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Dioxin May Promote Inflammation-Related Development of Endometriosis

Kaylon L. Bruner-Tran, Ph.D., Grant R. Yeaman, Ph.D., Marta A. Crispens, MD, Toshio M. Igarashi, M.D., Ph.D., and Kevin G. Osteen, Ph.D.

Women's Reproductive Health Research Center, Department of Obstetrics & Gynecology, Vanderbilt University School of Medicine, Nashville, TN, USA 37232

Abstract

Laboratory and population-based studies suggest that exposure to environmental toxicants may be one of several triggers for the development of endometriosis. We discuss evidence that modulation of the endometrial endocrine-immune interface could mechanistically link toxicant exposure to the development of this disease.

Capsule Summary: Environmental toxicant exposure induces an inflammatory-like endometrial response that may promote the development of endometriosis.

Keywords

endometriosis; dioxin; progesterone; inflammation; leukocytes

Introduction

Since the development of endometriosis is restricted to humans and other primates, the pathophysiology of this disease is often linked to the biological phenomenon of retrograde menstruation. Nevertheless, some degree of retrograde menstruation occurs in nearly all normally cycling women suggesting that genetic susceptibility and/or environmental factors must also contribute to the overall incidence of this disease. A potential role for endocrine disrupting environmental toxicants in the development of endometriosis first emerged with the demonstration that the incidence and severity of spontaneous disease was increased following chronic dietary exposure of a primate colony to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (1). Following the publication of this experimental report, several epidemiological studies in humans have examined the incidence of endometriosis following a known environmental exposure to TCDD or correlated incidence of disease to the body burden of TCDD or dioxin-like polychlorinated biphenyls (PCBs) (2–5). Of particular note, an increased incidence of endometriosis was identified among infertility patients in Belgium, a country in which high levels of TCDD have been documented in breast milk (3,6). These Belgium studies provide some of the most compelling population-based evidence that the development of endometriosis may be related to the influence of TCDD-like toxicants. However, results from other studies

Corresponding author: Kevin G. Osteen, Ph.D. Women's Reproductive Health Research Center, Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine, B-1100 Medical Center North, Nashville, TN 37232, Phone: 615-322-4196, Fax: 615-343-7913, e-mail: kevin.osteen@vanderbilt.edu.

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have been less conclusive (4,7,8), leaving some to surmise that there is no relationship between exposure to TCDD-like toxicants and the development of endometriosis (9,10).

One reason for the difficulty in associating toxicant measurements to the onset of reproductive tract disease may be that adult exposure has limited impact on the development of diseases such as endometriosis compared to early life exposures. It is becoming increasingly apparent that fetal and early developmental toxicant exposures perhaps are more relevant to disease processes since this is the time of maximum sensitivity to these agents. Although in vitro studies make it possible to examine the acute impact of TCDD exposure on human reproductive tract tissues and cells, appropriate animal studies are required to determine the long-term in vivo impact of developmental or neonatal exposure to this toxicant. For example, clinical experience with diethylstilbestrol, a potent pharmacological estrogen administered during pregnancy, suggests that the impact of an endocrine disrupting chemical may not become apparent until many years after the initial exposure (11,12). Additionally, developmental exposure to environmental toxicants can result in epigenetic modification of critical genes (13), including those that regulate the complex endocrine-immune interface within the adult reproductive tract. Whereas inflammatory processes are a normal part of endometrial physiology, environmental toxicants that modify either acute or systemic inflammatory processes could negatively affect reproductive success and potentially contribute to disease. Elucidating the potential impact of TCDD exposure on the development of endometriosis will require a combination of both carefully designed laboratory-based studies and retrospective analyses of appropriately controlled patient and disease-free populations. In this review, we will focus on emerging experimental evidence suggesting that the ability of TCDD exposure to limit progesterone sensitivity in the endometrium may promote the inflammatory-like endometrial microenvironment that has been linked to the pathophysiology of endometriosis.

Environmental TCDD Exposure

Numerous natural and man-made chemicals can interfere with the mammalian reproductive axis, resulting in decreased fertility, pregnancy loss and an increased incidence of reproductive tract disease (14). Among these chemicals, polyhalogenated aromatic hydrocarbons (PHAHs) are a family of persistent environmental toxicants that have been found to disrupt reproductive function in numerous animal species (15). The PHAH family includes polychlorinated dibenzo-p-dioxins (PCDDs, commonly called dioxins) and dibenzofurans (PCDFs). These chemicals demonstrate a high affinity for the aryl hydrocarbon receptor (AhR), an orphan nuclear receptor that is thought to mediate most of the toxic effects of these compounds. Non-ortho and mono-ortho PCBs also bind the AhR and produce dioxin-like effects. Although TCDD, the most toxic of the PHAH congeners, is only produced as an unwanted byproduct of manufacturing processes and incineration, PCBs have widespread commercial application and were heavily manufactured until being banned in the United States in 1977. However, manufacture of PCBs continued in other countries for an additional 10 years, and leakage of these toxicants into the environment from old electrical devices is still occurring. Additionally, since weather patterns lead to the spread of toxicants to areas far removed from the original site of contamination, human exposures to environmental toxicants remains an issue of increasing global concern. These chemicals are resistant to degradation and, due to their lipophilic nature, bioaccumulate and biomagnify at higher levels within the food chain (16). Thus, among various human or animal populations, ingestion of contaminated food and water is the primary source of dioxin-like toxicant exposure (17–19).

Although experimental studies often focus on a single toxicant, humans and animals are exposed to complex mixtures of chemicals. Therefore, the World Health Organization (WHO) developed a weighted scheme in order to determine toxicity of mixtures of dioxins and dioxin-like compounds (20). Using this scheme, TCDD is assigned a Toxic Equivalency Factor (TEF)

of 1. All other PHAHs are assigned a value based on their relative potency compared to TCDD. The amount of a given compound within a mixture is then multiplied by its TEF. Thus, the toxicity of a mixture of structurally-related toxicants, the Toxic Equivalency Quotient (TEQ), is obtained by adding together all of the TEFs. Using this calculation, it is possible to assess the effects of toxicant mixtures with a known TEQ value in order to more appropriately determine the effects of the environmental agents to which humans are actually exposed. For example, Birnbaum and colleagues (21) exposed pregnant rats to a mixture of PHAHs that approximated the presence of toxicants in the human food chain. These investigators found that while their toxicant mixture produced similar effects as that of TCDD alone, a higher dose of the mixture was required due to TCDD's greater efficiency of transfer to the offspring. While continued assessment of toxicant mixtures is highly desirable, there is still much value in studies assessing the impact of individual toxicants, particularly TCDD, since this highly toxic contaminant is so easily transferred from the maternal to the fetal and neonatal compartment (21,22).

TCDD and Endometrial Function

Among environmental contaminants acting through the AhR, TCDD (Fig. 1) has long been recognized as a prototypical disruptor of steroid action in reproductive tissues. Presently, it is known that human and animal exposure to TCDD not only affects steroid receptor levels and steroid-sensitive gene expression, but also can impact steroid metabolism and serum transport (23–25). Importantly, the ability of TCDD exposure to also modulate the local production and action of endometrial cytokines and chemokines broadly disrupts the mucosal immunology of the reproductive tract by redirecting elements of leukocyte trafficking and behavior (26–28). Disruption of the immune-endocrine interface potentially leads to the enhanced endometrial sensitivity to proinflammatory cytokines that has been noted in women with endometriosis (29). Given the inherent complexities of human exposure to multiple toxicants, our laboratory has focused on identifying key biological molecules and signaling processes that can be directly linked to disruption of endometrial function following exposure to a single toxicant, TCDD. Using an in vitro toxicant exposure model, our laboratory demonstrated that exposing primary co-cultures of adult human endometrial stromal and epithelial cells to TCDD decreases the ratio of progesterone receptor-B (PR-B) to PR-A in stromal fibroblasts and increases matrix metalloproteinase (MMP) expression in both cell types (29,30). It is important to note that the ability of TCDD to shift the PR-A/PR-B ratio in stromal cells may be only partially responsible for the elevated levels of cell-type specific MMP secretion we observed in our co-culture model. We have previously shown that differentiation-related loss of MMP-3 and MMP-7 expression in endometrial stromal and epithelial cells also requires stromal-epithelial cell communication via transforming growth factor- β (TGF- β)(31–34). Among TGF- β family members, progesterone-mediated expression of TGF- β 2 is not only involved in MMP regulation (31), but also serves a critical role as an anti-inflammatory immune modulator during pregnancy (35,36). Therefore, the ability of short-term TCDD exposure to inhibit endometrial expression of TGF- β 2 also contributes to the increase in MMP-3 and MMP-7 expression we have observed in vitro following toxicant exposure, even in the presence of progesterone (37). Thus, TCDD-mediated disruption of endometrial MMP expression involves both an alteration in stromal cell PR isotype expression and a reduced capacity for progesterone-mediated stromal-epithelial communication through TGF- β signaling.

Although both human and animal studies suggest that TCDD exposure can disrupt key elements of endometrial function in adults, a potential relationship between developmental exposure to this toxicant and altered adult endometrial function is also emerging. For example, we recently demonstrated in a murine model that developmental exposure to TCDD may alter the response of the reproductive tract to an additional toxicant exposure later in life. More specifically, compared to a single exposure to TCDD, mice receiving a combination of in utero and

prepubertal exposures exhibited a more pronounced reduction in uterine PR expression and differentiation-related TGF- β 2 expression (38). Given the rapid clearance rate of TCDD in mice (15,38), a cumulative effect of this toxicant in our model is unlikely. Additionally, evidence from a number of laboratories indicates that an early developmental exposure to a toxicant actually increases an individual's sensitivity to a second, later exposure (39,40). Importantly, the offspring of mice in our study described above (38) exhibited altered PR expression and disrupted fertility for at least three generations without additional toxicant exposure (41), suggesting that an inheritable, epigenetic alteration had occurred. Whether related to environmental toxicant exposure or not, the endometrium of women with endometriosis exhibits a similar loss of progesterone sensitivity and TGF- β 2 expression as we observed in our TCDD-exposed murine model (29,42,43). Certainly, accidental or experimental TCDD exposure during pregnancy has long been recognized to increase the risk of spontaneous abortion in humans and animals (15,44). Extending these observations, our murine studies suggest that in the absence of overt toxicant-mediated pregnancy loss, developmental and/or neonatal exposure to TCDD can significantly disrupt adult endometrial signaling, potentially impacting reproductive function for several generations.

TCDD and Inflammation-like Cell Behavior

Given the potent anti-inflammatory effects of progesterone (45–47), reduced sensitivity to this steroid could contribute to the autoimmune-like nature of endometriosis as well as to the more specific alterations in local and systemic immune cell behavior that have been reported in patients with this disease (48–56). Nevertheless, only recently has the loss of endometrial sensitivity to progesterone been recognized as a potential immunological component of the pathophysiology of endometriosis (29,34,42,57,58). During the secretory phase of each menstrual cycle, immune cells begin to actively migrate into the eutopic human endometrium and organize into distinct clusters of cells, presumably in preparation for the inflammatory-like process of menstruation (59). As progesterone levels fall, proinflammatory cytokines produced largely by these resident immune cells play a key role in the menstruation process leading to high levels of MMP expression and rapid endometrial tissue breakdown (60–62). Although the biology of the human endometrial mucosal immune system is complex, exposure to toxicants capable of disrupting progesterone's regulation of this system would likely impact menstruation, potentially affecting the development of endometriosis (54,63–69). Perhaps due to the occurrence of endometriosis only in primates and humans, the toxicology of menstrual disorders related to the development of this disease has not been studied extensively at the cellular level. Certainly, from animal studies, we now know that adult TCDD exposure can promote chronic inflammation (70,71) whereas, as noted above, a combination of in utero/developmental and prepubertal exposure to this toxicant can reduce uterine sensitivity to progesterone (38). Nevertheless, defining whether TCDD exposure can disrupt key elements of endometrial and immune cell behavior specifically affecting menstruation and the development of endometriosis will require the establishment of better in vitro and animal model systems.

Prior to menstruation, ovarian progesterone acts through both stromal-epithelial communication and stromal-immune cell communication to maintain endometrial stability by suppressing the ability of proinflammatory cytokines to stimulate the expression of multiple MMPs (72). Thus, to a large degree, menstruation represents the biological consequence of reduced progesterone support to specialized stromal cells within the endometrial functionalis, allowing for the initiation of an inflammatory-like process. At this juncture, it is not known whether endometrial immune dysfunction is related to altered stromal cell progesterone sensitivity or whether the progesterone insensitivity we have observed in these cells evolves as a consequence of chronic immune dysfunction. To approach this core issue, our laboratory has utilized human endometrial cells in a stromal-epithelial cell co-culture model (30). Using

this model, we have recently demonstrated that TCDD-mediated loss of stromal cell PR-B expression can be partially antagonized by co-addition of interleukin-1 receptor antagonist (IL-1ra) at the time of toxicant exposure (Fig. 2). The protective effect of IL-1ra in this co-culture model suggests that the ability of TCDD to disrupt PR isotype expression in stromal cells involves an inflammatory signal(s) from the adjacent epithelial cells. Interestingly, in the absence of experimental toxicant exposure, stromal cells obtained from the endometrium of patients with endometriosis exhibit a reduced in vitro sensitivity to progesterone and thus secrete higher levels of MMP-3 in response to an in vitro IL-1 α challenge (32,34). Taken together, these studies demonstrate that TCDD exposure shifts normal endometrial stromal cells to a phenotype that is similar to that observed in patients with endometriosis. Although the ability of TCDD exposure to trigger endometrial dysfunction is well-documented, it is important to note that the biological impact of TCDD-like toxicants is mediated by a signaling system that also functions in the metabolic degradation of xenobiotics (73,74). For example, molecular signaling in response to TCDD has been shown to up-regulate phase I and phase II detoxification genes in endometrial cells (73,75). Whereas a critical discussion of TCDD signaling in the endometrium is beyond the scope of this review, it is appropriate to briefly consider how exposure to this toxicant might disrupt the endometrial endocrine-immune interface at both the molecular and cellular level.

All TCDD-like ligands act via the AhR, a ligand-activated orphan nuclear receptor that is expressed in both somatic endometrial cells (76,77) and in immune cells (78–81). In each of these cell types, the unliganded AhR is present in the cytosol as a multiprotein complex bound to chaperone proteins. Upon ligand binding, the chaperone proteins dissociate, and the AhR translocates to the nucleus where it rapidly forms a heterodimer with the aryl hydrocarbon nuclear translocator (ARNT) protein. This heterodimeric AhR/ARNT complex binds to xenobiotic response elements (XREs) to alter expression of target genes (25,82). Although AhR/ARNT signaling may activate protective genes under certain circumstances, AhR/ARNT responsive genes also include a number of potent proinflammatory cytokines and chemokines, including interleukin-1, interleukin-8, tumor necrosis factor- α and RANTES (28,71,83). Therefore, exposure to AhR-binding toxicants could potentially contribute to a chronic pattern of inflammation-like signaling, possibly resulting in disruption of normal tissue function. To a degree, chronic activation of AhR signaling related to inflammation can be prevented due to ligand-induced degradation. For example, ligand-activated AhR can induce its own degradation through the 26S proteasome following receptor ubiquitination (84). Thus toxicant-mediated disruption of the endometrial immune-endocrine interface may be limited at the cellular level by both detoxification enzymes and ligand-mediated degradation of the AhR protein. Nevertheless, human and animal studies have clearly demonstrated that these protective mechanisms have a limited ability to prevent TCDD-mediated reproductive dysfunction.

TCDD and Inflammatory Endometrial Processes

The human endometrium is perhaps the most dynamic adult tissue, undergoing a development-like process that essentially replaces a functional organ system following tissue loss at each menstruation. Although exposure of isolated human endometrial cells or leukocytes to TCDD shifts each cell type to a more reactive proinflammatory phenotype (28–30,78,85), recent research indicates that AhR-mediated inflammation may also be part of normal endometrial physiology. As noted above, the glandular endometrium functions as part of the mucosal immune system and resident immune cells must contribute not only to steroid-dependent endometrial function but also to immune surveillance. For example, human and animal studies have demonstrated a critical role of AhR signaling during pregnancy and several endogenous AhR ligands have been recently proposed (86). Indeed, it has been suggested that an element of TCDD toxicity during pregnancy may be due to interference with normal, endogenous AhR-

mediated pathways (86). While more studies are clearly warranted to understand the role of AhR activation as a component of normal pregnancy, TCDD-mediated AhR signaling can be quite disruptive to endometrial function, potentially affecting both fertility and the development of reproductive diseases.

As noted above, progesterone exposure during the secretory phase of the menstrual cycle serves to down-regulate the endometrial MMP system, thus preventing endometrial breakdown prior to menstruation. Therefore, environmental toxicants that disrupt progesterone action essentially shift the endometrium toward the inflammatory-like microenvironment that normally promotes the cyclic process of endometrial tissue loss at menstruation. Although progesterone action alone may buffer certain inflammatory processes at the immune-endocrine interface of the endometrium, it is not surprising that the anti-inflammatory action of progesterone also includes the local production of other important immune modulators. In this regard, reduced endometrial expression of TGF β 2, an important Th2 cytokine, has been linked to recurrent pregnancy loss (36). Given the ability of TCDD to specifically inhibit both PR and TGF- β 2 expression in the endometrium (30,87), it is predictable that experimental exposure to this toxicant would trigger a more inflammatory-like endometrial microenvironment (88). Nevertheless, although the suppressive effect of TCDD exposure on adaptive immunity is well recognized (89), less is known about the disruptive impact that exposure to this toxicant might have on the acute inflammatory responses that could disrupt endometrial function and cause disease. In this regard, several investigators have suggested that members of the NF-kappa B signaling family (90) may play a critical role in triggering inflammatory processes associated with the development of endometriosis (91–93).

Similar to findings in numerous cancers, NF-kappa B has been shown to be constitutively active at ectopic sites of invasive endometrial growth within the peritoneal cavity (92,93). The NF-kappa B family promotes the expression of a wide array of genes that control cellular processes such as cell proliferation, adhesion, apoptosis, angiogenesis and the immune response (94). Activation of NF-kappa B involves the phosphorylation of inhibitory proteins (I-kappa Bs), resulting in dissociation, ubiquitination and proteasome degradation of I-kappa B (95,96). Following release from the NF-kappa B/I-kappa B complex, NF-kappa B translocates to the nucleus where it induces changes in gene transcription (97–99). As noted above, changes in the inflammatory state of the endometrium occur as a normal component of the menstrual cycle and several studies have noted cyclic changes in endometrial NF-kappa B activity (100–102). Although NF-kappa B becomes activated in response to a wide variety of stimuli, including microbial toxins, the effect of environmental toxicant exposure on NF-kappa B expression and activity has not been conclusively established. Interestingly, some studies, using isolated cell types, have suggested that AhR and NF-kappa B expression can be mutually co-repressive (103–105). In contrast, other studies using the mouse T cell lymphoma cell line (EL-4) indicate that TCDD activates NF-kappa B via dioxin response elements present within the FAS promoter (106). Whether single cell type models accurately represent in vivo tissue behavior remains unclear since TCDD-mediated activation of AhR in one cell type can dramatically affect the behavior of an adjacent cell type (107). For example, in the uterus of AhR knockout mice, Cooke and colleagues (108) have demonstrated a critical role for stromal AhR expression for the anti-proliferative effects of TCDD on adjacent epithelial cells. Our human endometrial studies, using stromal-epithelial co-cultures, also suggest that cell-cell communication is an important element of TCDD action in the reproductive tract (30). Certainly, the ability of TCDD exposure to disrupt progesterone-mediated stromal-epithelial communication suggests that exposure to this toxicant would likely affect inflammation-related NF-kappa B signaling in these cells. Supporting this assumption, the elevated NF-kappa B levels noted in endometrial tissue and isolated cells from endometriosis patients (109–111) are reduced following progestin-based treatments (28,112). Although more studies will be necessary to fully understand the potential disruptive role of TCDD on immune function in the

reproductive tract, it is intriguing to note that both PR knock-out and AhR knockout mice exhibit an enhanced acute inflammatory response (45,104,113).

Modeling Toxicant-mediated Development of Endometriosis

Humans and primates are the only mammals that spontaneously develop endometriosis, thus limitations associated with conducting human studies and the expense of utilizing primates have prompted investigators to pursue the development of small animal models that simulate the natural history of human endometriosis. Immune compromised mice, such as the athymic (nude) mouse, do not reject xenographic tissue, allowing human endometrial tissue to be introduced into the peritoneal cavity, mechanistically emulating retrograde menstruation. Nude mice exhibit a spontaneous mutation leading to thymic dysgenesis and consequential T cell deficiency, although they maintain normal NK and myeloid cell function and complement activity. Our laboratory developed an experimental model of endometriosis in which fragments of human endometrium obtained during the proliferative phase can be exposed to various biological agents prior to injection into the peritoneal cavity of ovariectomized and estrogenized nude mice (57,114,115). Since the initial phases of naturally occurring endometriosis remain largely unknown, our experimental model of endometriosis has also provided unique information in regard to both human and murine cell behavior during the initial phases of endometrial attachment and invasion into the peritoneal wall. For example, a critical part of successful ectopic lesion survival is the peritoneal wall vascular response to human tissue invasion. Thus, an increased ability of human tissue fragments to rapidly acquire a vascular supply within the peritoneal cavity would theoretically provide a survival advantage for efficient ectopic growth. To experimentally test this possibility, we compared the peritoneal wall vascular response in mice receiving human endometrium acquired from control tissue donors versus women with endometriosis. Within 24 hours of control human tissue injection into the murine peritoneal cavity, ectopic attachment to the peritoneal wall is evident, but a minimal local vascular response is noted on gross examination (Fig. 3A). In contrast, during this same time interval, peritoneal attachment of endometrial tissues acquired from women with endometriosis leads to a robust murine vascular response during early invasion (Fig. 3B). These findings suggest that eutopic endometrial tissue acquired from endometriosis patients possess a greater innate capacity to stimulate peritoneal blood vessel growth in our experimental disease model. Interestingly, exposure of control endometrial tissue to TCDD, prior to injection into the peritoneal cavity of recipient mice, leads to a similar host vascular response as observed with tissue acquired from endometriosis patients (Fig. 3C). Thus, in addition to increasing MMP expression in endometrial fragments (87), TCDD exposure also affects the vascular response at the site of endometrial invasion into the murine peritoneal wall. To quantitatively analyze our visual observations of vascularization in our experimental model, we examined microvascular density (MVD) via immunohistochemical localization of endoglin. MVD analysis has been validated as a useful predictor of tumor growth (116,117), and endothelial cell expression of endoglin has been suggested for clinical use as a prognostic indicator in women with endometrial carcinoma (118). Importantly, endoglin is only present in actively proliferating cells, and, thus, has been shown to be useful to identify neoangiogenesis occurring in at ectopic sites of endometriosis (119). Confirming our gross findings in our experimental model of endometriosis, control human tissues exposed to TCDD demonstrated both a greater level of endoglin staining and a higher MVD at the peritoneal invasion site compared to lesions established in the absence of toxicant exposure (Fig. 4).

An important question from our experimental endometriosis model is: what factor(s) affect the ability of human endometrial tissue to stimulate murine angiogenesis within the peritoneal cavity? Certainly, as noted throughout this manuscript, TCDD exposure creates an endometrial phenotype that becomes increasingly insensitive to progesterone while exhibiting more inflammatory-like behavior. In this regard, using our experimental nude mouse model, we have

examined the impact of TCDD on vascularization at ectopic sites. As shown in Figure 5, although pretreatment of control endometrial tissues with progesterone limits vascularization at the site of peritoneal wall invasion in mice (Fig. 5A), pretreatment of identical control human endometrial tissue with progesterone in the presence of TCDD results in active lesion establishment, including a robust and extensive degree of vascularization (Fig. 5B). Additionally, microscopic examination of the lesions established following progesterone pretreatment versus progesterone and TCDD pretreatment revealed distinct differences in the type and distribution of infiltrating murine neutrophils (Fig. 5C–E). In experimental lesions established without toxicant exposure, few neutrophils were present within the stromal compartment but rather were located in the lumen of glands (Fig. 5E), as is commonly seen in normal secretory phase human endometrium. In contrast, neutrophils were numerous in the stromal compartment of TCDD-exposed tissues (Fig. 5F), and immunofluorescent staining indicates the neutrophils are of murine origin (data not shown). Although more studies are needed, the patterns of neutrophil infiltration may be significant since murine studies have shown that neutrophils migrating into the uterus of PR null mice become highly activated and rapidly degranulate (45). Additionally, neutrophils can trigger inflammation and are also a major source of vascular endothelial growth factor (VEGF) (120–125). In previous experiments, we have shown that blocking the actions of human tissue derived VEGF can prevent the establishment of lesions in our experimental disease model (126). Taken together with our previous studies, new data described herein suggest that TCDD-mediated loss of endometrial PR-B expression (30) may also affect the behavior of neutrophils migrating to the site of endometrial tissue invasion, perhaps increasing the angiogenic response during a critical period of ectopic survival.

Future Directions

In order for endometriosis to become established via retrograde menstruation, fragments of endometrial tissues must have the capacity to attach and invade into the surface of the peritoneal wall or peritoneal organs, rapidly develop a vascular supply and subsequently proliferate under the influence of estrogen. Given that each of these biological processes is critical to the establishment of this disease, it is important to understand the potential impact of environmental toxicant exposure on each individual process. Since direct human studies are not possible, our experimental endometriosis model has been invaluable to identifying key bioactive factors expressed by the invading human tissue, the invaded host murine tissue, or both during the initial disease process (56,109,127,128). Nevertheless, a limitation of the nude mouse model is the immune-compromised status of the murine host. Although the altered immune system of nude mice allows for the survival of xenografted human tissue, this model does not allow examination of the adaptive arm of the immune system during establishment of experimental endometriosis. Thus, we are currently utilizing Recombinant Activating Gene 2/common cytokine receptor γ chain (γ c) double null mice (Rag2 γ (c)) (129) in order to explore the interaction of both human endometrial tissues and human immune cells at the site of peritoneal invasion. Using these animals, future studies should provide a much broader understanding of the both the role of specific immune cell population on the disease process, as well as the cross talk between endometrial and immune cells during establishment of endometriosis. Additionally, as noted earlier, a woman's risk for the development of endometriosis may also relate to toxicant exposure that may have occurred within her fetal and/or neonatal environment. Future studies, using the Rag2 γ (c) experimental endometriosis model as well as our developmental TCDD exposure model (38) should allow us to further examine the mechanisms by which TCDD exposure at different stages of life may alter the endometrial endocrine-immune interface, potentially affecting the development of endometriosis.

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Figure 1.

Chemical structure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD is made of two aromatic rings (green) joined through a pair of oxygen atoms (red) with four chlorine atoms (yellow) attached at positions 2, 3, 7, and 8. Four hydrogen atoms are shown in blue.

Figure 2. Western analysis of PR-A and PR-B expression in normal proliferative endometrial stromal cells. Stromal cells were cultured 5 days in the presence of epithelial cells and treated with 1 nM estradiol, 0–10 nM TCDD with or without interleukin-1 receptor antagonist. PR isoforms were detected using a single murine monoclonal antibody (PgR1294) as previously described (26).

Figure 3. Gross appearance of experimental endometriosis 24 hrs after injection of normal proliferative endometrium treated with 1 nM estradiol (A), estradiol treated tissue from a woman with endometriosis (B), or endometrium from a normal woman treated with estradiol and TCDD prior to injection. Experimental endometriosis was established in nude mice as previously described (12,13).

Figure 4. Immunohistochemical localization of endoglin in normal secretory human endometrium (A) and ectopic endometrium removed during the secretory phase from a patient with endometriosis (B). Endoglin is also present in human endometrium growing as ectopic lesions in nude mice (C-D). Tissues in (C) were treated with 1 nM estrogen and 500 nM Progesterone prior to injection into mice. Tissues in (D) were treated with E+P and 10 nM TCDD prior to injection. Magnification, 200x

Figure 5. Gross and microscopic photomicrographs of experimental endometriosis in nude mice. Experimental disease was established as previously described (12,13) using human endometrium from a disease-free donor. Organ cultures were maintained for 24 hrs with 1 nM E and 500 nM P (Panels A, C, E) or EP+ 10 nM TCDD (Panels B, D, F) prior to introduction into mice. Panels A and B show gross appearance; note the larger more vascularized lesion established by TCDD-exposed tissue (Panel B) compared to a lesion established following EP-treatment (Panel A). Low-power micrographs (40x) show the different histological appearance of the lesion established by the EP-treated tissue (Panel C) compared to the lesion established by the TCDD-exposed tissue (Panel D). Epithelial glands [E] are present in both lesions (Panels C and D). Lesions established by EP-treated tissues (Panel C) but not EP+TCDD treated tissues (Panel D) show clusters of mononuclear cells [MC]. These are seen at higher magnification in Panel E (200x) and the insert (400x), and are morphologically consistent with lymphocytes. The gland lumens in lesions established by EP-treated tissues (Panel C) but not EP+TCDD treated tissues (Panel D) contain neutrophils, determined by immunofluorescent staining to be

of human origin (not shown). Frequently associated with capillaries, neutrophils are more abundant in the stroma of the TCDD-treated tissues (Insert, Panel F (400x); indicated by the arrows) and are largely of murine origin.