

Environmental immune disruption: a comorbidity factor for reproduction?

Sherry E. Rier, Ph.D.

Division of Environmental Sciences, University of Maine at Machias, Machias, Maine

Objective: To review the evidence on exposure to environmental contaminants and immune system disruption, and how this has been demonstrated or hypothesized to impact reproductive health and fertility.

Design: Review of literature.

Result(s): Exposure to environmental contaminants including polyhalogenated aromatic hydrocarbons, heavy metals, and other hormone disrupting chemicals are associated with a wide spectrum of effects on the reproductive, immune, and endocrine systems. Of particular importance is the potential impact of environmental chemicals on the mucosal immune system of the human female reproductive tract. Immune cells within the reproductive tract produce cytokines and chemokines in response to estrogen and progesterone, thereby influencing various reproductive processes including ovulation, sperm migration, fertilization, implantation, endometrial remodeling, and immune response to infectious challenge. Recent research in animals and humans indicates a potential association between exposure to dioxins, endometriosis, and disruption of the immune system. Studies have shown that rhesus monkeys exposed to dioxins with elevated serum levels of certain toxic coplanar PCBs and an increased total serum toxic equivalency had a high prevalence of endometriosis, and the severity of disease correlated with serum concentrations of PCB77. Dioxin-exposed animals with endometriosis showed long-term alterations in immunity associated with elevated levels of dioxin and specific coplanar dioxin-like congeners.

Conclusion(s): Perspectives on the potential mechanism(s) of toxicity induced by environmental chemicals in endometriosis and other reproductive diseases, important knowledge needs, potential animal models, and considerations integral to future studies are discussed. (Fertil Steril® 2008;89:e103–8. ©2008 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, environmental toxicants, dioxin, TCDD, PCBs

The focus of this article is to review available evidence concerning the potential effect of environmental chemicals on the immune system and how this has been demonstrated or hypothesized to impact reproductive health and fertility. The general human population has hundreds of different environmental chemicals in blood and tissues. Persistent organic pollutants of concern for immune-reproductive toxicity include polyhalogenated aromatic hydrocarbons, heavy metals, and other hormonally active chemicals. Little is known about the effect of exposure to environmental contaminants; however, exposure to dioxins has been implicated in the pathogenesis of the reproductive disease, endometriosis; for review, see (1, 2). Animal studies have shown that exposure to dioxins is associated with adverse female reproductive effects including reduced fertility, ability to maintain pregnancy, and decreased litter size over 3 generations (3); anovulation, ovarian dysfunction and suppression of the estrous cycle (4); as well as alterations in mammary gland development following in utero and lactational exposure (5). In addition to effects on the reproductive system, dioxin also adversely affects immunocompetence in rodents such as suppression of T-cell-dependent antibody responses, decreased

lymphocyte antitumor cytolytic activity, and increased production of inflammatory cytokines, tumor necrosis factor alpha (TNF- α) and interleukin (IL)-6, by peritoneal and peripheral blood leukocytes [for review, see (6)]. The etiology of endometriosis and infertility are unknown, but studies suggest that immune mechanisms contribute to the disease processes active in these conditions.

DIOXIN AND DIOXIN-LIKE COMPOUNDS

Evidence indicates that the actions of TCDD and dioxin-like chemicals are mediated by the aryl hydrocarbon receptor (AhR) (7), a basic region/helix-loop-helix transcription factor, whose natural ligand is not known. Following receptor activation, the receptor–ligand complex is translocated to the nucleus via the AhR nuclear translocator (ARNT) where DNA binding occurs, resulting in transcriptional activation. Target genes include cytochrome P-450 and genes involved in cellular growth, differentiation, and inflammation (8, 9). Various dioxin congeners can act additively via specific binding of the AhR, and their potency relates to AhR affinity; therefore, these chemicals are classified as “dioxins.” Non-ortho and some mono-ortho polychlorinated biphenyls (PCBs) are also AhR agonists and contribute significantly to the toxicity of complex mixtures of environmental polyhalogenated aromatic hydrocarbons (PHAHs) (10, 11). TCDD and dioxin-like polychlorinated dibenzo dioxins (PCDDs)

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Reprint requests: Sherry E. Rier, Ph.D., Division of Environmental Sciences, University of Maine at Machias, 9 O'Brien Avenue, Machias, Maine 04654 (FAX: 207-255-1390; E-mail: srier@maine.edu).

and polychlorinated dibenzo furans (PCDFs) are produced as unwanted byproducts of many industrial and combustion processes, whereas PCBs were used extensively in a broad range of commercial applications. Dioxins are resistant to degradation and, because of 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 (7, 10, 12–14). In industrialized countries, exposure to environmental PHAHs has led to serum TCDD levels of one to five parts per trillion (PPT, lipid adjusted) and body burdens estimated at 25 PPT TCDD (lipid adjusted) toxic equivalents (TEQs) in individuals with no overt exposure. In these populations, TCDD contributes approximately 15% of the body burden of total dioxins, whereas dioxin-like PCDDs, PCDFs, and PCBs comprise about 85% of the dioxin body burden. 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 biologic 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 via disruption of endocrine and immune responses in susceptible humans and animals (14, 15). One such candidate disease is endometriosis.

THE POTENTIAL IMPACT OF ENVIRONMENTAL CHEMICALS ON THE UNIQUE MUCOSAL IMMUNE SYSTEM OF THE FEMALE REPRODUCTIVE TRACT

Of particular importance is the potential impact of environmental chemicals on the mucosal immune system of the human female reproductive tract (FRT). The tissues of the FRT contain a full spectrum of immune cells embedded in the tissue architecture composed primarily of stromal and epithelial endocrine cells. Reproductive processes are regulated by sex hormones in concert with bioactive mediators (cytokines, growth factors, and remodeling enzymes) produced by immune and endocrine cells. The FRT is comprised of histologically distinct regions including the fallopian tube, uterine endometrium, endocervix, ectocervix, and vaginal mucosa. Previous studies have shown that RT leukocytes are primarily composed of T cells and granulocytes with fewer numbers of macrophages and B cells (16). The phenotype and function of RT leukocytes change in numbers and orientation during the menstrual cycle in response to estrogen and progesterone. This unique mucosal immune system must maintain a delicate balance to confer protection against pathogens, whereas sex hormones down-regulate adaptive immunity to meet the constraints of procreation. T-cell aggregates, comprised of a B-cell core surrounded by CD8+ T cells and a mantle of macrophages develop in endometrium during the secretory phase (17). CD8+ T-cell cytolytic activity and CD4 expression by T-cells is suppressed in endometrium during the secretory phase (18, 19). Suppression of immune responses in endometrium during the progesterone-dependent secretory phase likely confers protection for

the semiallogenic fetus and for establishment of pregnancy. Disruption of the hormonally dependent immune system of the FRT is a potential target of environmental chemicals.

The specific receptor for TCDD and dioxin-like chemicals is present in reproductive and neuroendocrine tissues. 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 (20, 21). Other animal studies indicate that AhR-responsive genes function in reproductive processes within the uterine endometrium. In the rabbit model, there are AhR mRNA changes in the uterus during the pro-inflammatory, invasive processes of implantation and the progesterone-mediated immunosuppression of pregnancy maintenance (22). The mRNA of AhR and ARNT are constitutively expressed in uterine and ectopic endometrium from women with and without endometriosis (23, 24). TCDD treatment of human endometrial explants results in increased expression of AhR mRNA and the mRNA of the dioxin-responsive gene P-4501A1 (25). In ovarian endometriosis, the expression of AhR mRNA is increased (26), and P-4501A1 mRNA is increased 8.7 times in ectopic tissue relative to uterine endometrium (24). Taken together, these reports suggest that the endometrium of animals and humans is a target tissue for the dioxin's action.

ANIMAL STUDIES ON DIOXINS EXPOSURE AND ENDOMETRIOSIS

Recent studies indicate an association between dioxins exposure endometriosis and immune system disruption. Previous work in nonhuman primates demonstrates that dietary exposure to 5 PPT or 25 PPT TCDD for 4 years results in a dose-dependent increase in spontaneous endometriosis 10 years postexposure in the rhesus monkey (27). In agreement with these findings, Yang et al. (28) observed that exposure to 5 or 25 PPT TCDD for 1 year results in a higher survival rate of endometrial implants, and an increased implant size in animals fed 25 PPT TCDD using a model of surgically induced endometriosis in the cynomolgous monkey. In rodents, treatment with TCDD or the dioxin-like 2,3,4,7,8-pentachlorobiphenyl for 12 weeks produces a dose-dependent increase in the size of surgically induced endometrial lesions in an estrogen-responsive mouse model of endometriosis (29). This effect may be AhR-mediated because treatment of mice with nondioxin-like compounds has no effect on the growth of endometriotic sites. Recent work has shown that TCDD promotes the establishment of ectopic endometrial lesions in a nude mouse model of endometriosis by disrupting progesterone regulation of the expression of matrix metalloproteinases (MMPs), a family of enzyme isoforms involved in tumorigenic invasive processes and tissue remodeling during endometrial proliferation and menstruation (30). Importantly, dioxin alters progesterone responses in endometrium. TCDD treatment of human endometrial cells results in reduced expression of progesterone receptor B and increased expression of MMPs similar to endometrial tissues from women with

endometriosis (31). Developmental exposure to TCDD in mice results in altered expression of uterine progesterone receptors similar to endometrial tissues from women with endometriosis (32).

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 dietary TCDD exposure (33). Similar to the general human population and laboratory animals, TCDD-treated and control animals were exposed to dioxin-like PCBs via their diet or other environmental sources. Importantly, 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. Dioxin-treated animals had aggressive disease in intestines, liver, spleen, bladder, and lung (unpublished observations). These data suggest a potential involvement of an increased body burden of dioxin-like PCB compounds in the etiology of endometriosis in rhesus monkeys. 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. The body burden of PCDDs and PCDFs and total PHAH compounds is 2- to 20-fold higher in the general human population than in TCDD-exposed monkeys with endometriosis. Total dioxin-like PCB concentrations of 290 PPT (lipid adjusted) found in TCDD-treated animals (33) is somewhat higher compared with lipid-adjusted levels of 100–190 PPT reported in humans (12, 13, 34). In the most recent risk characterization of dioxin and related compounds by the World Health Organization, endometriosis is considered a low-dose biologic effect of dioxins exposure occurring at or near human background levels (35).

HUMAN STUDIES ON DIOXINS EXPOSURE AND ENDOMETRIOSIS

Early small hospital-based case-control studies on dioxins exposure and endometriosis were inconclusive [for review, see (1, 2)]. Several studies did not evaluate blood levels of all dioxins in women with and without endometriosis (36–38). One study determined total dioxins TEQ in blood; however, 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 (39). In addition, the assay used to quantify total TEQ may be subject to suppression by blood levels of nondioxin-like PCBs. However, recent work has shown that serum levels of dioxins are increased in women with peritoneal endometriosis and deep endometriotic lesions compared with fertile control women without disease (40). Moreover, increased serum levels of dioxin-like PCB congeners, combined with elevated levels of nondioxin-like PCBs, were detected in Italian women with endometriosis (41). Thus, evidence is accumulating to support the hypothesis that environmental dioxins play a role in the pathophysiology of endometriosis.

DIOXINS EXPOSURE, ENDOMETRIOSIS, AND IMMUNE SYSTEM DISRUPTION

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. Increased mitogen-stimulated TNF- α secretion and decreased cytolytic activity against NK-sensitive target cells by peripheral blood leukocytes was found in these monkeys 13 years after termination of TCDD treatment (42). Changes in immune status in TCDD-treated animals correlated with elevated serum concentrations of TCDD and dioxin-like PCB126. Immune cells in blood and peritoneal fluid from women with endometriosis also produce elevated levels of TNF- α and other inflammatory cytokines [for review, see (43)]. Moreover, immune cells in peritoneal fluid of women with endometriosis exert decreased NK cytolytic activity, an immune process that may limit the growth of ectopic lesions. 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, and implantation of endometrial fragments.

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

The initiating event(s) in the development of endometriosis is unknown; however, the effect of dioxins to stimulate pro-inflammatory cytokines could lead to the establishment of the major characteristics of endometriosis. Animal experiments and in vitro studies have shown changes in peritoneal levels of cytokines. Activation of this inflammatory cytokine network in the extrauterine environment may result in COX-2 induction and increased PGE₂ synthesis in ectopic endometrium postulated to lead to chronic localized inappropriate estrogen production, suppression of progesterone responses, mis-expression 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, neo-vascularization, and leukocyte recruitment, including MCP-1, VEGF, IL-6, and IL-8. Importantly, TCDD induces the direct activation of genes involved in cell cycle and death, including the TNF/TNFR family members via the direct response element (DRE) in the promoter region (44). A greater number of ectopic endometrial cells may survive, thrive, and disseminate because 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 likely lead to the sequelae of endometriosis, such as pelvic pain, adhesion formation, and infertility. TCDD and dioxin-like PHAHs may exert effects on the pathophysiology of endometriosis through a number of pathways, including: [1] activation of pro-carcinogens, [2] altered synthesis and metabolism of estradiol, [3] altered production of pro-inflammatory growth factors or cytokines, and [4] mis-expression of remodeling

enzymes. These findings have led to the postulation that exposure to dioxins is associated with immune disruption, which promotes endometriosis by inducing chronic inflammation, excess estrogen formation, activation of pro-carcinogens, and altered progesterone responses in endometrium.

KEY RESEARCH DIRECTIONS

Recent advances in the detection and quantification of individual PHAH congeners in biologic samples have made it possible to assess 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. 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 obstetric history of pregnancy and breast feeding, body mass index, and age. Epidemiologic studies must also account for other exposures to estrogen-like compounds including dietary phytoestrogens and estrogenic nondioxin-like PCBs. Although preliminary work suggests a potential involvement of exposure to dioxins in the pathogenesis of endometriosis, much work remains to clearly define cause and effect, to determine the timing and dose of dioxins exposure associated with reproductive toxicity, as well as to define critical periods of exposure such as during gestation and puberty. Progress in this area will be important in the translation of science to the clinic, leading to new treatments for disease prevention and intervention, including reduction of the body burdens of dioxins and other hormonally active environmental chemicals. Recent research already indicates that targeting chronic inflammation and excess estrogen formation using inhibitors of TNF- α and aromatase expression may be effective treatments for endometriosis (45, 46).

CRITICAL RESEARCH TOOLS

Endometriosis occurs exclusively in menstruating species, including humans and nonhuman primates, with spontaneous development in rhesus monkeys closely resembling human disease (47). 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 (28, 48). In addition, rodent and rabbit models of disease have been employed using surgical autotransplantation of endometrium [reviewed in (49)]. Use of immune-deficient mice bearing human eutopic and ectopic endometrium offers a significant

advantage over previous animal models as a short-term model of disease, and will generate results that are more readily generalized to humans. When designing studies to investigate the role of dioxins in the pathogenesis of endometriosis, it is important to use 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 nude mouse model, 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. (28), 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. Dioxins may increase inflammation at these sites via 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 does not occur in these animals. In vitro and in vivo models must be developed that mimic the microenvironment of reproductive tract tissues at critical time periods. Bioassays must be developed to test the effect of individual and combinations of chemicals on specific end points such as aromatase activity, cytokine secretion, progesterone receptor expression, and markers of cell growth and differentiation. Other environmental chemicals, acting individually or synergistically, may also be capable of inducing endometriosis or other reproductive diseases, suggested by recent work demonstrating higher PCBs in combination with increased phthalate esters in South Indian women with endometriosis (50).

In conclusion, 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. 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 via stimulation of chronic inflammation, potentially leading to enhanced estrogen synthesis and disruption of progesterone-dependent remodeling responses that normally limit the development of endometriosis. These findings are relevant to human endometriosis, and other reproductive diseases, 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 environmental contaminants and endometriosis, and elucidate the mechanism of action of these toxicants in the pathophysiology of this disease.

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