells were incubated in DMEM/F12 medium containing 2.5% FCS for 48 h. The supernatants were collected, and IL-8 were quantified by ELISA. Data are mean ± SEM for three replicates. * P < 0.05 compared to the control.
by which the retrograde endometrium is eliminated with difficulty, and leads to the vicious cycle of endometrium attachment.

Leukocytes, including macrophages and their numerous products, may be involved in the onset and development of endometriosis (Vinatier et al., 1996). The infiltrated increased macrophages in PC not only accumulated in ectopic tissues but also harbour in peritoneum (Khan et al., 2004). In view of this, we postulated that the interaction of these cells might aggravate peritoneal inflammation. So we constructed the co-culture systems of ESC-U937 and HPMC-U937, respectively, to mimic the peritoneal local inflammation. The present data revealed that the co-culture of HPMC with U937 cells secreted significantly more IL-8 suggesting that the mesothelial cells covering the PC might be a potential source of IL-8, and that the cross-talk between the mesothelial cells and the macrophages improves synthesis of IL-8.

It has been found in the present study that estradiol or TCDD alone inhibited U937 cells to secrete IL-8 in a dose-dependent manner, and the combination of them had no synergic effect on IL-8 release. Surprisingly, when the co-culture of HPMC and U937 cells was exposed to TCDD, the secretion of IL-8 was up-regulated. Estradiol had no obvious stimulatory effect on IL-8 secretion of the co-culture, but when combined with TCDD, IL-8 level in supernatant was much reduced. The underlying mechanism of this change in IL-8 secretion is not known. The phenomenon might correlate directly with elaborate regulation of chemokines in the process of inflammation. The secretion pattern of chemokine in the co-cultured cells was affected by estradiol and TCDD. IL-8 secretion was inhibited, whereas RANTES and MCP-1, which are involved in the recruitment of monocytes, were induced (data not shown), leading to the fulfillment of a bimodal recruitment involving a switch from neutrophil to monocyte.

Retrograde menstruation and estrogen are requirements in the origin of peritoneal endometriosis. The results of the present study show that estradiol and TCDD can coordinate to evoke and aggravate inflammatory response of the endometriotic focus-associated cells in vitro by stimulating pro-inflammatory cytokine secretion (e.g. IL-8), and lead to a persistent and serious inflammation. The findings may give a new insight into the mechanisms of estrogen and TCDD interaction in endometriosis. The present study helps us to understand the pathogenesis of eutopic tissue, and to design a regimen for endometriosis.

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