Environmental Dioxins and Endometriosis

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ABSTRACT

Endometriosis is a common gynecologic problem of unknown etiology. Estrogen dependence and immune modulation are established features of this disease, and environmental contaminants have been suggested to play a role in the pathobiology of this disease as well. Previous work in nonhuman primates has shown that exposure to the dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is associated with an increased prevalence and severity of endometriosis. Further animal experiments have implicated dioxin and dioxin-like compounds in this disease. Rodent studies support the plausibility of a role of environmental contaminants in the pathophysiology of endometriosis, although a convincing mechanistic hypothesis has yet to be advanced. Small hospital-based case-control studies have failed to provide compelling evidence for or against an association of environmental contaminants and endometriosis. Herein we review evidence that dioxin and dioxin-like compounds are potent modulators of immune and endocrine function critical to the pathobiology of endometriosis. Furthermore, perspectives on the potential mechanism(s) of dioxin and dioxin-like compound–induced toxicity in endometriosis, important knowledge needs, potential animal models for endometriosis studies, and considerations integral to future human case-control studies are discussed.

KEYWORDS: endometriosis, environmental dioxins, TCDD, dioxin-like polychlorinated biphenyls

Endometriosis is classically defined as the growth of endometrial glands and stroma at extrauterine sites. It is a prevalent gynecologic disorder that may be present in 10% of reproductive-aged women.[1] Often accompanied by chronic pelvic pain, infertility, and adhesion formation, endometriosis is responsible for more than 100,000 hysterectomies each year and the annual health care costs attributable to this disease exceed $1 billion in the United States alone.[2] Development of endometriosis appears to be closely related to a woman's history of exposure to estradiol, and progesterone exposure may minimize disease in some women.[3,4] Traditional medical therapies consist of hormonal or surgical treatments that limit the action of endogenous estrogen.

The etiology of endometriosis is unknown. However, it is widely accepted that endometriosis arises from the aberrant implantation and proliferation of retrograde endometrial fragments deposited into the peritoneal cavity[5] or differentiated from a primitive progenitor cells lining the abdominal cavity and internal organs.[6,7] Of the foregoing causes, backflow of menstrual contents into the pelvic cavity has been suggested to account for the majority of documented cases of endometriosis. A number of lines of evidence lend support to this theory. First, endometriosis is found more often in women with outflow defects of the uterine cavity,[8] and second, partial obstruction of the cervical os in baboons resulted in endometriosis in all animals within 3 months.[9] Although retrograde menstruation or bleeding into the peritoneal cavity during menstruation is widely accepted as a major contributing factor in the pathogenesis of endometriosis, it is a common phenomenon occurring in approximately 70 to 90% of women.[10] Hence, factors other than simply access of endometrial contents to the pelvis

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via retrograde menstruation are thought to contribute to the development and progression of endometriosis.

Endometrial cells destined to become endometriotic implants are thought to be different from normal endometrial cells, a notion that is supported by a number of observations. Endometrial cells from women with endometriosis survived transplantation in athymic nude mice longer than normal proliferative phase endometrium from women without endometriosis,[11] suggesting that these cells are functionally distinct. Endometriotic cells express P-450 aromatase and are therefore capable of de novo estrogen synthesis, whereas eutopic endometrium from disease-free women does not express aromatase[12] or expresses it at lower levels. Furthermore, dysregulation of interleukin-6 (IL-6), which inhibits endometrial growth, and its soluble receptor (IL-6sR) has been shown in human endometriotic tissue.[13,14] The critical event(s) or biochemical change(s) that ultimately leads to immune-endocrine disruption and the establishment of endometriosis, however, remains an enigma.

DIOXIN AND DIOXIN-LIKE COMPOUNDS

Studies have suggested that environmental contaminants may be important in the development of endometriosis. One of the primary classes of xenobiotics of concern is that of dioxin and the dioxin-like compounds. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a prototype for the polyhalogenated aromatic hydrocarbons (PHAHs), which have a common mechanism of action and spectrum of effects.[15–19] Evidence indicates that the actions of TCDD and dioxin-like chemicals are mediated by the aryl hydrocarbon receptor (AhR), which functions as a basic helix-loop-helix transcription factor. Similar to the steroid hormone receptor family of proteins, the AhR acts as a signal transducer and transcription factor. Following receptor activation, the receptor-ligand complex is translocated via the AhR nuclear translocator (ARNT) to the nucleus, where DNA binding occurs resulting in transcriptional activation. Target genes include cytochrome P-450 and genes involved in cellular growth, differentiation, and inflammation. Other polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) substituted in the 2,3,7,8 lateral positions also have high binding affinity for the AhR and elicit toxic effects similar to those of TCDD. Moreover, non-ortho and some mono-ortho polychlorinated biphenyls (PCBs) are AhR agonists and contribute significantly to the toxicity of complex mixtures of environmental PHAHs. Various dioxin congeners can act additively via specific binding of the AhR and their potency is related to AhR affinity; therefore, these chemicals are classified as "dioxins."

TCDD and dioxin-like PCDDs and PCDFs are produced as unwanted by-products of many industrial and combustion processes, whereas PCBs were used extensively in a broad range of commercial applications. Dioxins are resistant to degradation and, due to their lipophilic nature, bioaccumulate and biomagnify at higher trophic levels of the food chain. TCDD and related PHAH congeners have been identified in human serum and accumulate in tissues.[17,20–22] In the general population, ingestion of contaminated foods is the most likely source of TCDD and other PHAHs, but accidental exposure has also occurred in the workplace, during wartime, and as a result of industrial accidents. In industrialized countries, exposure to environmental PHAHs has led to serum TCDD levels of 1 to 5 lipid-adjusted parts per trillion (PPT-LIPID) and body burdens estimated at 25 TCDD equivalents (TEQs) PPT-LIPID in individuals with no overt exposure.[23] In these populations, TCDD contributes approximately 15% of the body burden of total dioxins while dioxin-like PCDDs, PCDFs, and PCBs constitute about 85% of the dioxin body burden.[22] Although the toxic effects of TCDD in animals are unequivocal, its effects in humans are less clear. However, studies indicate that humans exhibit many of the biological effects of dioxins observed in animals. It has therefore been postulated that increased concentrations of TCDD and dioxin-like chemicals in blood and tissues may participate in disease pathogenesis through disruption of endocrine and immune responses in susceptible humans and animals. One such candidate disease is endometriosis.

DIOXIN AND DIOXIN-LIKE COMPOUNDS AND ENDOMETRIOSIS IN NONHUMAN PRIMATES

Rier et al[24] provided the first report of an association between chronic TCDD exposure and endometriosis in the rhesus monkey. Animals were exposed to 0, 5, or 25 PPT TCDD in their feed for approximately 4 years, and animals were assessed at surgical laparoscopy 10 years after termination of TCDD treatment. This study demonstrated that TCDD-treated animals spontaneously developed endometriosis and that there was a significant dose-dependent increase in the incidence and severity of endometriosis in TCDD-exposed monkeys. More recently, Yang et al[25] surgically induced endometriosis in the cynomolgus monkey to investigate the effects of TCDD exposure on the survival and growth of ectopic endometrium. Fragments of endometrium were autotransplanted to the pelvic cavity of animals dosed 5 days a week with 0, 1, 5, or 25 PPT TCDD. The growth and survival of implants were assessed by laparoscopy at 1, 3, and 6 months and at 1 year. Consistent with previous work in the rhesus monkey,[24] exposure to 5 or 25 PPT TCDD for 1 year resulted in a higher survival rate of implants and an increased implant size in animals fed 25 PPT TCDD. In contrast, the implant size was decreased in animals fed 1 PPT TCDD. These effects were not observed prior to 1 year of treatment. Thus, this group con-

cluded that (1) TCDD facilitates the survival of endometrial implants, (2) TCDD exerts a bimodal effect on endometrial implant growth, and (3) TCDD exposure does not affect endometrial implant survival and proliferation until after 1 full year of exposure.

In recent work, serum concentrations of TCDD, 19 dioxin-like PHAH congeners, and lipids were determined in the colony of TCDD-exposed rhesus monkeys 13 years after termination of TCDD exposure.[26] Similarly to the general human population and laboratory animals,[22,27] TCDD-treated and control animals were exposed to dioxin-like PHAH through their diet or other environmental sources. Notably, the animals in this study with elevated serum levels of dioxin-like PCB77 and PCB126 and an increased total serum TEQ had a high prevalence of endometriosis. The severity of the disease correlates with the serum concentration of PCB77 but not the serum TCDD level. These data suggest a potential involvement of an increased body burden of dioxin-like PCB compounds in the etiology of endometriosis in rhesus monkeys. Thirteen years after exposure, the body burden of dioxins in TCDD-treated monkeys is similar to or lower than that in the general human population.[26] Thus, it is important to consider the implications of this finding for human health, particularly with regard to the average human PHAH burden and the prevalence of endometriosis in humans. Exposure to these chemicals is of global concern and is the subject of international negotiations toward a regulatory treaty. In Japan, Yoshida et al[28] reported that safety against endometriosis cannot be guaranteed given the present dioxin exposure of certain at-risk populations, such as local residents living near incinerators or heavy fish consumers. In the most recent risk characterization of dioxin and related compounds by the U.S. Environmental Protection Agency, endometriosis is considered a low-dose biological effect of dioxin exposure occurring at or near human background levels.[23]

DIOXIN AND DIOXIN-LIKE COMPOUNDS AND ENDOMETRIOSIS IN RODENTS

Rodent studies demonstrate that treatment with TCDD or the dioxin-like furan chemical 2,3,4,7,8-pentachlorodifuran produces a dose-dependent increase in the size of surgically induced endometrial lesions.[29,30] In contrast, treatment of mice with non–dioxin-like compounds has no effect on the growth of endometriotic sites, suggesting that dioxin-like environmental toxicants are more likely to contribute to the pathogenesis of endometriosis than the non–dioxin-like ones. However, a number of factors affect the efficacy of TCDD treatment to support endometrial implants. TCDD treatment prior to surgical induction of disease, subacute dosing, plasma estrogen levels, and ovarian function appear to be important to the dioxin-mediated effects in this rodent model. Regression of the endometrial implants has been observed when mice are treated with TCDD and high levels of exogenous estrogen after surgical induction of disease[31] or when the TCDD dose (>10 μg/kg) is toxic to the ovaries.[32] Overall, these data suggest a potential role of dioxins in the pathogenesis of endometriosis.

HUMAN STUDIES OF EXPOSURE TO DIOXINS AND ENDOMETRIOSIS

In light of the animal evidence, Koninckx et al[33] called for additional studies of the link between dioxins and endometriosis, noting a high prevalence of endometriosis in infertile Belgian women and high dioxin concentrations in breast milk in this country.[34] Since that time, four hospital-based case-control studies have been conducted investigating a relationship between endometriosis and exposure to dioxins. In Israel, Mayani et al[35] reported that more infertile women with endometriosis have a detectable serum TCDD concentration than infertile women without endometriosis. Dioxin was detected in the blood of 18% of infertile women with endometriosis as compared with 3% of infertile controls. In another study, Boyd et al[36] reported similar levels of TCDD and dioxin-like PCDDs and PCDFs in women with endometriosis and in a control group (fertile women). However, this study did not surgically confirm the absence of disease in the control group and failed to account for factors thought to influence the body burden of lipophilic dioxins, including obstetrical history. In an ongoing study, the association between endometriosis and exposure to dioxin is being investigated in TCDD-exposed women living in Seveso, Italy.[37] Unfortunately, this study was unable to define endometriosis surgically. Thus, the true prevalence of endometriosis in this cohort will remain unknown until the development of a nonsurgical test for endometriosis.

A weakness of the foregoing studies is that they have not included an assessment of the dioxin-like PCBs, major contributors to total body burdens of dioxins. In a Belgian study, Pauwels et al[38] found that a nonsignificant increased risk of endometriosis is associated with elevated blood levels of total TCDD equivalents (TEQ) using an AhR-dependent bioassay system. Dioxin activity may have been underestimated in this study because the presence of high blood levels of non–dioxin-like PCBs, likely to be present in some Belgians following an accidental PCB exposure in this country,[39] inhibit dioxin-mediated activity in this assay.[40] Accurate quantification of blood dioxin activity using this assay requires prior extraction of noncoplanar PCBs. As with other human work, this study was too small to detect differences in the levels of blood dioxins among women with and without endometriosis, if they were indeed present. Hence, the human data neither confirm nor refute the hypothesis that environmental dioxins play a role in the pathophysiology

of endometriosis. Future studies are needed to confirm this notion in human populations.

POTENTIAL MECHANISM(S) OF DIOXIN AND DIOXIN-LIKE COMPOUND ACTION IN ENDOMETRIOSIS

The accumulated literature clearly establishes endometriosis as a pleiotropic disease involving immune-endocrine disruption and aspects of cellular adhesion, implantation, neovascularization, and proliferation. TCDD and dioxin-like PHAHs may exert effects on the pathophysiology of endometriosis through a number of pathways, including (1) activation of procarcinogens, (2) altered synthesis and metabolism of estradiol, (3) altered production of proinflammatory growth factors or cytokines, and (4) misexpression of remodeling enzymes (Fig. 1).

Dioxin-induced effects are mediated by its tissue-specific receptor, the AhR. Recent studies using AhR-deficient mice indicate that the AhR and AhR-responsive genes play an important role in immune system development, in reproductive success, and in the metabolism of foreign compounds.[41,42] Other animal studies indicate that AhR-responsive genes function in reproductive processes within the uterine endometrium. In the rabbit model, localization of AhR messenger RNA (mRNA) changes during the proinflammatory, invasive processes of implantation and the progesterone-mediated immunosuppression of pregnancy maintenance.[43] The AhR protein is detected in human uterine and ectopic endometrium,[44] and the mRNAs of AhR and ARNT are constitutively expressed in these tissues from women with and without endometriosis.[12,45] TCDD treatment of human endometrial explants results in increased expression of AhR mRNA and the mRNA of the dioxin-responsive gene *P-4501A1*.[46,47] In ovarian endometriosis, the expression of AhR mRNA is increased[48] and *P-4501A1* mRNA is increased eightfold in ectopic tissue relative to uterine endometrium.[49] Endometriotic cells exhibit increased expression of P-450 aromatase, indicating that lesions are capable of de novo estrogen synthesis.[12] In addition, regression of endometriosis has been observed following treatment with an aromatase inhibitor in a woman with severe postmenopausal disease resistant to traditional treatments.[50] These findings led to the postulation that dioxins may promote endometriosis through the induction of P-450 isoenzyme expression and increased formation of catechol estrogens, resulting in chronic exposure of the endometrium to growth-promoting estrogen. Dioxins may also exert

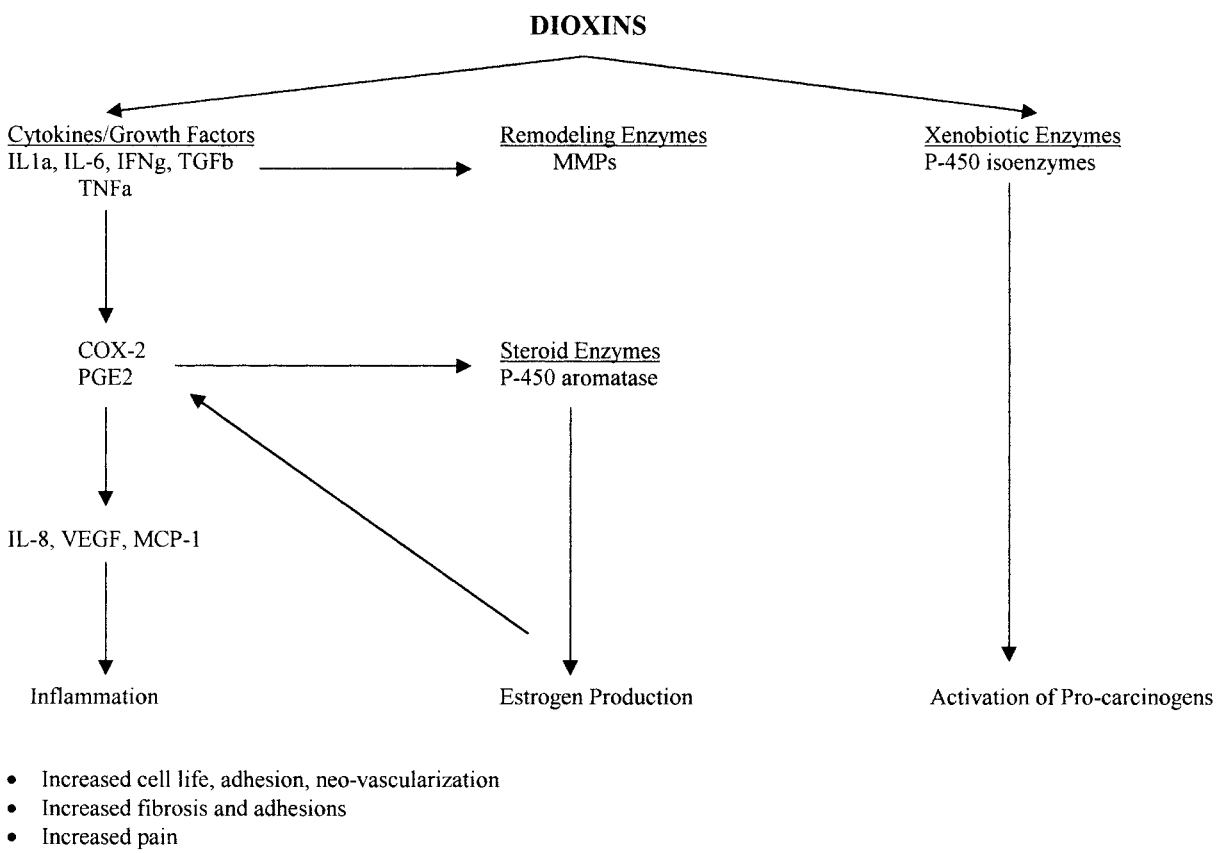

Figure 1 Proposed mechanism of TCDD-induced toxicity in endometriosis. Exposure to TCDD and dioxin-like PCBs may promote endometriosis via activation of procarcinogens and stimulation of chronic inflammation leading to enhanced estrogen synthesis and disruption of progesterone-dependent remodeling responses that normally limit the development of endometriosis.

direct toxic effects on the endometrium via activation of procarcinogens, resulting in disruption of cellular proliferation, hormone synthesis, and metabolism.

Dioxin's ability to induce inflammation could play a role an important role in the pathogenesis of endometriosis. Normal uterine endometrial growth and remodeling are regulated by sex hormones in concert with bioactive mediators produced by immune and endocrine cells, including the proinflammatory cytokines (IL-1, IL-6, tumor necrosis factor-α [TNF-α], interferon-γ [IFN-γ]), transforming growth factors (TGF-α, TGF-βs), and remodeling enzymes (matrix metalloproteinases, MMPs).[51,52] Immune dysregulation, evidenced by decreased clearance of retrograde endometrial cells and aberrant production of cytokines by peritoneal leukocytes and endometrial cells, has been proposed as a mechanism that allows the implantation and growth of ectopic endometrium. TCDD and dioxin-like PHAHs may promote endometriosis via chronic stimulation of the expression and activity of the proinflammatory cytokines, involved in the cyclic regulation of endometrial remodeling, proliferation, and cell death. Inflammatory mediators and their metabolic by-products, shown to be elevated in women with endometriosis, are potent regulators of cellular proliferation and programmed cell death.[53,54] Furthermore, a direct cause and effect between chronic inflammation and carcinogenesis are established,[55] suggesting a rational mechanism by which inflammation leads to aberrant tissue proliferation.

Among the host of growth-promoting cytokines produced during chronic inflammation, several lines of evidence suggest that TNF-α is a key factor in dioxin toxicity and the pathogenesis of endometriosis. TCDD or TNF-α administration in rodents enhances leukocyte inflammatory responses and cellular infiltration of macrophages and neutrophils into the peritoneal cavity following antigen challenge.[56] TCDD-induced peritoneal hyperinflammation can be blocked by neutralization of endogenous TNF activity. Acute TCDD exposure in rodents increases peritoneal and peripheral blood (PB) leukocyte production of TNF-α.[57–59] The ability of dexamethasone or TNF antibody to reverse the mortality and weight loss associated with treatment with TCDD and endotoxin is consistent with a role for inflammatory responses and enhanced expression of TNF-α in TCDD toxicity.[60]

Peritoneal hyperinflammatory responses to red blood cells found in rodent models of TCDD exposure may represent a process similar to enhanced peritoneal inflammatory responses observed in endometriosis. In women with endometriosis, peritoneal leukocytes are activated and secrete increased levels of TNF-α and IL-6.[61–63] Moreover, ectopic endometrial cells exhibit aberrant expression of IL-6 and IFN-γ compared with uterine cells from women without disease.[13,14,64,65] Increased levels of proinflammatory mediators, chemotactic and neovascularization factors TNF-α, IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), and vascular endothelial growth factor (VEGF), are present in the peritoneal fluid of women with mild disease.[51] Ectopic endometrium appears to be capable of producing high levels of active TNF-α protein because this tissue expresses increased levels of TNF-α converting enzyme (TACE) protein.[66] Administration of recombinant TNF binding protein-1 (TBP-1) inhibits the growth of experimentally induced endometriosis in the rat.[67] Studies have shown that TNF-α increases the adhesion of endometrial stromal cells to peritoneal mesothelium[68] and promotes angiogenesis.[69] Thus, TCDD may target PB, peritoneal, and endometrial leukocyte populations, inducing chronic expression of TNF-α and other inflammatory mediators and resulting in increased adhesion, vascularization, and proliferation of endometriotic cells. Dioxins may affect the expression of TNF-α via the induction of an inflammatory cytokine network because the region of DNA that recognizes the ligand-activated AhR, the dioxin-response element or DRE, is present in the genes of potent inducers of TNF-α, including IL-1b, IL-6, and IFN-γ.[70,71]

Consistent with the notion of TCDD-induced immune-mediated pathology in endometriosis, TCDD-exposed rhesus monkeys exhibit severe disseminated endometriosis and long-term immune alterations. Thirteen years after termination of TCDD treatment, this study found that exposure to dioxins is associated with increased mitogen-stimulated TNF-α secretion and decreased cytolytic activity by PB leukocytes.[72] Changes in immune status in TCDD-treated animals correlated with elevated serum concentrations of TCDD and dioxin-like PCB126. These findings suggest a relationship between exposure to dioxins, severe endometriosis, and altered immune responses of potential importance to endometrial growth, elimination of retrograde endometrial fragments, implantation, and pregnancy maintenance.

It has also been suggested that TCDD promotes endometriosis through alterations in tissue remodeling processes. TCDD promoted the establishment of ectopic lesions of human endometrium in a nude mouse model of disease by disrupting progesterone regulation of expression of MMPs, a family of tissue remodeling enzymes involved in tumorigenic invasive processes and remodeling of the endometrium.[52,73–76] Estrogen promotes the establishment of the endometriotic lesions in this model, whereas progesterone inhibits lesion formation by suppression of endometrial MMP activity. Treatment of cultured human endometrial explants with estradiol maintains cell-specific MMP expression in vitro and spontaneously promotes the establishment of ectopic peritoneal lesions in vivo when the explants are injected into recipient animals. In contrast, treatment with progesterone in conjunction with estradiol suppresses both in vitro MMP secretion and in vivo lesion formation. Treatment with TCDD plus estradiol increased both the number and size of the lesions compared with

estrogen treatment alone, and treatment with TCDD in the presence of estradiol and progesterone disrupted the ability of progesterone to block lesion formation and MMP expression. These results provide evidence linking the progesterone- and cytokine-dependent regulation of MMP secretion in human endometrial tissue with the establishment of an endometriosis-like disease in the nude mouse and demonstrate the ability of TCDD to disrupt this regulation.

The initiating event(s) in the development of endometriosis is unknown; however, the effect of dioxins on inflammation, proliferation, and differentiation could explain the major pathognomonic features of endometriosis (Fig. 1). Emerging evidence strongly argues that endometriosis is associated with chronic inflammation characterized by increased expression of proinflammatory cytokines by endometriotic cells and leukocytes that regulate endometrial growth, remodeling, and immunity. Activation of this inflammatory cytokine network in the extrauterine environment results in cyclooxygenase 2 (COX-2) induction and increased prostaglandin E_2 (PGE_2) synthesis in ectopic endometrium, postulated to lead to chronic estrogen production, suppression of progesterone responses, misexpression of remodeling enzymes (MMPs), and an extended cell life of endometriotic cells. Chronic inflammation contributes to enhanced lesion formation by inducing the expression of mediators of adhesion, neovascularization, and leukocyte recruitment, including MCP-1, VEGF, IL-6, and IL-8. A greater number of ectopic endometrial cells may survive, thrive, and disseminate as a result of inhibition of apoptosis and suppression of leukocyte cytolytic activity. Increased sensitization of pain receptors by increased prostaglandins and enhanced fibrosis mediated by abnormal immune responses probably lead to the sequelae of endometriosis, such as pelvic pain, adhesion formation, and infertility.

Taken together, current data suggest that TCDD may affect the pathophysiology of endometriosis by modulation of immune and endocrine function; however, the specific mechanism of dioxin-mediated toxicity in the pathogenesis of endometriosis remains unclear. The accumulated evidence supports the hypothesis that exposure to TCDD and dioxin-like PCBs promotes endometriosis through activation of procarcinogens and stimulation of chronic inflammation leading to enhanced estrogen synthesis and disruption of progesterone-dependent remodeling responses that normally limit the development of endometriosis.

PERSPECTIVE ON ANIMAL MODELS OF ENDOMETRIOSIS

Endometriosis occurs exclusively in menstruating species, including humans and nonhuman primates, with spontaneous development in rhesus monkeys closely resembling human disease.[24,77,78] Thus, the monkey is the most appropriate, yet expensive, animal model for the study of disease pathogenesis. Because spontaneous endometriosis in monkeys appears to develop over a period of 7 to 10 years, surgical induction of disease has been employed by suturing fragments of endometrial tissue at ectopic sites or seeding the peritoneal cavity with minced fragments of endometrial tissue.[25,79] In addition, rodent and rabbit models of disease have been employed utilizing surgical autotransplantation of endometrium[77] or injection of human endometrial tissue into the peritoneal cavity of immune-deficient mice.[73] The demonstration that heterotransplants of animal and human tumors maintain their tumorigenicity, morphology, and hormone responsiveness in immune-deficient mice suggests that these animals may prove to be useful models for the study of human endometriosis.[80,81] Several teams of investigators have examined immune-deficient mice, either athymic nude mice[11,74,82] or severe combined immune deficient mice (SCID),[83,84] as short-term models of endometriosis. Therefore, we suggest that immune-compromised mouse models bearing human eutopic and ectopic endometrium will provide advantages over previous rodent models and generate results that are more readily generalizable to humans.

Surgical induction of endometriosis in rodents has been used to investigate the effect of environmental contaminants on the establishment of endometrial implants, survival of existing implants, and mechanisms of contaminant action.[77] However, rodent models of endometriosis are fraught with a number of significant limitations. The estrous cycle in rodents is significantly shorter than that in humans and is characterized by hormonal differences such as an abbreviated luteal phase compared with humans. Although rodents do not go through a process of menstruation, they do have a process of reorganization of the uterine epithelium. Moreover, circulating levels of estradiol in rodents are markedly lower than in humans. In spite of these limitations, rodents offer significant advantages over other animal models related to the significant cost of using nonhuman primates and the long time frame to disease onset in nonhuman primates. Mechanistic studies in humans are not practical as endometriosis is already well established by the time a diagnosis is made, and it is not possible to complete experiments to investigate the mechanism of toxicant effects on the pathogenesis of endometriosis. Thus, animal models continue to be important for the study of the pathobiology of endometriosis.

Studies in humans and nonhuman primates have shown that endometriosis is a dynamic disease with periods of development, progression, and regression.[85,86] Lesions change in appearance over time and disappear at some sites, and other lesions progress in severity. When designing studies to investigate the role of dioxins in the pathogenesis of endometriosis, it is important to utilize animal models that most closely resemble human disease. Currently, experimentally induced endometriosis

in nonhuman primates and rodents is the most practical model of disease. However, disease induction by peritoneal seeding of endometrial tissue in the monkey and the immune-compromised mouse models, rather than autotransplantation of endometrium, may be critical to mimic the human disease process. Studies that employ surgical induction by autotransplantation of endometrium in monkeys and rodents are limited in scope because they test the effect of dioxins on established disease only, not the capacity to form and remodel lesions. As described by Yang et al,[25] lesions of autotransplanted endometrium regress over time in control and TCDD-treated animals; therefore, these studies may document the effect of toxicant exposure on normal growth inhibition processes at ectopic sites. The microenvironment at the ectopic site that receives the endometrial tissue must be considered. TCDD may increase inflammation at these sites through leukocyte infiltration and activation, priming the site for acceptance or rejection of endometrial tissue. In addition, one cannot investigate the effect of dioxins on biochemical mechanisms active in the disease process in rodents with surgically induced lesions because menstruation and endometriosis do not occur in these animals.

FUTURE STUDIES AND DATA GAPS

Advances in the detection and quantification of individual PHAH congeners in biological samples have made it possible to assess the total PHAH body burden in humans and animals, a critical step in evaluating an association between exposure to dioxins and an increased prevalence of endometriosis. Future studies are expected to exploit this advance to assess the health impact of PHAH body burdens in exposed cohorts and the general population. However, long-term prospective studies in human populations must be carefully designed and will be severely hampered until the development of (1) a serum biomarker for endometriosis and (2) accurate biomarkers of human exposure to dioxins. Progress in this area of research would greatly strengthen our ability to investigate the relationship between exposure to dioxins and an increased prevalence of endometriosis in humans. Furthermore, human studies must include surgical confirmation of endometriosis and control for factors that influence the body burden of dioxins including obstetrical history of pregnancy and breast-feeding, body mass index, and age. Epidemiologic studies must also account for other exposure susceptibility factors, such as dietary phytoestrogens and estrogenic non–dioxin-like PCBs. Animal studies will be required to examine the effects of dioxins on the pathophysiology of endometriosis. Although preliminary work suggests a potential involvement of exposure to dioxins in the pathogenesis of endometriosis, much work remains to define cause and effect clearly and to understand the potential mechanism of toxicity.

CONCLUSIONS

Endometriosis remains a common gynecologic problem of unknown cause that is associated with significant morbidity. Evidence from animal studies, nonhuman primates and rodents, suggests that endometriosis is associated with exposure to TCDD as well as dioxin-like PCBs. These findings are relevant to human endometriosis because exposure of the general human population to these compounds has been documented in the literature. Additional studies in humans and animals are warranted to investigate the potential association between exposure to these toxicants and endometriosis and elucidate the mechanism of action of dioxins in the pathophysiology of this disease.

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REFERENCES

1. Wheeler JM. Epidemiology and prevalence of endometriosis. Infertil Reprod Med Clin North Am 1992;3:545–549
2. Carlson K, Miller B, Fowler FJ. The Maine women's health study: I. Outcomes of hysterectomy. Obstet Gynecol 1994; 83:556–565
3. Halme J, Stovall D. Endometriosis and its medical management. In: Wallach EE, Zacur HA, eds. Reproductive Medicine and Surgery. St. Louis: Mosby; 1995:695–710
4. Eskenazi B, Warres M. Epidemiology of endometriosis. Obstet Gynecol Clin North Am 1997;24:235–258
5. Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 1927;14:422–469
6. Suginami H. A reappraisal of the coelomic metaplasia theory by reviewing endometriosis occurring in unusual sites and instances. Am J Obstet Gynecol 1991;165:214–218
7. Fujii S. Secondary mullerian system and endometriosis. Am J Obstet Gynecol 1991;165:219–225
8. Olive DL, Henderson DY. Endometriosis and mullerian anomalies. Obstet Gynecol 1987;69:412–415
9. D'Hooghe TM, Bambra CS, Suleman MA, et al. Development of a model of retrograde menstruation in baboons (*Papio anubis*). Fertil Steril 1994;62:635–638
10. Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol 1984;64:151–154
11. Zamah NM, Dodson MG, Stephens LC, et al. Transplantation of normal and ectopic human endometrial tissue into athymic nude mice. Am J Obstet Gynecol 1984;149:591–597
12. Noble LS, Simpson ER, Johns A, Bulun SE. Aromatase expression in endometriosis. J Clin Endocrinol Metab 1996;81: 174–179
13. Zarmakoupis PN, Rier SE, Maroulis GB, Becker JL. Inhibition of human endometrial stromal cell proliferation by interleukin 6. Hum Reprod 1995;10:2395–2399

14. Rier SE, Zarmakoupis PN, Hu X, Becker JL. Dysregulation of interleukin-6 responses in ectopic endometrial stromal cells: correlation with decreased soluble receptor levels in peritoneal fluid of women with endometriosis. J Clin Endocrinol Metab 1995;80:1431–1437
15. Whitlock JP. Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. Annu Rev Pharmacol 1990; 30:251–277
16. Safe S. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 1990;21:51–88
17. Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 1994;24:87–149
18. Hankinson O. The aryl hydrocarbon receptor complex. Annu Rev Pharmacol 1995;35:307–340
19. Van den Berg M, Birnbaum L, Bosveld ATC, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106:775–792
20. Schecter A, Stanley J, Boggess K, et al. Polychlorinated biphenyl levels in the tissues of exposed and nonexposed humans. Environ Health Perspect 1994;102:149–158
21. Schecter A, Furst P, Furst C, et al. Chlorinated dioxins and bibenzofurans in human tissue from general populations: a selective review. Environ Health Perspect 1994;102:159–171
22. DeVito MJ, Birnbaum LS, Farland WH, Gasiewicz TA. Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. Environ Health Perspect 1995;103:820–831
23. Farland W, Schaum J, Winters D, et al. USEPAs risk characterization of dioxin and related compounds. Organohalogen Compd 2000;48:248–251
24. Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fundam Appl Toxicol 1993;21:433–441
25. Yang JZ, Agarwal SK, Foster WG. Subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin modulates the pathophysiology of endometriosis in the cynomolgus monkey. Toxicol Sci 2000;56:374–381
26. Rier SE, Turner WE, Martin DC, et al. Serum levels of TCDD and dioxinlike chemicals in rhesus monkeys chronically exposed to dioxin: correlation of increased serum PCB levels with endometriosis. Toxicol Sci 2001;59:147–159
27. Vanden Heuvel JP, Clark GC, Tritscher AM, Lucier GW. Accumulation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in liver of control laboratory rats. Fundam Appl Toxicol 1994;23:465–469
28. Yoshida K, Ikeda S, Nakanishi J. Assessment of human health risk of dioxins in Japan. Chemosphere 2000;40:177–185
29. Cummings AM, Metcalf JL, Birnbaum L. Promotion of endometriosis by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats and mice: time-dose dependence and species comparison. Toxicol Appl Pharmacol 1996;138:131–139
30. Johnson KL, Cummings AM, Birnbaum LS. Promotion of endometriosis in mice by polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls. Environ Health Perspect 1997; 105:750–755
31. Yang JZ, Foster WG. Continuous exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin inhibits the growth of surgically induced endometriosis in the ovariectomized mouse treated with high dose estradiol. Toxicol Ind Health 1997;13:15–25
32. Matsui KA, Okamura S, Yamashita K, Fujii-Kuriyama Y, Yasuda M. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on surgically induced endometriosis in mice and the role of Ah receptor. Organohalogen Compd 2000;49:345–348
33. Koninckx PR, Braet P, Kennedy SH, Barlow DH. Dioxin pollution and endometriosis in Belgium. Hum Reprod 1994; 9:1001–1002
34. WHO Report. Levels of PCBs, PCDDs and PCDFs in Breast Milk: Result of WHO Coordinated Inter-Laboratory Quality Control Studies and Analytical Field Studies. WHO Environmental Series, 1989
35. Mayani A, Barel S, Soback S, Almagor M. Dioxin concentrations in women with endometriosis. Hum Reprod 1997;12: 373–375
36. Boyd JA, Clark GC, Walmer DK, Needham LL, Lucier GW. Endometriosis and the environment: biomarkers of toxin exposure. Presented at the Endometriosis Workshop; May 15–17, 1995; National Institutes of Health, Bethesda, MD
37. Eskenazi B, Mocarelli P, Warner M, et al. Seveso Women's Health Study: a study of the effects of 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin on reproductive health. Chemosphere 2000;40:1247–1253
38. Pauwels A, Brouwer A, Weyler J, Schepens PJC. The risk of endometriosis and exposure to dioxins and polychlorinated biphenyls: a case-control study of infertile women. Hum Reprod 2001;16:2050–2055
39. Van Larebeke N, Hens L, Schepens P, et al. The Belgian PCB and dioxin incident of January–June 1999: exposure data and potential impact on health. Environ Health Perspect 2001; 109:265–273
40. Brown DJ, Chu M, Overmeire IV, Chu A, Clark GC. Determination of rep values for the CALUX bioassay and comparison to the WHO TEF values. Organohalogen Compd 2000; 48:211–214
41. Fernandez-Salguero P, Pineau T, Hilbert DM, et al. Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. Science 1995;268:722–726
42. Abbott BD, Schmid JE, Pitt JA, et al. Adverse reproductive outcomes in the transgenic Ah receptor–deficient mouse. Toxicol Appl Pharmacol 1999;155:62–70
43. Hasan A, Fischer B. Hormonal control of arylhydrocarbon (AhR) expression in the rabbit uterus. Anat Embryol 2001; 204:189–196
44. Yeaman GR, Collins JE. Arylhydrocarbon receptor expression in human female reproductive tract tissues and peritoneal fluid. Abstract presented at Endometriosis 2000 7th Annual World Congress; May 15, 2000; London
45. Igarashi T, Osuga U, Tsutsumi O, et al. Expression of Ah receptor and dioxin-related genes in human uterine endometrium in women with or without endometriosis. Endocr J 1999;46:765–772
46. Bofinger DP, Feng L, Chi L, et al. Effect of TCDD exposure on CYP1A1 and CYP1B1 expression in explant cultures of human endometrium. Toxicol Sci 2001;62:299–314
47. Pitt JA, Feng L, Abbott BD, et al. Expression of AhR and ARNT mRNA in cultured human endometrial explants exposed to TCDD. Toxicol Sci 2001;62:289–298
48. Khorram O, Garthwaite M, Golos T. Uterine and ovarian aryl hydrocarbon receptor (AhR) and aryl hydrocarbon receptor nuclear translocator (ARNT) mRNA expression in benign and malignant gynaecological conditions. Mol Hum Reprod 2002;8:75–80
49. Bulun SE, Zeitoun KM, Kilic G. Expression of dioxin-related transactivating factors and target genes in human eu-

topic endometrial and endometriotic tissues. Am J Obstet Gynecol 2000;182:767–775
50. Takayama K, Zeitoun K, Gunby RT, et al. Treatment of severe postmenopausal endometriosis with an aromatase inhibitor. Fertil Steril 1998;69:709–713
51. Rier SE, Yeaman GR. Immune aspects of endometriosis: relevance of the uterine mucosal immune system. Semin Reprod Endocrinol 1997;15:209–220
52. Osteen KG, Bruner KL, Sharpe-Timms KL. Steroid and growth factor regulation of matrix metalloproteinase expression and endometriosis. Semin Reprod Endocrinol 1996;14:247–255
53. Watson AJ. Chemopreventive effects of NSAIDs against colorectal cancer: regulation of apoptosis and mitosis by COX-1 and COX-2. Histol Histopathol 1998;13:591–597
54. Yeh WC, Hakem R, Woo M, Mak TW. Gene targeting in the analysis of mammalian apoptosis and TNF receptor superfamily signaling. Immunol Rev 1999;169:283–302
55. Weitzmann SA, Gordon LI. Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. Blood 1990; 655–663
56. Kerkvliet NI, Oughton JA. Acute inflammatory response to sheep red blood cell challenge in mice treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): phenotypic and functional analysis of peritoneal exudate cells. Toxicol Appl Pharmacol 1993;119:248–257
57. Clark GC, Taylor MJ, Tritscher AM, Lucier GW. Tumor necrosis factor involvement in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin–mediated endotoxin hypersensitivity in C57BL/6J mice congenic at the Ah locus. Toxicol Appl Pharmacol 1991; 111:422–431
58. Moos AB, Oughton JA, Kerkvliet NI. The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on tumor necrosis factor production by peritoneal cells. Toxicol Lett 1997;90:145–153
59. Moos AB, Baecher-Steppan L, Kerkvliet NI. Acute inflammatory response to sheep red blood cells in mice treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: the role of proinflammatory cytokines, IL-1 and TNF. Toxicol Appl Pharmacol 1994;127:331–335
60. Taylor MJ, Lucier GW, Mahler JF, et al. Inhibition of acute TCDD toxicity by treatment with anti–tumor necrosis factor antibody or dexamethasone. Toxicol Appl Pharmacol 1992; 117:126–132
61. Halme J. Release of tumor necrosis factor-alpha by human peritoneal macrophages in vivo and in vitro. Am J Obstet Gynecol 1989;161:1718–1725
62. Rier SE, Parsons AK, Becker JL. Altered interleukin-6 production by peritoneal leukocytes from patients with endometriosis. Fertil Steril 1994;61:294–299
63. Rana N, Braun DP, House R, et al. Basal and stimulated secretion of cytokines by peritoneal macrophages in women with endometriosis. Fertil Steril 1996;65:925–930
64. Klein NA, Pergola GM, Rao-Tekmal R, Dey TD, Schenken RS. Enhanced expression of resident leukocyte interferon gamma mRNA in endometriosis. Am J Reprod Immunol 1993;30:74–81
65. Tseng JF, Ryan IP, Milam TD, et al. Interleukin-6 secretion in vitro is up-regulated in ectopic and eutopic endometrial stromal cells from women with endometriosis. J Clin Endocrinol Metab 1996;81:1118–1122
66. Gottschalk C, Malberg K, Arndt M, et al. Matrix metalloproteinases and TACE play a role in the pathogenesis of endometriosis. Adv Exp Med Biol 2000;477:483–486
67. D'Antonio M, Martelli F, Peano S, Papoian R, Borrelli F. Ability of recombinant human TNF binding protein-1 (r-hTBP-1) to inhibit the development of experimentally-induced endometriosis in rats. J Reprod Immunol 2000;48:81–98
68. Zhang RJ, Wild RA, Ojago JM. Effect of tumor necrosis factor-alpha on adhesion of human endometrial stromal cells to peritoneal mesothelial cells: an in vitro system. Fertil Steril1993;59:1196–1201
69. Leibovich SJ, Polverini PJ, Shepard HM, et al. Macrophage-induced angiogenesis is mediated by tumour necrosis factor-alpha. Nature 1987;329:630–632
70. Lai ZW, Pineau T, Esser C. Identification of dioxin-responsive elements (DREs) in the 5′ regions of putative dioxin-inducible genes. Chem Biol Interact 1996;100:97–112
71. Lai ZW, Hundeiker C, Gleichmann E, Esser C. Cytokine gene expression during ontogeny in murine thymus on activation of aryl hydrocarbon receptor by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Mol Pharmacol 1997;52:30–37
72. Rier SE, Coe CL, Lemieux AM, et al. Increased tumor necrosis factor alpha by peripheral blood leukocytes from TCDD-exposed rhesus monkeys. Toxicol Sci 2001;60:327–337
73. Bruner KL, Rodgers WH, Korc M, et al. Transforming growth factor-β mediates the progesterone suppression of an epithelial metalloproteinase by adjacent stroma. Proc Natl Acad Sci U S A 1995;92:7362–7366
74. Bruner KL, Rodgers WH, Gorstein F, Osteen KG. Suppression of matrix metalloproteinases inhibits establishment of ectopic lesions by human tissue in nude mice. J Clin Invest 1997;99:2851–2857
75. Osteen KG, Keller NR, Feltus FA, Melner MH. Paracrine regulation of matrix metalloproteinase expression in the normal human endometrium. Gynecol Obstet Invest 1999;48(suppl 1):2–13
76. Bruner-Tran K, Rier SE, Eisenberg E, Osteen KG. The potential role of environmental toxins in the pathophysiology of endometriosis. Gynecol Obstet Invest 1999;48:45–56
77. Vernon MW. Animal models in endometriosis research. Infertil Reprod Med Clin North Am 1992;3:565–581
78. Fanton JW, Golden JG. Radiation-induced endometriosis in *Macaca mulatta*. Radiat Res 1991;126:141–146
79. D'Hooghe TM, Bambra CS, Raeymaekers BM, et al. Intrapelvic injection of menstrual endometrium causes endometriosis in baboons (*Papio cynocephalus* and *Papio anubis*). Am J Obstet Gynecol 1995;173:125–134
80. Povlsen CO, Fialkow PJ, Klein E, et al. Growth and antigenic properties of a biopsy-derived Burkitt's lymphoma in thymus-less (nude) mice. J Cancer 1973;11:30–39
81. Merenda C, Sordat B, Mach JP, Carrel S. Human endometrial carcinomas serially transplanted in nude mice and established in continuous cell lines. Int J Cancer 1975;16:559–570
82. Bergqvist A, Jeppsson S, Kullander S, Ljungberg O. Human uterine endometrium and endometriotic tissue transplanted into nude mice. Morphologic effects of various steroid hormones. Am J Pathol 1985;121:337–341
83. Aoki D, Katsuki Y, Shimizu A, Kakinuma C, Nozawa S. Successful heterotransplantation of human endometrium in SCID mice. Obstet Gynecol 1994;83:220–228
84. Awwad JT, Sayegh RA, Tao XJ, et al. The SCID mouse: an experimental model for endometriosis. Hum Reprod 1999; 14:3107–3111
85. D'Hooghe TM, Bambra CS, Isahakia M, Koninckx PR. Evolution of spontaneous endometriosis in the baboon (*Papio anubis*, *Papio cynocephalus*) over a 12-month period. Fertil Steril 1992;58:409–412
86. Redwine DB. Age-related evolution in color appearance of endometriosis. Fertil Steril 1987;48:1062–1063