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REVIEW ARTICLE

# Rodent Models of Experimental Endometriosis: Identifying Mechanisms of Disease and Therapeutic Targets

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**Abstract:** ***Background*:** Although it has been more than a century since endometriosis was initially described in the literature, understanding the etiology and natural history of the disease has been challenging. However, the broad utility of murine and rat models of experimental endometriosis has enabled the elucidation of a number of potentially targetable processes which may otherwise promote this disease.

***Objective*:** To review a variety of studies utilizing rodent models of endometriosis to illustrate their utility in examining mechanisms associated with development and progression of this disease.

***Results*:** Use of rodent models of endometriosis has provided a much broader understanding of the risk factors for the initial development of endometriosis, the cellular pathology of the disease and the identification of potential therapeutic targets.

***Conclusion*:** Although there are limitations with any animal model, the variety of experimental endometriosis models that have been developed has enabled investigation into numerous aspects of this disease. Thanks to these models, our understanding of the early processes of disease development, the role of steroid responsiveness, inflammatory processes and the peritoneal environment has been advanced. More recent models have begun to shed light on how epigenetic alterations contribute to the molecular basis of this disease as well as the multiple comorbidities which plague many patients. Continued developments of animal models which aid in unraveling the mechanisms of endometriosis development provide the best opportunity to identify therapeutic strategies to prevent or regress this enigmatic disease.

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## 1. INTRODUCTION

Descriptions of endometriosis, defined as the presence of endometrial glands and stroma outside the uterus, can be found in medical literature at least as early as the mid-1800's [1, 2]; however, it would be several decades later before the name "endometriosis" was coined. In his landmark paper, Dr. John Sampson [3] suggested development of endometriosis was due to ectopic implantation of refluxed menstrual tissue [3]. Although research studies continue to support the "retrograde menstruation" theory as one mechanism by which endometriosis can develop [4-6], deposition of menstrual tissue within the peritoneal cavity cannot account for all incidences of disease. Additionally, retrograde menstruation is common among reproductive age women [7-9]; however, only about 10% of women develop endometriosis, suggesting other mechanisms are also involved. Alternative explanations for the occurrence of endometriosis include coelomic metaplasia [10-12] and peritoneal activation of embryonic cell rests [13, 14]. The latter theory likely explains the unusual and rare occurrence of endometriosis in men [15, 16]. Recently, ectopic endometrial tissue has been described in human fetuses which has been theorized to develop as a consequence of ectopic localization of primitive endometrial tissue during organogenesis [17, 18]. Other factors likely affecting an individual's risk for development of endometriosis include: genetic predisposition, immune dysregulation and/or a history of environmental toxicant exposure (reviewed by [19]). More recently, Brosens and colleagues have postulated a role for neonatal menstruation, as a

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consequence of early onset steroid responsiveness, in developing endometriosis as an adolescent [20].

While generally considered a benign condition, endometriosis exhibits cancer-like features and can spread throughout the peritoneal cavity and to distal sites. Endometriosis is frequently physically debilitating, as patients often suffer from chronic pelvic pain, dyspareunia, dysmenorrhea and subfertility. Unfortunately, most women with this disease also exhibit one or more co-morbidities including adenomyosis, adhesive disease and inflammatory conditions such as interstitial cystitis and inflammatory bowel disease [21-23]. However, understanding the natural history of endometriosis, as well as the myriad of equally poorly understood co-morbidities, has proven elusive, despite extensive research in each disease area. Given our lack of understanding of the etiology of endometriosis, treatment options for women with this disease remain limited and generally involve a combination of hormonal manipulation and surgery to remove diseased tissue. Unfortunately, the side effects of hormonal therapy for endometriosis cause many women to abandon this treatment; nevertheless, surgical treatment alone is frequently non-curative and many women suffer recurrence [24-32]. Thus, identifying better diagnostic and treatment strategies for this disease is a major focus of many endometriosis research laboratories.

Endometriosis is rare in non-menstruating species, suggesting that shedding of endometrial tissue may be a causative factor as originally proposed by Sampson [3]. Indeed, numerous studies have demonstrated that the transfer of endometrial tissue to the peritoneal cavity can lead to the development of ectopic endometrial lesions. For example, in the mid-1950s, Sampson's retrograde menstruation theory was experimentally examined in women *via* intraperitoneal injection of their own menstrual tissue several months prior to a scheduled surgery for fibroids. This study identified ectopic peritoneal lesions in 2 of 13 women (15%), providing support for the retrograde menstruation theory [4]. Given the obvious limitations and ethical considerations of human experimentation, current endometriosis researchers rely heavily on non-human primate or rodent models in order to investigate elements of disease pathophysiology.

## 2. THE MODELS

Among various animals utilized for experimental endometriosis, rodent models have a number of advantages relative to other species. They are cost effective due to their small size and large litters while their short gestation enables transgenerational analysis. Additionally, the wide availability of genetic knock-out mice, antibodies against murine and rat proteins and knowledge of the murine genome make these animals extremely useful for the study of many diseases, including endometriosis. As shown in Table **1**, many different model systems have been developed in mice and rats, each with unique features that are valuable for targeted studies. Importantly, rodent models of endometriosis are often utilized for preclinical testing in an attempt to identify new therapeutic agents. The use of experimental endometriosis models for therapeutic testing has recently been reviewed [33, 34]; thus, these studies will not be extensively considered here. Rather, in this review, we will highlight the most commonly used rodent models of endometriosis (Table **1**) with an emphasis on studies exploring disease-related mechanisms. We will also explore the use of animal models that have provided insight into the development of comorbidities common among women with endometriosis. Significantly, using appropriate experimental endometriosis models for identifying therapeutic *targets* is a critical step prior to the examination of a treatment regimen aimed at any particular pathway or protein.

### 2.1. Experimental Endometriosis in Immunocompromised Mice

Examination of early events associated with endometriosis (ie, endometrial characteristics which promote ectopic implantation and survival), requires an appropriate model system. Therefore, several laboratories, including ours, have utilized chimeric mouse models of endometriosis in which human endometrial tissue can be introduced into immunocompromised mice (for example, [35-38]). Immunocompromised mice lack a fully competent immune system; therefore, these animals do not mount an immune response against *human* tissue xenografts; therefore, these mice are valuable for examining multiple cellular pathways associated with development of endometriosis. Immunocompromised mouse strains include athymic (nude) mice, Severe Combined Immunodeficient (SCID) mice and rag2γ(c) (Recombinant Activating Gene 2/common cytokine receptor γ chain (γc) double null) mice. The capacity to examine human endometrial cells and tissues growing *in vivo* make chimeric models of experimental endometriosis particularly useful for pre-clinical testing, which is critical to developing better therapeutic agents. Importantly, experimental endometriosis can be established using tissues obtained from women with and without endometriosis *via* endometrial biopsy, collection of menstrual effluent or from surgical specimens (for example, [36, 39-41]). By utilizing tissues acquired from women with and without endometriosis, phenotypic differences between control and disease-altered eutopic endometrium can be incorporated into the experimental design. For example, a therapeutic treatment directed at the endometrial tissue phenotype of an endometriosis patient can be initiated *in vitro* prior to introduction into mice, enabling targeting of specific cellular processes which may be involved in early lesion establishment.

#### *2.1.1. Experimental Endometriosis in Nude Mice*

Athymic nude mice have a spontaneous mutation in the forkhead box N1 (*Foxn1*) gene resulting in an underdeveloped thymus and severely compromised number of T cells [42-44]. Although homozygous nude mice have a normal complement of B cells, the lack of T cells prevents complete B cell maturation. These features render nude mice suitable for receiving transplantation of human xenografts and, not surprisingly, these animals have been widely used for cancer studies. Zamah and colleagues were the first to report experimental endometriosis in nude mice [41]. For their study, human endometrial tissues (eutopic and ectopic) were minced and injected into the peritoneal space of nude mice. Some mice were treated with estrogen, allowing examination of the impact of this steroid on lesion growth. In this study, the majority of animals developed a disease resembling en-

**Table 1. Rodent models of experimental endometriosis.**

| Model | Features | Benefits | Limitations | Examples |
|---|---|---|---|---|
| **Immunocompromised Models** | | | | |
| ***Athymic Nude***<br>Spontaneous mutation of *Foxn1* (Chromosome 11) | Lack T cells, compromised B cell function | Accepts human xenografts; useful for early lesion establishment studies | Age-related compensatory immunity | [36, 41] |
| ***Congenic SCID***<br>Spontaneous autosomal recessive mutation of *Prkdc* (DNA repair enzyme) (Chromosome 16) | Lack B and T cells | Accepts human xenografts; fertility preserved; useful for early lesion establishment studies | Age-related compensatory immunity; high susceptibility to opportunistic infections | [45] |
| ***Rag2γ(c)***<br>targeted double mutation obtained by crossing gamma c knockout and Rag2 deficient mice (C57Bl/6 background) | Lack B, T and NK cells | Lack of age-related compensatory immunity allows for longer duration of studies; ability to graft human immune cells allows for natural experimental model | Animals with more aggressive behavior | [38, 46, 47] |
| **Autologous Models** | | | | |
| ***Rat surgical model*** | Uterine tissues sewn to arteries of small intestine | Animal maintains normal immune function | Require animal undergo surgery | [48-50] |
| ***Injection Models*** | Minced uterine tissues are injected IP or SC | Peritoneal injections more closely mimic retrograde menstruation; either donor or recipient can be treated allowing for large permutation of experimental setups | Ectopic tissue can be difficult to locate; variable success rates in disease establishment | [51] |
| ***C57bl/6 Green Fluorescent Protein*** | Transgenic mice that express GFP are used as endometrial donors | Lesions visualized by fluorescence | | [52] |
| **Transgenic knock-out models**<br>**-PRKO**<br>**-ERKO** | Mice transgenic for loss of steroid receptor expression. | Role of specific genes can be examined | | [51, 53] |
| **EDC-Exposure Models**<br>**-TCDD**<br>**-BPA** | Female mice exhibit uterine progesterone resistance; subfertility<br>Ectopic disease | Useful to examine the uterine phenotype associated with endometriosis | Unidentified effects of toxicant exposure may additionally impact disease processes. | [54-57] |
| **Multigenerational Models** | | | | |
| ***Rat Surgical Model***<br>***Toxicant Exposed Model*** | Daughters fertility impacted<br>Daughters and sons fertility impacted | Useful for epigenetic studies and comorbidity analysis<br>Useful for epigenetic studies and comorbidity analysis | | [58]<br>[54, 59] |

dometriosis; however, the most extensive disease was noted in mice receiving both estrogen and ectopic tissues from women with endometriosis.

Since this first report, the nude mouse model of experimental endometriosis has been used extensively by our group as well as by many other investigators to examine aspects of establishment and progression of this disease within the peritoneal cavity. For our model of experimental endometriosis using nude mice [36], human endometrial tissue is obtained by biopsy during the proliferative phase of the menstrual cycle and initially maintained as an organ culture. Following overnight incubation in media containing estradiol, the minced endometrial tissue fragments are washed in physiological saline to remove residual blood and mucous and tissues are subsequently injected into mice either intraperitoneally or subcutaneously along the ventral midline. Recipient mice can be left intact or ovariectomized and implanted with slow-release estradiol capsules thereby mimicking the growth-promoting environment of the human proliferative phase. As shown in Fig. (**1**), ectopic peritoneal lesions which establish in mice are similar in appearance (both gross and microscopic) to the spontaneous disease occurring in women.

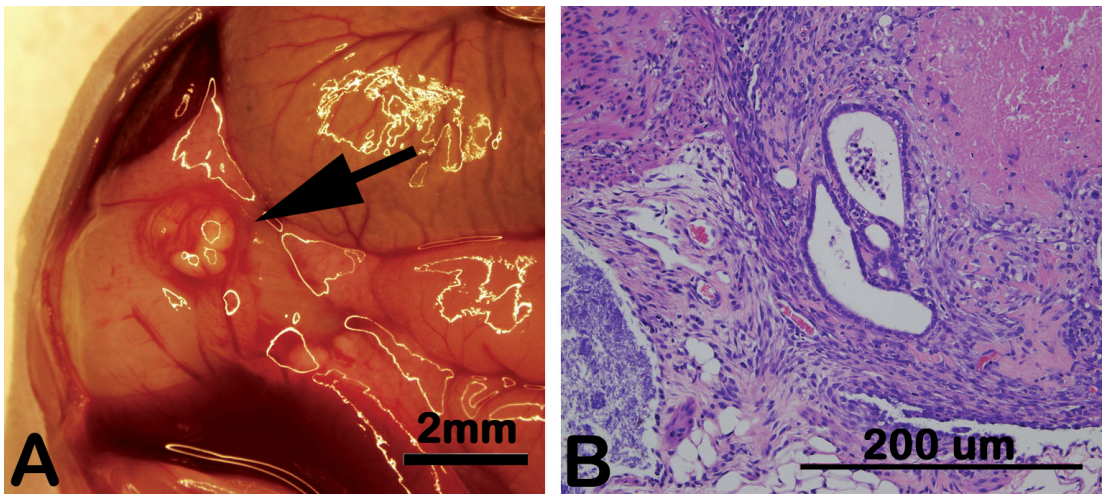


**Fig. (1). Experimental human endometriosis in a nude mouse.** Proliferative phase human endometrial tissue readily establishes ectopic lesions in a nude mouse. Prior to injection of endometrial fragments, mice are ovariectomized and provided a slow-release estradiol capsule to mimic the human proliferative phase. Lesions developing in mice are markedly similar to human disease both macroscopically (**A**) and microscopically following hematoxylin and eosin staining (**B**). Results are representative of numerous (N>25) experiments using different human samples.

### *2.1.2. Experimental Endometriosis in SCID Mice*

Severe Combined Immunodeficiency (SCID) is observed in humans as well as many other species [60]. This genetic disorder results in dysfunctional T and B lymphocytes leading to an inability of the adaptive immune system to mount or sustain an appropriate immune response. In mice, the condition results from a rare, recessive mutation leading to limited activity of a DNA repair enzyme (Prkdc or "protein kinase, DNA activated, catalytic polypeptide"). Loss of this enzyme is associated with a failure of humoral and cellular immune systems to mature. The mutation was first described in BALB/c-Ighb mice in 1980 by Bosma and associates at Fox Chase Cancer Center [61]. As summarized in Table **1**, SCID mice exhibit an impaired ability to make T or B lymphocytes and cannot either efficiently fight infections or reject tumors. For these reasons, SCID mice are also suitable models for establishing experimental endometriosis using human endometrial tissues. The earliest study using SCID mice for endometriosis research was Aoki *et al*. [45]. These investigators established experimental human endometriosis in both SCID and nude mice; and reported the latter to be a superior model system due to a significantly greater survival rate of human tissues in the SCID animals. However, these animals are more prone to disease and opportunistic infections compared to nude mice.

### *2.1.3. Experimental Endometriosis in Rag2γ(c) Mice*

Significantly, both SCID mice and nude mice develop extrathymic immunity over time [62, 63]; thus, experimental endometriosis studies utilizing these animals must be completed before 3 months of age, severely limiting the utility of these animals for studies over extended periods of time. For this reason, studies of prolonged duration require a different model. Recombinant Activating Gene 2/common cytokine receptor γ chain (γc) double null mice (rag2γ(c)) are more completely immunosuppressed compared to nude or SCID mice, demonstrating an absence of B cells, T cells, NK cell activity and all lymphocytes (Table **1**). These mice were first described by Mazurier *et al*. [47] who crossed the common gamma c knockout mouse (developed by [64]) with the recombinase activating gene-2 (rag2) deficient mouse (developed by [65]). Rag2γ(c) mice do not demonstrate the same age-related compensatory immunity as other immunocompromised mice and, surprisingly, these animals are more disease resistant than SCID mice. Thus, xenographic studies conducted in rag2γ(c) mice can be of much longer duration than studies conducted in nude or SCID mice. Greenberg and Slayden [38] were the first to report the xenotransplantation of endometrial tissues into rag2γ(c) mice. In their study, human endometrium was introduced into the peritoneal cavity of mice which were then subjected to multiple artificial menstrual cycles. Their study revealed that endometriotic-like disease was present in the majority of mice even after four artificial 28 day cycles and provided strong support for the use of these mice for long-term endometriosis studies.

Equally significant, rag2γ(c) mice also accept human immune cells without rejection, allowing these cells to be examined in conjunction with experimental endometriosis [46]. As will be discussed in detail below, the ability to additionally assess the role of human immune cells in establishment or prevention of experimental disease is an important advantage of studies using rag2γ(c) mice compared to studies conducted with either the nude or SCID mouse.

### *2.1.4. Kidney Capsule Model*

Although experimental endometriosis using chimeric murine models is most commonly conducted with intraperitoneal injection of human endometrial tissues, other approaches also have been utilized to an advantage. An important adaptation arising from chimeric mouse models has been the establishment of renal grafts in which human tissue, including reproductive tissues, are placed under the kidney capsule [66, 67]. Endometrial tissue growing under the kidney capsule allows easier identification and excision com-

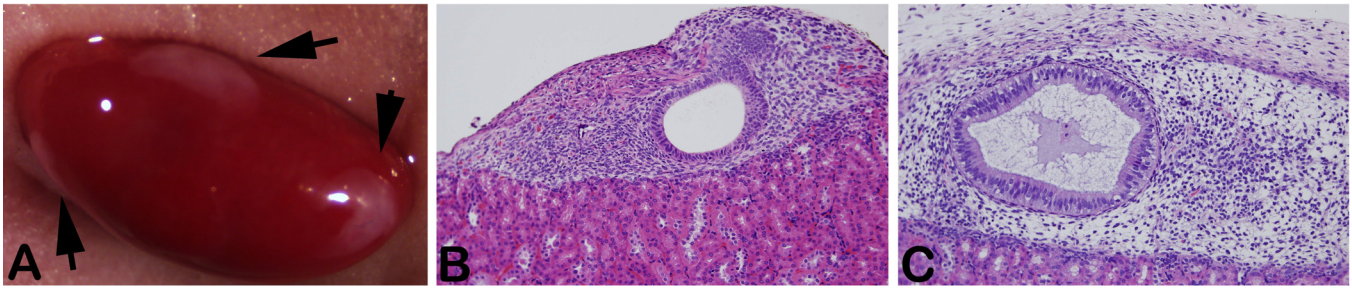


**Fig. (2). Human endometrium growing under the kidney capsule of nude mice.** Human endometrial stromal and epithelial cells are isolated and recombined in collagen and placed under the kidney capsule. Tissues are readily visible on gross examination (arrows) (**A**). Following treatment of the animal with estradiol (**B**) or estradiol plus medroxyprogesterone acetate (**C**) lesions exhibit proliferative or secretory phenotypes, respectively. Results are representative of multiple (N=5) experiments utilizing different human tissues.

pared to the random sites of attachment following intraperitoneal injection. An additional advantage of the kidney capsule is its high vascularity, which enhances survival of ectopic tissues. The kidney capsule lends itself not only to insertion of whole tissues (explants), but also enables isolation and recombination of endometrial cells within a collagen matrix. [67]. Renal grafting of various combinations of endometrial cells allows a more precise examination of each endometrial cell type during growth and differentiation *in vivo*. Importantly, we have found that control human endometrial tissues growing at this site in mice remain responsive to ovarian steroids and exhibit classic orphological appearance and biochemical differentiation in response to progesterone treatments (Fig. **2**).

### 2.2. Immunocompetent Rodent Models

Endometriosis is recognized to be a steroid-dependent disease largely occurring during the reproductive years of a woman's life; however, defects within the immune system are also a critical component of disease pathophysiology (reviewed by [68]). Although long considered an autoimmune disease, recent research indicates that cell-mediated immunity is also disrupted in endometriosis. Thus, a major disadvantage of experimental endometriosis models established in immunocompromised mice is the limited ability to examine the role of the immune system in development and progression of disease. Despite the ability of rag2γ(C) mice to accept both human tissue and immune cells, the short-lived nature of circulating human immune cells in these animals remains an important drawback of immunological studies using this experimental model. Therefore, immunocompetent rodent models remain essential to examining the influence of the immune system on development and progression of experimental endometriosis.

#### *2.2.1. Rat Surgical Model*

One of the earliest *in vivo* experimental endometriosis models developed was the rat surgical model, first reported by Vernon and Wilson [50]. In this experimental model, the investigators removed one uterine horn and dissected the endometrial tissue into 2-mm squares. Four uterine squares were subsequently autotransplanted to the arterial cascades of the small intestine of the same animal. Although not useful for examining early events of endometriosis development due to the need for surgical induction of ectopic growth, the rat surgical model has provided significant insight into endometriosis-associated infertility (reviewed by [49]).

#### *2.2.2. Syngeneic Mouse Model*

More recently, a number of groups have utilized syngeneic mouse models of endometriosis, in which the uterus of one animal is removed during estrus (natural or induced), minced and injected intraperitoneally into one or two recipient mice [51, 69-72], an approach that is similar to the studies noted above in immunocompromised mice. Syngeneic murine models have several potential advantages over the rat surgical model. First, peritoneal seeding of uterine fragments is more similar to retrograde menstruation in women. Second, either the donor or recipient animal can receive therapeutic intervention or be otherwise manipulated prior to induction of disease. Finally, the availability of an extensive number of transgenic mice, in which specific genes can be either eliminated or overexpressed, make these animals ideal for studying the role of specific pathways in the development and progression of endometriosis and other diseases.

### 2.3. Toxicant Exposure Models

Although the role of the environment in the development of reproductive dysfunction continues to be debated, endometriosis researchers have long considered the likelihood that environmental toxicants may be one trigger for disease development. As a consequence of industrialization, humans and other animals are exposed to a wide array of manmade toxicants, many which act as endocrine disrupting chemicals (EDCs) [73, 74]. Among human exposures to various EDCs, interest in exposure to TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin, commonly known as dioxin) arose from a primate study published by Rier *et al*. [75], which suggested that exposure to this chemical family may increase the risk for developing endometriosis. TCDD along with a number of dioxin-like PCBs (polychlorinated byphenols) bind the aryl hydrocarbon receptor (AhR), an orphan nuclear receptor that has been suggested to play a role in normal uterine function [76, 77]. Inappropriate activation of this receptor by environmental toxicants not only disrupts steroid action but also promotes inflammatory processes that may be linked to endometriosis [78, 79]. Nevertheless, although numerous epidemiological studies have been undertaken in order to assess the adult body burden of TCDD or various PCBs in relation to the presence of absence of endometriosis; these studies

reach conflicting conclusions. For example, although several epidemiological studies suggested that blood and/or tissue levels of various environmental endocrine disruptors, correlated to the presence of endometriosis in women (for example, [80-84]; other studies failed to find an association (for example, [85-89]). At this juncture, the difficulty in definitively determining a causative role of toxicant body burden and endometriosis is perhaps related to both the variable toxicity of a given agent as well as the significant difficulty in identifying the presence or absence of endometriosis within a study population [90-92]. Therefore, a number of researchers have utilized rodent models of toxicant exposure to explore the potential role of these compounds in the development of endometriosis.

#### *2.3.1. Adult Toxicant Exposure Models*

In a series of studies, Birnbaum and colleagues explored the influence of an adult exposure to TCDD and other EDCs on the progression of ectopic lesions using an experimental endometriosis model in mice similar to the rat surgical model described above. Initially, this group compared the rat and mouse surgical models with regard to growth and progression of experimental disease in response to TCDD exposure [93]. For these studies, adult animals (Sprague-Dawley rats and B6C3F1 mice) were treated with TCDD prior to surgical induction of endometriosis as well as following surgery. Although TCDD treatment negatively impacted disease and reproductive endpoints in both animal models, the enhanced impact of TCDD on immune function in mice led the authors to conclude that these animals may be more appropriate for future toxicity studies. A follow-up study from this group similarly examined other polyhalogenated aromatic hydrocarbons on endometriotic proliferation using the same mouse model. Their second study found that lower doses of TCDD (1 and 3 ug/kg bw) and 4-PeCDF (100 ug/kg bw) significantly enhanced the growth of experimental endometriosis while PCB 126 had no impact on lesion growth. Interestingly, they also found that the highest dose of TCDD had no impact on lesion growth, which the authors suspected was due to ovarian toxicity. Finally, nondioxin-like compounds, PCB 153 and 1,3,6,8-TCDD had no impact of experimental disease, supporting the hypothesis that TCDD and dioxin-like PCBs primarily promote endometriosis *via* AhR binding [94].

Although human and murine responses to these toxicants are not identical, our group demonstrated that an acute exposure of human endometrial tissue to TCDD subsequently promoted ectopic lesion development in our nude mouse model of experimental endometriosis [95]. As will be discussed later, direct, *in vitro* toxicant exposure models using adult human reproductive tract cells and tissues can be useful to identify cellular pathways disrupted by EDC exposures in addition to rodent models.

Nevertheless, attempting to link the presence or absence of endometriosis to acute or chronic EDC exposures during adulthood misses the potential role of early life toxicant exposures to the risk of adult onset diseases, including endometriosis [59, 74]. Compared to adult exposure, toxic effects of EDCs are greater when exposures occur earlier in life, before the onset of puberty while exposures occurring during the neonatal period are recognized as being the most damaging [96].

#### *2.3.2. Developmental Exposure Models*

As stated above, the negative effects of EDCs may be more severe when exposures occur during early life, either in utero or prior to puberty. Therefore, it is critical that appropriate developmental exposure models be employed in order to fully understand the impact of TCDD or other toxicants on adult development of endometriosis and related co-morbidities. For example, the Birnbaum laboratory exposed both pregnant rats and mice to TCDD on embryonic day 8 (E8) and then surgically induced endometriosis in adult offspring followed by a second exposure to TCDD. This study confirmed their previous studies demonstrating murine models of experimental endometriosis exhibit a higher level of sensitivity to TCDD compared to rats. More importantly, this study revealed that a prior developmental exposure to TCDD enhanced the effect of a *secondary adult exposure* to this same toxicant [55].

In our model system of developmental toxicant exposure, pregnant C57BL/6 mice are exposed to a single TCDD dose (10μg/kg) by gavage on E15.5, when organogenesis is complete. In contrast to the Birnbaum group, we initially did not attempt to induce experimental endometriosis, rather our goal was to examine the uterine phenotype of the adult offspring following early life toxicant exposure. We found that adult female offspring (F1 mice) of mice exposed to TCDD during development exhibit reduced uterine progesterone responsiveness, a phenotype associated with endometriosis in women [97]. These mice, like women with endometriosis, exhibit subfertility and an increased risk of delivering preterm [54, 98]. Thus, these animals exhibiting an "endometriosis-like" uterine phenotype have been very valuable in examining co-morbidities associated with this disease, which will be discussed below. More recently, we have used these animals to examine development of experimental endometriosis using the syngeneic mouse model described previously.

In addition to the dioxin-like EDCs, other toxicants have been shown to affect the development of endometriosis. For example, Signorile and colleagues exposed balbc mice to the EDC bisphenol A (BPA) during pre- and perinatal development [57]. Specifically, animals were treated with a high or low dose of BPA daily throughout pregnancy and until PND7. At 3 months of age, mice were euthanized and the peritoneal cavity and selected tissues examined, revealing endometriosis-like structures within the abdominal fat of a subset of BPA exposed mice. Significantly, histological examination revealed these lesions contained both glands and stroma. Since mice do not menstruate, these findings provide preliminary evidence, and a potential model system, to investigate activation of embryonic cell rests or stem cells by EDCs as biologically feasible pathway leading to the development of endometriosis.

## 3. THE MECHANISMS

In large part, current research efforts in the field of endometriosis are focused on the development of new and better clinical therapies for women with this condition. However, designing more effective therapeutic strategies requires

that we develop a deeper understanding of the trigger mechanisms driving the development and progression of endometriosis. Detailed below are examples of mechanistic studies, utilizing the models described above, which attempt to identify the pathogenic mechanisms associated with development of endometriosis.

### 3.1. Early Lesion Establishment and Vascularization

Successful establishment of endometriosis is a multi-step process requiring the rapid occurrence of peritoneal attachment, extracellular matrix degradation and neovascularization. In order to examine early events associated with ectopic attachment of endometrial tissues, our laboratory utilized the chimeric human/mouse model system. Using this model, we explored the potential role of matrix metalloproteinases (MMPs) in the invasive establishment of experimental endometriosis in nude mice [36, 99]. The MMPs are highly regulated enzymes that are necessary for normal and pathologic tissue remodeling, including the cellular migration and matrix restructuring associated with wound-healing or tumor metastasis [100]. Our studies demonstrated that blocking endometrial MMP expression or action prevented the establishment of human endometrial growth at ectopic sites within the peritoneal cavity of nude mice [36].

Following the physical attachment of endometrial tissues to ectopic sites, survival of lesions requires the rapid establishment of a vascular supply and, in a collaborative study, we demonstrated that blocking angiogenesis is effective in preventing lesion development [101]. In a follow-up study focused on very early disease, we demonstrated that vascular development is apparent by 24 hrs with extensive vascularization occurring about 5 days post-injection [102]. Importantly, we noted that peritoneal attachment and acquisition of a vascular supply occurs more rapidly following experimental disease establishment with eutopic endometrial tissues acquired from women with endometriosis, suggesting that the endometrial phenotype associated with endometriosis patients exhibits a greater innate capacity to stimulate peritoneal blood vessel growth in our model [79]. Furthermore, acute exposure of disease-free control endometrial tissue to TCDD, prior to injection into the peritoneal cavity of recipient mice, leads to a similar host vascular response within the peritoneal cavity as observed with tissue acquired from endometriosis patients [79]. Thus, in addition to stimulating MMP expression in endometrial fragments [103], TCDD exposure also promotes a vascular response at the site of endometrial invasion into the murine peritoneal wall, suggesting that this toxicant may promote lesion development *via* multiple mechanisms.

In order to specifically examine the individual cellular responses within the human endometrium to TCDD, we have begun to utilize organ-on-chip models [104]. These microscaled models enable compartmentalized, heterogeneous cell culture systems that better recapitulate *in vivo* anatomy and allow the direct evaluation of paracrine and endocrine crosstalk among cell types. Using such a system, in conjunction with the *in vivo* models described herein, should provide detailed information with regard to the responses of individual cells to TCDD as well as the whole tissue response within the peritoneal cavity.

Although the phenotype of endometrial tissue fragments significantly contributes to disease pathogenesis, recent studies provide evidence that the peritoneal phenotype also plays a critical role in endometriosis. Moreover, recent studies suggest that the eutopic endometrial phenotype works in concert with peritoneal inflammation, synergistically promoting ectopic endometrial growth. For example, in response to retrograde menstruation, there is a large influx of immune cells into the peritoneal cavity, which in healthy women would clear the dead and dying menstrual debris. However, in women with endometriosis, both heavier menstrual bleeding and immune cell dysfunction may reduce the capacity of the innate immune system to scavenge refluxed tissues. Equally important, inflammatory mediators produced at inappropriate levels likely impact the invasive and neoangiogenic processes that are necessary for establishment of endometriosis [68, 105]. Menstrual debris may also stimulate pro-inflammatory cytokine production as a consequence of inflammasome activation.

The inflammasome is a family of multiprotein complexes that are expressed by macrophages and other immune cells. These components act as immune system receptors and sensors and regulate the inflammatory response associated with both infectious microbes and damaged-associated host proteins. To date, four inflammasome complexes have been identified; each having a distinct protein composition which are formed in a stimulus-dependent manner (reviewed [106]). Among these, the NLRP3 (NLR Family Pyrin Domain Containing 3) complex is the most well-studied and is present in a variety of normal and disease states [107]. All of the inflammasome complexes cleave pro-IL-1β, leading to its activation and promoting an inflammatory cascade. Activation of this system in the context of endometriosis may inadvertently promote tissue repair rather than tissue clearing [108]. This acute inflammatory response system can also activate the local vasculature, further promoting lesion survival. To this end, using the nude mouse model of experimental endometriosis, we found that vessels present within lesions revealed anastomosis had occurred between human and murine endothelial cells [101], suggesting that the human tissue produces chemoattractants that promote growth of the murine vasculature. Supporting these findings, chimeric human/murine vessels were also identified by Alvarez-Gonzalez *et al*. in a SCID mouse model of human experimental endometriosis in [109]. This latter study confirmed that the chimeric vessels were functional, with a circulating blood supply established by 3 weeks. Clearly, development of a vascular supply is essential for ectopic endometrial survival. Therefore, a large number of studies have utilized murine models to examine the potential therapeutic value of angiogenesis inhibitors [101, 110-117]. Collectively, these studies indicate that both natural and pharmaceutical agents that inhibit angiogenesis can impede maintenance and growth of experimental endometriosis and support investigation of angiostatic agents as potential therapeutic agents for women with this disease.

### 3.2. Steroid Action in Endometriosis

Normal endometrial function is largely dependent on the sequential action of the ovarian steroids estrogen and progesterone [118]. Following menstruation, rising levels of estra-

diol act to induce endometrial regrowth and estrogen levels remain high even after ovulation when progesterone levels increase to induce endometrial maturation. Progesterone not only acts on the endometrium to promote differentiation in preparation for pregnancy, but is also a potent anti-inflammatory steroid. If nidation does not occur, progesterone levels will begin to decline, resulting in a loss of endometrial steroid support and culminating in the acute inflammatory process of menstruation. Discussing the multiple cellular processes within the endometrium that are controlled by ovarian steroids is beyond the scope of this review examining experimental models of endometriosis. Nevertheless, researchers interested in the pathogenesis of endometriosis have long recognized the critical roles of the sex steroids in endometrial function whether within the uterus or at ectopic sites of growth. Exposure to estrogen is one of the principal endocrine risk factors for developing endometriosis [19], largely as result of this steroid's ability to promote rapid endometrial proliferation.

In contrast to estrogen action, progesterone exposure (ie, pregnancy or combined oral contraceptives) acts to reduce proliferation and induce cellular maturation, thus serving as a negative risk factor for development of endometriosis [19, 119]. However, numerous studies have demonstrated that women with endometriosis exhibit altered expression of a variety of genes and proteins that are normally regulated by the sex steroids [120, 121] revealing potential therapeutic targets [122, 123]. Importantly, both steroids act *via* their respective receptors, primarily progesterone receptor-B (PR-B) and estrogen receptor-α (ESR1). Additionally, PR-A, a truncated isoform of PR-B, can act as a dominant repressor of PR-B [124, 125] and has been found to be overexpressed in endometriosis [103, 126, 127]. Similarly, estrogen receptor-β (ESR2) is overexpressed in women with endometriosis and appears to play a role in disease development [119, 128, 129].

Estrogen's ability to promote endometrial proliferation in both eutopic [118] and ectopic [19] sites of growth has made this steroid an important target of investigation. For example, using a syngeneic mouse model of endometriosis, [51] the Korach laboratory demonstrated estradiol-mediated signaling was required in the development of endometriosis-like lesions. Specifically, this group utilized estrogen receptor knockout (αERKO and βERKO) mice to explore the influence of expression or absence of each receptor in the ectopic lesions as well as within the recipient animal. Their studies demonstrated that, compared to wild-type donors, the number of successful lesions was dramatically reduced in mice receiving minced uterine tissue from either αERKO or βERKO mice, which they surmise was related to an inability of αERKO uteri to acquire a vasculature in response to estradiol. Interestingly, the ER status of the recipient animal had little impact on lesion establishment.

Unlike estrogen, progesterone exposure (ie, pregnancy) is known to reduce the risk of endometriosis. For this reason, experimental models of endometriosis have been useful for preclinical studies examining the potential benefit of various progestins. A potential confounding issue with progestin-based therapy for endometriosis is that many patients with this disease exhibit sensitivity to progesterone [97, 130]. As noted earlier, our studies more than a decade ago demonstrated that the reduced response to progesterone action in endometrial tissue acquired from endometriosis patients is associated with increased expression of MMPs and an enhanced capacity to establish experimental endometriosis [130]. A later study revealed that although endometrial tissues from women with endometriosis established as experimental disease in nude mice were resistant to regression by progesterone, treatment with tanaproget, a selective PR-B agonist, effectively reduced disease burden [131]. Additionally, experimental endometriosis using transgenic mice in which one of the major steroid receptors has been deleted further underscores the important and complex relationship between estrogen and progesterone action and endometriosis. Using a syngeneic endometriosis model with wild-type and progesterone receptor knockout (PRKO) mice, Bulun and colleagues demonstrated that the presence of PR in ectopic uterine tissue was essential to prevent estrogen-mediated proliferation and survival of lesions. The authors concluded that resistance to progesterone of human endometriosis may be a consequence of reduced PR expression or function [53]. Taken together, these data suggest that normalizing progesterone action within the endometrium would effectively reduce a woman's risk for developing endometriosis.

### 3.3. Inflammatory Response in Women with and Without Endometriosis

The presence of menstrual debris within the peritoneal cavity is generally accepted as a *mechanical* factor in a woman's risk for the development of endometriosis. Nevertheless, an important, but, unanswered question is why only certain women develop this disease despite retrograde menstruation being a common phenomenon among reproductive age women [9] One explanation may be the intrinsic differences in progesterone responses noted between endometrial tissues of patients compared to women without endometriosis. However, as reviewed by Brosens *et al*. [132], alterations within the eutopic endometrium of women with endometriosis are not limited to reduced progesterone responsiveness. Indeed, alterations in expression of angiogenic factors, identification of nerve fibers and expression of inflammatory cytokines have been demonstrated to be altered in patients compared to disease-free women. Clearly, these differences likely promote either the development of endometriosis or play a role in co-morbidities associated with this disease.

In our laboratory, we identified elevated expression of IL-1α and IL-1β in endometrial tissues acquired from women with endometriosis which was associated with an enhanced ability to establish experimental disease in our chimeric nude mouse model [129]. Indeed, an important advantage of immunocompromised mice is the ability to compare endometrial tissues from women with and without endometriosis in an *in vivo* system. Our published data using this model, in which human endometrial fragments are introduced into the peritoneal cavity of immunocompromised mice, demonstrated an invasive advantage of eutopic endometrium obtained from women with endometriosis [37, 79]. Specifically, although endometrial tissues from women with and without endometriosis are equally capable of establish-

ing ectopic disease, tissues from women with endometriosis establish larger lesions that are more quickly able to establish a vasculature [102]. Significantly, we found that peripheral immune cells obtained from control women limited the growth of experimental endometriosis [46]; however, in contrast, a similar immune cell preparation obtained from women with endometriosis enhanced the development of experimental disease (Fig. **3** and Table **2**). These data suggest an important phenotypic difference *in immune cells*, in addition to endometrial tissue, obtained from women with and without endometriosis.

The hyperinflammatory peritoneal environment observed in association with endometriosis in women has prompted a number of studies in rodents examining the efficacy of anti-inflammatory agents. Many of these studies, targeting specific inflammatory cytokines, were recently reviewed by Nothnick and Alali [105] and will not be detailed here. However, in contrast to targeting a specific inflammatory cytokine, a number of recent studies have explored the utility of dietary modulators of inflammation for the treatment of endometriosis. For example, using the rat surgical model of endometriosis, Akyol *et al*. [133] demonstrated that omega-3 fatty acids (found in fish oil) reduced experimental disease burden in association with a reduction in peritoneal levels of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and vascular endothelial growth factor (VEGF). Using a similar approach, İlhan *et al*. [134] treated rats with surgically induced endometriosis with a mixture of sea buckthorn and St. John's wort oils. These compounds have been used for many years in folk medicine for the treatment of a wide array of inflammatory disorders [135-138], but had not previously been examined with regard to endometriosis. In their study, İlhan *et al*. (2016) found that following 4 weeks of treatment with the oil mixture, rats in the treated group exhibited significantly fewer endometriotic lesions and adhesions compared to vehicle treated rats. Reduction in disease burden was associated with decreased peritoneal levels of IL-6, VEGF and TNF-α. Given the side effects of traditional pharmacologic approaches for the treatment of endometriosis, these studies support the exploration of anti-inflammatory diets for women with this disease.

## 4. DRIVERS OF CO-MORBIDITIES ASSOCIATED WITH ENDOMETRIOSIS

As stated above, women with endometriosis frequently exhibit serious co-morbidities, but the drivers of these disease associations remain unclear. However, experimental rodent models of endometriosis have begun to reveal that inflammation may be contributing to epigenetic changes that play an important role in understanding the relationship between endometriosis and other diseases/conditions.

### 4.1. Epigenetics

Epigenetic marking of DNA, unlike genetic mutations, alters gene expression without changing the DNA sequence. Epigenetic modification is one mechanism by which DNA accessibility is controlled, altering the ability of the cellular machinery to activate transcription. The most well-studied

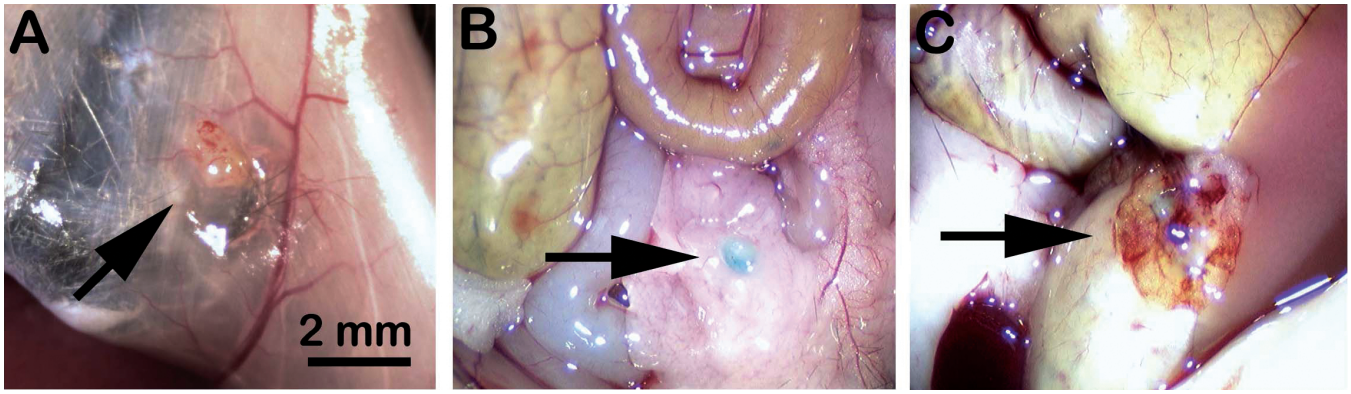


**Fig. (3). Experimental human endometriosis in rag2γ(c) mice.** Proliferative phase human endometrial tissue growing as ectopic, intraperitoneal lesions in rag2γ(c) mice that were ovariectomized and implanted with slow release estradiol capsules (A-C). Mice received no immune cells (A), immune cells from a disease-free donor (B) or cells from an endometriosis patient (C). Prior to injection, immune cells were incubated with CarboxyFluoroscein Succinimidyl Ester (CSFE), resulting a blue-green appearance. Representative results of multiple (N=3) experiments using different human tissues are shown.

**Table 2. Impact of immune cell phenotype on experimental endometriosis.**

| Mouse Treatment | Tissue Type/Cell Type | Percent w/ Disease | Avg Total Vol Lesions/Animal |
|---|---|---|---|
| **E** | Normal/None | 100% | 2.9 |
| **E** | Normal/Normal | 44% | 0.3 |
| **E** | Normal/Endometriosis | 100% | 3.3 |

epigenetic mechanisms are methylation and acetylation of DNA and histones [139]. Importantly, although these marks can be reversible, they are stable and, when occurring within the germline, these changes can be inherited [140-142]. During development, epigenetic reprogramming of individual cells results in cellular differentiation specific for each organ system, despite each cell having the same DNA. Thus, it is the epigenetic marks that ultimately determine each cell's fate and allows the formation of a complex, multi-organ animal from the same genetic blueprint. Additionally, epigenetic modifications accumulate as we age, largely in response to our own choices (diet, activity level) or where we live (environmental exposures). These accumulated epigenetic marks are primarily why we become susceptible to age-related diseases. Additionally, environmental factors that affect epigenetic modification of the germ cells have the potential to contribute to an *offspring's* phenotype [141]. Significantly, the presence of aberrant epigenetic patterns can cause developmental abnormalities and are associated with the etiology of certain human diseases [143]. As will be discussed below, recent research suggests that epigenetic changes, perhaps driven by inflammation related to environmental toxicant exposure, may promote the development of endometriosis and/or associated comorbidities.

### 4.2. Infertility

Infertility impacts up to 40% of women with endometriosis [19], but whether infertility is a function of inflammation that is associated with the disease or a consequence of intrinsic endometrial defects or both remains unclear. In an effort to address this question, Stilley *et al*. [58] used the rat surgical model of endometriosis to examine the impact of ectopic disease on fertility. Perhaps not surprisingly, fertility in animals with surgically induced endometriosis was significantly compromised, while fertility was not compromised in sham operated rats (hemi-hysterectomy, but no ectopic lesions). Furthermore, this study identified a number of ovarian alterations (fewer ovarian follicles and corpora lutea with luteinized unruptured follicles), poor preimplantation embryo development and spontaneous abortion in rats bearing endometriosis-like lesions compared the sham rats. Finally, this study also identified the same reproductive abnormalities in the *daughters* of rats with endometriosis-like lesions, suggesting that exposure to the inflammatory environment of this disease during in utero development may lead to permanent epigenetic changes in the offspring.

Using our developmental toxicant exposure model [54, 98], we initially reported that exposure of pregnant C57bl/6 mice to TCDD led to adult daughters (F1 females) with a uterine phenotype similar to that of women with endometriosis [98]. More specifically, we found that F1 females exhibit reduced uterine PR expression and loss of progesterone-sensitive TGF-β2 (transforming growth factor β) expression, proteins essential for establishment and maintenance of pregnancy. Thus, we were not surprised to find that subfertility was also common among F1 females mated to control breeder males. Approximately 50% of female F1 mice failed to exhibit signs of pregnancy (weight gain/nipple prominence) despite multiple matings and observation of vaginal plugs (4+). In addition to subfertility, F1 females which achieved pregnancy also exhibited a high rate of spontaneous preterm birth [54], a pregnancy outcome that has recently been linked to endometriosis [132]. Importantly, F2-F4 female mice continued to exhibit an endometrial phenotype similar to women with endometriosis, even though these animals were not subjected to additional toxicant exposure. Specifically, although the animals in our study did not have experimental endometriosis, we identified both multigenerational (F1-F2) and transgenerational (F3-F4) occurrence of reproductive disorders that are similar to those encountered by endometriosis patients [54].

Finally, we have also examined whether the male offspring of mice exposed to TCDD during pregnancy could transfer the endometriosis phenotype to his female progeny. Similar to our findings in F1 females, F1 males also exhibit a hyper-inflammatory phenotype and reduced fertility [144]. Importantly, the *daughters* of F1 males (F2P females) exhibited the same reproductive abnormalities identified in F1 females and their offspring, including reduced PR expression, subfertility and increased risk of spontaneous PTB (Table **3**). Our animal studies, taken with the studies by other groups described above, strongly support a developmental origin of endometriosis. Equally important, our findings suggest that the environmental exposure history of either parent can lead to a transgenerational risk for the development of endometriosis. Understanding the potential role of toxicant-mediated inflammation may provide important insight enabling targeted therapies that reduce disease risk.

### 4.3. Adenomyosis

Adenomyosis, the presence of endometrial glands and stroma embedded within the uterine muscle, is frequently identified in women undergoing hysterectomy as a surgical treatment for endometriosis [145]. Adenomyosis, like endometriosis, has been associated with reduced fertility, pelvic pain, heavy menstrual bleeding and dysmenorrhea [22, 146]. The causes of adenomyosis are currently unknown, although both human and animal studies have suggested a role of inflammatory processes in the development of this disease [59, 147-149]. Furthermore, the occurrence of adenomyosis as a co-morbidity in women with endometriosis, fibroids and menorrhagia, supports a potential role of estrogen action and inflammation in the pathogenesis of this disease as well.

**Table 3. Impact of paternal TCDD exposure on pregnancy outcomes in his adult daughter (F2P).**

| Mouse History | Pregnancy | Pregnancy *Full-Term* | Outcome *Preterm* |
|---|---|---|---|
| **Control** | 15/15 (100%) | 100% | 0% |
| **F2P Female** | 7/19 (37%) | 71% | 29% |

In our TCDD exposure model, we recently reported the transgenerational occurrence of adenomyosis in mice exhibiting the endometriosis-like uterine phenotype as a consequence of developmental toxicant exposure of F1 animals [147]. Within this recent study, we conducted a retrospective analysis of uteri from TCDD exposed F1 female mice and two generations of their offspring to determine whether histological evidence of adenomyosis was present. Although none of the control mice examined exhibited adenomyosis, we identified deep, adenomyotic lesions in the majority of mice with a history of direct (F1-F2) or indirect (F3) TCDD exposure. Since we have demonstrated that F1-F4 mice exhibit a "hyperinflammatory" systemic phenotype, the occurrence of adenomyosis in these animals provides additional support for inflammatory mechanisms in the promotion of this disease.

As stated above, in addition to inflammation, several studies have reported the promotion of adenomyosis by estrogen. For example, Koike *et al*. [150] exposed pregnant mice (ICR/Jcl, CLEA) to 0.01 mg ethinyl estradiol (EE2 )/kg per day or vehicle (olive oil) by gavage from day 11 to 17 of gestation. Adult female offspring (F1 mice) were either treated with the same dose EE2 or to vehicle twice a week until 20 weeks of age. The control female offspring were also exposed to either vehicle of 0.01 mg EE2. All mice were euthanized at 7 months. Adenomyosis was common in in the EE2 -exposed uteri, and incidence of ectopic glands and serous cysts were significantly increased in the prenatally EE2 -exposed ovaries as compared with respective controls. This study suggests that continuous EE2 exposure can promote the development of adenomyosis. Using a very different model, Otto *et al*., [151] also demonstrated an important role of estrogen in the development of adenomyosis. This study examined the influence of the estrogen modulating compounds Faslodex and cetrorelix in SHN mice. SHN mice have been found to develop adenomyosis after pituitary grafting, which leads to an altered endocrine profile. The GnRH antagonist cetrorelix and the estrogen receptor antagonist Faslodex, which negatively interfered with estrogen-mediated signaling, completely inhibited development of adenoymosis, whereas danazol, was slightly less effective in inhibiting disease in SHN mice [151].

### 4.4. Adhesive Disease

Abdominal adhesions are fibrous bands of scar tissue that fuse two or more abdominal organs to each other and/or the peritoneum. Although adhesions most commonly occur as a complication of abdominal surgery; de novo formation may also occur in the absence of surgery as a consequence of inflammatory conditions within the abdomen [152]. For this reason, it is perhaps not surprising that compared to the general population; women with endometriosis are at a higher risk of both surgery-associated adhesions and spontaneous development of adhesive disease [153]. The basic pathophysiology of postsurgical adhesion development is known to involve inflammatory processes that occur during normal wound healing. Certainly, macrophages and neutrophils play key roles in the initiation of inflammation related to wound healing by releasing both proinflammatory cytokines and proangiogenic factors. Women with endometriosis are known to exhibit dysregulated immune cell function, which likely contributes to an enhanced inflammatory response and increased risk of developing adhesive disease [154]. In our chimeric nude mouse model, we examined the development of adhesive disease in association with experimental endometriosis [154, 155]. As reported in our published studies, we noted a cooperative effect of the presence of endometrial tissue fragments on the development of post-surgical adhesions when tissue fragments were introduced into the peritoneum within 16 hours of ovariectomy. Sham surgery (removal of the fat pad surrounding ovary, but not the ovary) or saline injection in the absence of human tissue *was not* associated with increased adhesive disease [154, 155]. Thus, within the peritoneal cavity, immune cell responses to injury, similar to infection or radiation therapy, may promote formation of adhesions [156]. Interestingly, we found that adhesions did not form when an identical experiment was conducted in rag2γ(c) mice (Table **4**), animals which are more severely immunocompromised compared to nude mice. Nude mice exhibit loss of T cell function, but other immune cells are functional. In contrast, rag2γ(c) mice lack functional receptors for multiple interleukins, including IL-2, IL-4, IL-7, IL-9, and IL-15. As a consequence, B and T cell maturation are both compromised. Further, rag2γ(c) mice lack functional NK cells. An absence of adhesions in these animals suggests an additional role of one or more of these cytokines and/or immune cell types in post-surgical adhesion development. Interestingly, enhanced expression of IL-4 and IL-15 has been observed in patients with endometriosis [157, 158], raising the possibility that these cytokines might contribute to *de novo* adhesions in these women.

In studies using our developmental TCDD exposure model, we made the incidental observation that adhesions were rare in control mice at necropsy, but commonly observed F1-F3 mice. Therefore, in order to prospectively determine whether developmental TCDD exposure promotes the development of endometriosis-associated adhesive disease, we established a syngeneic endometriosis model in control and F1 mice within 16 hours of a surgical ovariectomy similar to the approach used with the chimeric adhesion model described above [154, 155]. These studies revealed that 100% of F1 mice developed adhesive disease following ovariectomy and injection of uterine fragments, regardless of the origin of the tissue (control or F1 uterus). In

**Table 4. Influence of mouse genotype on endometriosis-associated adhesions.**

| Mouse Genotype | N | Percent w/ Lesions | Avg Total Vol Lesions/Animal | Percent w/Adhesions | Adhesion Score |
|---|---|---|---|---|---|
| **Nude** | 16 | 91% | 2.5 | 81% | 3.2 |
| **Rag2γ(c)** | 12 | 100% | 2.7 | 0 | -- |

contrast, only 30% of control mice subjected to ovariectomy and uterine tissue injection (control or F1 tissue) developed adhesions [59]. These data further suggest that alterations in inflammatory responses within the peritoneal environment may be the most important driving force in the development of post-surgical adhesive disease.

### 4.5. Pain

Endometriosis-associated pain is a significant contributor to morbidity; however, studies correlating pain to extent or location of disease has not yielded advances in treatment (reviewed by [159]). Significantly, recent studies have illustrated that women with endometriosis exhibit "central sensitization", a phenomenon by which pain itself promotes sensitivity to subsequent painful stimuli. Over time, a patient develops significant sensitivity to any stimuli, feeling more pain with less stimulation. Ultimately, even stimuli which should not be painful (*i.e*. a light touch) can trigger pain as a consequence of central sensitization [160, 161]. Central sensitization is particularly refractory to treatment; thus, animal models which can advance our understanding of this condition are desperately needed.

Using the rat surgical model described above, Berkley and colleagues have demonstrated that ectopic endometrial tissues become innervated and lead to vaginal hyperalgesia [48]. Significantly, inflammation appears to be a key player in the development of innervated lesions [160]; thus several groups are actively using various rodent models to test whether blocking the actions of the proinflammatory agent prostaglandin E2 can reduce experimental disease as well as associated pain [34, 162]. For example, using the chimeric rag2γ(c) mouse model, Arosh *et al*. [162] demonstrated that selective inhibition of EP2/EP4 not only inhibited survival of ectopic lesions, but also inhibited innervation of lesions and suppressed inflammation of dorsal root ganglia neurons. Significantly, following treatment with the EP2/EP4 inhibitors, animals in this study exhibited a reduction in pelvic pain, as assessed by stimulating the pelvic floor with von-Frey filaments (a standard nociception assay [163]).

## CONCLUSION

Despite a century of research, the pathogenesis of endometriosis remains poorly understood. Nevertheless, the use of a variety of rodent models of experimental endometriosis has enabled significant advancement in our understanding of disease processes necessary for development of this disease. Although these models have limitations and cannot completely recapitulate the human disease process, studies described herein and in other recent reviews [33, 34, 164, 165] demonstrate the significant contribution of rodent models in providing a broader understanding of the complex and interactive roles of the endometrial phenotype, the peritoneal microenvironment, host angiogenic responses and the competency of the immune system which collectively determine an individual's risk of developing endometriosis. Examination of inflammatory events within the peritoneal cavity in concert with identifying genetic and epigenetic alterations associated with endometriosis is expected to further enhance our understanding of why only certain women develop this condition. Clearly, developing new and more effective therapies will require a more complete understanding of the mechanisms which promote this disease. Furthermore, identifying specific factors (phenotypic, genetic and/or epigenetic) which predispose an at risk individual to the development of endometriosis is critical to ultimately preventing the development of this disease as well as its many comorbidities.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

None of the authors have any conflicts of interests pertaining to the content of this manuscript.

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