

The non-human primate model of endometriosis: research and implications for fecundity

A.G. Braundmeier and A.T. Fazleabas¹

Department of Obstetrics and Gynecology (MC808), College of Medicine, University of Illinois at Chicago, 820 South Wood Street, Chicago, IL 60612, USA

¹Correspondence address. E-mail: asgj@uic.edu

ABSTRACT: The development of an animal model of endometriosis is crucial for the investigation of disease pathogenesis and therapeutic intervention. These models will enhance our ability to evaluate the causes for the subfertility associated with disease and provide a first-line validation of treatment modulators. Currently rodents and non-human primate models have been developed, but each model has their limitations. The aim of this manuscript is to summarize the current findings and theories on the development of endometriosis and disease progression and the effectiveness of therapeutic targets using the experimental induced model of endometriosis in the baboon (*Papio anubis*).

Key words: animal model / endometriosis / endometrium / primate / uterine / pathologies

Introduction

Endometriosis is a gynecological condition that affects 1 out of every 10 women of reproductive age (Eskenazi and Warner, 1997). This condition is traditionally defined as the development of endometrial glands and stroma outside of the uterine cavity, but this definition has broadened to include the development of any endometrial cell type (glands or stroma) outside of the uterine cavity (Clement, 2007). Laparoscopic evaluation followed by histological confirmation is the gold standard for diagnosis of endometriosis, but the accuracy of the diagnosis is highly dependent upon surgical and pathological expertise.

Currently there are three theories that describe the etiology of endometriosis. The first is the embryonic rest theory. This theory proposes that at puberty there is activation of cells of Mullerian duct origin at various sites in the pelvic cavity (Batt and Smith, 1989; Batt *et al.*, 1990). This theory supports the finding of rare cases in which endometriosis was found in males (Witz, 1999). The second theory is that of coelomic metaplasia. This theory states that substances in menstrual fluid can induce peritoneal tissues to form endometrial cells, suggesting that there is a factor found in menstrual fluid that is a precursor for the disease (Meyer, 1919). The third theory, and the most widely accepted, is the Sampson hypothesis of retrograde menstruation (Sampson, 1927). This theory states that endometrial fragments are displaced into the peritoneal cavity through a process known as retrograde menstruation. Retrograde menstruation occurs in the majority (70–90%) (Blumenkrantz *et al.*, 1981; Eskenazi and Warner, 1997) of women, but only a small percentage (10%) of

these women develop endometriosis, which suggests that the peritoneal environment or the endometrium of women with endometriosis may be altered compared with that of healthy women (Sampson, 1927).

Clinically, endometriosis presents itself with symptoms such as chronic pelvic pain, dysmenorrhea, dyspareunia and subfertility (Verkauf, 1987). The initiation of endometriosis is difficult to evaluate because at the onset of clinical symptoms many women already have significant established diseases (Eskenazi and Warner, 1997). Treatment therapies for endometriosis involve both pharmacological and surgical intervention either individually or combined (Valle, 2002). Medical treatments available for endometriosis include oral contraceptives, GnRH analogs, progesterone analogs and aromatase inhibitors. Surgically, endometriosis has been treated by the excision or ablation of endometriotic lesions and the removal of adhesions in the peritoneal cavity. Several reports have debated the effectiveness of either medical or surgical therapy for the treatment of infertility and chronic pain (Kettel and Murphy, 1989; Barbieri, 1992; Shaw, 1992; Howard, 1993; Hughes *et al.*, 1993; Vercellini *et al.*, 1997; Moghissi, 1999; Harrison and Barry-Kinsella, 2000; Lessey, 2000; Wilson *et al.*, 2000; Chwalisz *et al.*, 2002; Abbott *et al.*, 2003; D'Hooghe, 2003; Donnez *et al.*, 2003; Garry, 2004). Currently, there is no universally acceptable, standard treatment protocol for endometriosis, hence the treatment is individualized for each patient with mixed outcomes.

The identification of molecules involved with the pathogenesis of endometriosis or strategic therapies for treatment is difficult in a

spontaneous model of endometriosis because of the many unknown and uncontrollable factors. Furthermore, in women, time from the onset of disease to diagnosis is between 8–11 years (Sinaii *et al.*, 2002). The establishment of an animal model with experimentally inducible endometriosis would allow investigators to characterize factors involved at the early onset of disease and throughout disease progression allowing for the evaluation of molecular changes that cause infertility and chronic pelvic pain. The experimentally induced model also allows for the investigation of the efficacy of therapeutic intervention strategies.

Animal models of endometriosis

Immunocompromised rodents have been used for the development of an animal model of endometriosis by surgically implanting human endometrial tissue into the peritoneal wall (Zamah *et al.*, 1984; Bruner *et al.*, 1997; Awwad *et al.*, 1999). Another version of the rodent model transplants endometrial tissue from genetically similar mice to induce disease (Rossi *et al.*, 2000). Although the use of rodents is a cost-effective animal model that does allow for the generation of specific knock-outs, the disadvantages are numerous. Ectopic lesions are very small and are not physiologically similar to those found in advanced stages of human disease. Also the use of immunocompromised rodents eliminates the investigation of any immunoregulatory component involved in the pathogenesis of disease. Additionally, these animals do not develop spontaneous disease since they are a non-menstruating species. In contrast though, the rodent model would be cost-effective for preliminary efficacy trials of medical therapies.

Several non-human primates (NHP) have been used for the development of experimental endometriosis: Japanese macaque, pigtailed macaque, rhesus monkeys and baboons (Story and Kennedy, 2004). The use of NHP is advantageous for the study of endometriosis because it provides a phylogenetically similar animal model to the human, and this use of NHP allows for the evaluation of disease pathogenesis as well as therapeutic targets. However, the use of NHP is very costly and supply is very limited. Of all the NHP models developed, the baboon (*Papio anubis*) is the most favorable because of its size and similar reproductive anatomy. Unlike rodents and other primate models of disease, baboons have a menstrual cycle similar to humans in both duration and endometrial remodeling (Hendrickx, 1971). Baboons also have similar changes in the eutopic endometrium during the window of uterine receptivity and a similar type of placentation as that seen in humans (Hendrickx, 1971). In addition, baboons also develop spontaneous endometriosis in which ectopic lesions resemble those of humans (Merrill, 1968; Folse and Stout, 1978). Endometriosis can also be induced in baboons by injecting autologous menstrual effluent into the pelvic cavity (D'Hooghe, 1997; Fazleabas *et al.*, 2002). The intraperitoneal injection of menstrual tissue mimics the normal physiological process of retrograde menstruation and allows investigators to study disease progression from the initial onset of disease. In baboons, we are able to perform evaluative laparoscopies and collect biological samples prior to the induction of disease and then compare these control samples to post-inoculation samples of the same animal (Fazleabas *et al.*, 2002). The employment of each animal to serve as its own control reduces inter-animal variability in experimental designs and reduces

the animal numbers required per study. The body size of baboons allows for multiple and complex surgical procedures and repeated collection of measurable biological samples such as blood, tissue and peritoneal fluid. Because laparoscopic evaluation can also cause inflammation of the peritoneal cavity (D'Hooghe *et al.*, 1999), it is important that animals induced with disease be compared with healthy control animals which have also undergone the same number of laparoscopic procedures at the same intervals for each study.

Therefore, the baboon provides an excellent model to study the pathogenesis of endometriosis, the identification of molecules in multiple physiological systems that are affected by the presence of ectopic peritoneal lesions and the effectiveness of novel therapeutic strategies. This review will summarize the current findings and ongoing studies, utilizing the experimentally induced baboon model of endometriosis.

Pathogenesis of ectopic endometrium

Although endometriosis was first described nearly a century ago, the understanding of the pathogenesis of endometriosis remains unclear. The key to understanding the pathogenesis of endometriosis is to evaluate molecular changes associated with the peritoneal lining and the sites of ectopic lesion invasion during the initial stages of development. These studies are impossible to perform in humans because disease is always established prior to clinical symptoms. Using an induced model of disease, we are able to test Sampson's theory of endometriosis by investigating the early molecular events that are necessary for the establishment of ectopic endometrial lesions. The initial step for the development of ectopic lesions is the attachment of menstrual endometrium to the peritoneal lining followed by the invasion of the menstrual tissue through the peritoneal surface and finally the establishment of a blood supply.

Attachment

The process of retrograde menstruation has been shown to increase several inflammatory mediators in the peritoneal fluid of baboons. It has been reported that in peritoneal fluid of baboons, peritoneal cells expressed higher levels of tumor necrosis factor (TNF- α), transforming growth factor (TGF- β_1) and intracellular adhesion molecule ICAM-1, and the peritoneal fluid contained higher levels of TGF- β_1 and interleukin (IL-6) (D'Hooghe *et al.*, 2001). These inflammatory mediators have the capacity to enhance the binding of menstrual tissue to the peritoneal lining of abdominal structures.

In vitro studies have challenged the previous concept that ectopic lesions invade at locations only where the peritoneal surface has been damaged (Koks *et al.*, 1999). Multiple studies have shown that human menstrual tissue fragments cannot only attach but also invade peritoneal cell monolayers (Witz *et al.*, 1999, 2003; Debrock *et al.*, 2002). However, these studies found that the invasion of menstrual tissue through peritoneal monolayers was not dependent on the presence of endometriosis, indicating that the peritoneal environment is important for normal clearance of menstrual tissue.

If retrograde menstruation occurs in the majority of human females and NHP, then why is not there a higher prevalence of endometriosis? The efficient immunological clearance of menstrual tissue or a

decreased immuno-tolerance may explain why the prevalence of endometriosis is not greater than that observed. It has been reported that the peritoneal fluid from women with endometriosis have decreased natural killer and macrophage activity (Oosterlynck *et al.*, 1991), but elevated levels of IL-1, IL-6, IL-10, p40, TNF- α and TGF- β have also been reported (Punnonen *et al.*, 1996; Mazzeo *et al.*, 1998; Harada *et al.*, 1999; Gazvani and Templeton, 2002). In baboons, TNF- α , TGF- β , CD3 and HLA-DR were expressed on a larger number of cells within the peritoneal fluid following the induction of endometriosis compared with pre-inoculatory controls (D'Hooghe *et al.*, 2001).

Invasion

The increased levels of cytokines in the peritoneal fluid of both women and NHP with endometriosis may aid the invasion of ectopic lesions by activating tissue remodeling enzymes known as matrix metalloproteinases (MMPs). The expression profile for MMPs in the human endometrium throughout the menstrual cycle and their regulation by ovarian steroid hormones has been well documented (Rodgers *et al.*, 1993, 1994; Singer *et al.*, 1999; Curry and Osteen, 2001). Increases in these mediators of inflammation may support the remodeling of the peritoneal mesothelium which would allow for the invasion and subsequent growth of endometrial fragments. Our laboratory has reported that MMP-7 levels are increased in baboon ectopic endometrium (Fazleabas *et al.*, 2002), and these data are supported by human studies (Bruner-Tran *et al.*, 2002). The inhibition of MMP action may be a beneficial therapeutic target for the reduction of ectopic lesion development.

Angiogenesis and maintenance of disease

The development of ectopic endometrium is heavily dependent on the establishment of a sufficient blood supply at the site of invasion and its maintenance by steroid hormones. The establishment of angiogenesis can be seen macroscopically at sites of ectopic endometrial invasion. In both humans and baboons, vascular endothelial cell growth factor (VEGF) levels are elevated in eutopic and ectopic endometrium (Donnez *et al.*, 1998; Fujishita *et al.*, 1999; Tan *et al.*, 2002; Takehara *et al.*, 2004; Gashaw *et al.*, 2006). Additionally, CYR61 (an angiogenic factor important for cell proliferation, development and tumorigenesis) has been shown to be up-regulated in both the eutopic and ectopic endometrium (Absenger *et al.*, 2004; Gashaw *et al.*, 2006). These data indicate that the enhanced expression of angiogenic factors in the eutopic endometrium may attribute to the development of ectopic lesions in the peritoneal cavity.

Ectopic endometrial lesions are steroid hormone responsive. In human and baboon endometriotic tissues, there is an up-regulation of aromatase, estrogen receptor-2 (ESR2) expression and downregulation of progesterone receptor beta (PGR- β) (Noble *et al.*, 1996, 1997; Brandenberger *et al.*, 1999; Attia *et al.*, 2000; Fazleabas *et al.*, 2003; Jackson *et al.*, 2007). The up-regulation of estrogen biosynthesis in ectopic endometrium has been shown to occur through alterations in the methylation patterns of ESR2 and steroidogenic factor 1 (Xue *et al.*, 2007a, b). It has also been shown that ectopic endometrial stromal cells fail to convert estradiol (biologically active estrogen) to estrone because of reduced expression of 17- β -hydroxysteroid dehydrogenase type 2 in ectopic endometrial epithelial cells (Zeitoun *et al.*,

1998). This enzyme is regulated by PGR, and the reduction of PGR in ectopic lesions may in turn cause the dysregulation of 17- β -hydroxysteroid dehydrogenase type 2 expression (Cheng *et al.*, 2007). The dysregulation of steroid synthesis enzymes and steroid receptors allows for ectopic lesion development by maintenance of cell proliferation through the use of exogenous steroids and endogenous synthesis of estrogen. The ability of these lesions to regulate their own steroid hormone production within their local environment renders them hormonally independent from the eutopic endometrium (Attar *et al.*, 2009; Bulun *et al.*, 2009).

Infertility

There are many factors that contribute to endometriosis-associated subfertility: immune dysfunction, distortion of the pelvic anatomy, poor oocyte quality and fertilization rates, and the dysregulation of the genetic profile of the eutopic endometrium, during the window of receptivity, resulting in decreased implantation success (Ayers *et al.*, 1987; Halme *et al.*, 1987; Mills *et al.*, 1992; Burney *et al.*, 2007). The identification of dysregulated genes in the eutopic endometrium could reveal potential precursors for the development of endometriosis and also therapeutic targets for infertility treatment.

The power of microarray technology allows the identification of genes that are dysregulated in endometriotic tissue among several physiological pathways. Several genes have been found to be dysregulated in the eutopic endometrium of women with endometriosis, and these studies have been validated in both the rodent and baboon models of induced endometriosis (Eyster *et al.*, 2002; Giudice *et al.*, 2002; Giudice, 2004; Matsuzaki *et al.*, 2005; Flores *et al.*, 2007; Mettler *et al.*, 2007; Pan *et al.*, 2007; Wren *et al.*, 2007; Gaetje *et al.*, 2008). In general, these studies have consistently found that the following genes are dysregulated in eutopic endometriotic tissue: aromatase, endometrial bleeding factor, hepatocyte growth factor, 17- β -hydroxysteroid dehydrogenase, HOXA10, HOXA11, leukemia inhibitory factor, MMPs 3, 7 and 11, tissue inhibitors of metalloproteinases, progesterone-receptor isoforms, complement 3, glutathione peroxidase, catalase, thrombospondin 1, VEGF, integrin $\alpha_v\beta_3$ and glycodelin. These genes have been associated with regulating processes such as embryonic attachment and invasion, angiogenesis, endometrial tissue remodeling, fetal immunological tolerance and uterine steroid hormone responsiveness, which are all physiological functions vital for successful embryonic implantation (summarized in Fig. 1).

Immunological genes

It is known that endometriosis induces an inflammatory environment in the peritoneal cavity of women (Oosterlynck *et al.*, 1991, 1994; D'Hooghe *et al.*, 2001; Gazvani and Templeton, 2002; Umezawa *et al.*, 2009), but does the presence of ectopic lesions also induce an inflammatory environment in the eutopic endometrium? Microarray analysis of endometrium from women with or without endometriosis has revealed several genes that are dysregulated during the secretory stage are important for successful implantation (Burney *et al.*, 2007). This study reported that MUC-1 and osteopontin, facilitators of embryo adhesion, and glycodelin, immune regulator of maternal fetal tolerance (Bolton *et al.*, 1987), were down-regulated during the secretory phase of the menstrual cycle. Studies from our laboratory

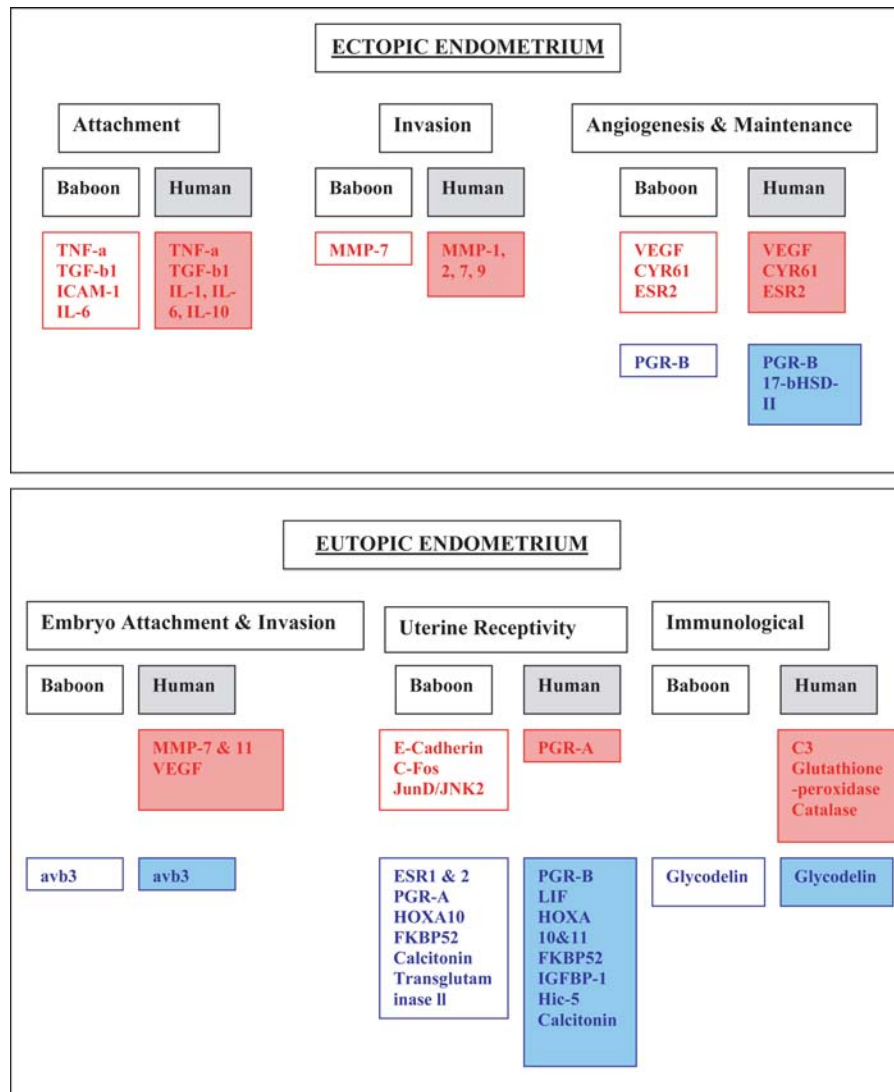


Figure 1 Gene dysregulation in ectopic and eutopic endometrium in baboons and women with endometriosis. All references can be found within the manuscript. Red denotes upregulated genes, blue denotes downregulated genes. Abbreviations: TNF, tumour necrosis factor; TGF, transforming growth factor; ICAM, intracellular adhesion molecule; IL, interleukin; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; ESR, oestrogen receptor; PGR, progesterone receptor; HSD, hydroxysteroid dehydrogenase; LIF, leukaemia inhibitory factor; FKBP, regulator of progesterone responsiveness; IGFBP, insulin-like binding protein; CYR, cysteine-rich angiogenic inducer; Hic-5, progesterone responsive gene; avb3, integrin avb3.

have corroborated these findings using a simulated model of pregnancy in the baboon. We have found that in normal endometrium, glycodelin levels increase in response to the embryonic signal CG (chorionic gonadotrophin); however, glycodelin induction by CG failed in endometriotic eutopic tissue (Fazleabas *et al.*, 2003).

This study prompted our laboratory to investigate other immunological genes that may be dysregulated in the eutopic endometrium during the window of receptivity. Using a focused microarray, we analyzed eutopic endometrium from control and diseased animals and found that there was no significant change in several immunological factors during the window of receptivity in eutopic endometriotic tissue (Hastings *et al.*, 2006). These data indicate that although endometriosis does not alter the expression of immune factors, it impairs

the function of immunoregulatory proteins to adequately suppress the activity of endometrial T cells to sustain proper embryo implantation.

Steroid hormone responsiveness

The coordinated changes that occur in the endometrium throughout the menstrual cycle are regulated by the ovarian steroid hormones estrogen (E2) and progesterone. Hormonal action is mediated through their interaction with their respective receptors on both endometrial stromal and epithelial cells. Using the baboon model of induced endometriosis, our laboratory was able to identify the dysregulation of estrogen and progesterone responsive genes as well as ESR1 and ESR2 and PGR-A and PGR-B during disease progression.

In healthy baboons without endometriosis, eutopic endometrial stromal expression of ESR1 was primarily evident during the window of receptivity, but in endometriotic animals, this increase was not observed (Jackson *et al.*, 2007). The decrease in expression was seen primarily in the stromal cells 6 months after the induction of disease. No changes were observed in the glandular epithelial layer through disease progression. The expression pattern of ESR2 was also altered in the presence of disease. During the window of receptivity, ESR2 was decreased in the eutopic endometrium of diseased animals compared with controls, and this decrease was evident in both the glandular and stromal compartments at 6 months of disease. The decrease in ESR1 and ESR2 expression, in diseased animals, renders the endometrium unresponsive to estrogen stimulation and as a consequence PGR induction, which is necessary during the window of receptivity. These changes are not apparent at the onset of disease (<6 months) but rather after adequate establishment of disease (>6 months), indicating that ectopic lesions altered the expression of ESR in the eutopic endometrium.

Following the early onset of disease, c-FOS, an estrogen-responsive gene, was highly up-regulated, in addition to junD and JNK2 (Hastings *et al.*, 2006). These genes are important for controlling cell cycle and cellular fate by repressing gene expression or silencing. One of the targets of AP-1 transcription factors is PGR isoform A (Shemshedini *et al.*, 1991). The early expression of this family of transcription factors may affect the responsiveness of the endometrium to ovarian steroids during the window of receptivity throughout disease progression.

The expression of the PGR-A and PGR-B is important during the window of receptivity for successful implantation. During the progression of endometriosis, it has been reported that the eutopic endometrium becomes resistant to progesterone action by the dysregulation of a number of progesterone-responsive genes (Hic-5, glycodeilin, IGFBP-1, HOXA10, calcitonin) and progesterone chaperone genes (FKBP52) (Kao *et al.*, 2003; Burney *et al.*, 2007; Jackson *et al.*, 2007, 2009; Kim *et al.*, 2007; Aghajanova *et al.*, 2009). Using our baboon model of endometriosis, we examined the eutopic endometrial expression of PGR-A and PGR-B and downstream targets of progesterone action. We found a decrease in PGR-A immunolocalization in glandular epithelial cells, although there was no change in the immunolocalization of PGR-B. In stromal cells, there was no difference in the immunolocalization of PGR-A or PGR-B, but interestingly, the ability of these cells to respond to PR stimulation was decreased (Kim *et al.*, 2007). This decrease was attributed to the finding that FKBP52, a regulator of progesterone responsiveness, was diminished at later time points (>6 months) of disease (Jackson *et al.*, 2007), a finding that has been also confirmed in women with endometriosis (Hirota *et al.*, 2008).

In addition to the characterization of the steroid receptor profiles in diseased eutopic endometrium, it has also been reported that HOXA10, a downstream target of PR which is also down-regulated in FKBP52 null mice (Tranguch *et al.*, 2005, 2007), was decreased in endometriotic tissue from women and baboons (Taylor *et al.*, 1999; Kim *et al.*, 2007). In baboons, the decrease of HOXA10 expression resulted in a decrease of target gene integrin B3 and an increase of empty spiracles homolog 2 (Kim *et al.*, 2007). The dysregulation of HOXA10 in endometriosis may be the result of the methylation state of the gene. In baboons with disease, the F1 region of HOXA10 was hypermethylated compared with disease-free animals.

In women with severe endometriosis, HOXA10 expression was also associated with hypermethylation of all three regions of the HOXA10 gene (Gui *et al.*, 1999). These studies indicate that endometriosis may affect post-transcriptional modifications as a method of gene silencing or activation.

HOXA10 has also been shown to be important in endometrial stromal cell decidualization, in that null mice are infertile due to a decidualization defect (Benson *et al.*, 1996). The role of HOXA10 during the process of stromal cell decidualization was examined by investigating the potential HOXA10 regulation of insulin-like growth factor-binding protein-1 (IGFBP-1), a major marker for decidualization in the baboon. Endometrial stromal cells isolated from baboons with endometriosis have shown increased expression of IGFBP-1 following decidualization treatment (Kim *et al.*, 2007), indicating a hyper response to decidualization. In contrast, human endometrial stromal cells from diseased women have shown reduced IGFBP-1 secretion (Klemmt *et al.*, 2006). Both of these studies indicate that there is an altered decidualization response in eutopic endometrial stromal cells from diseased endometrium.

The importance of FKBP52 for embryonic implantation was proven by the use of FKBP52 null mice. When FKBP52 was deleted, the levels of HOXA10, Indian hedgehog (Ihh) and calcitonin (CALC) were also reduced during the window of receptivity (Tranguch *et al.* 2005, 2007; Yang *et al.*, 2006). Our laboratory evaluated the localization of calcitonin and calcitonin-modulated proteins, E-cadherin and transglutaminase II in the eutopic endometrium during the window of receptivity in baboons with induced endometriosis to determine whether these proteins were downregulated as a result of decreased FKBP52 expression. We found that in diseased animals, calcitonin staining was decreased in both endometrial epithelial and stromal cells, whereas E-cadherin staining was increased in endometrial epithelial cells, and transglutaminase-II staining was decreased in endometrial stromal cells compared with disease-free healthy controls (Jackson *et al.*, 2009). These data support the theory that, during disease progression, the eutopic endometrium has the ability to become resistant to progesterone actions probably as a consequence of impaired PGR function directly or indirectly by the attenuated expression of critical chaperone proteins such as FKBP52 and Hic-5 (Aghajanova *et al.*, 2009).

The aberrant dysregulation of the steroid receptors and their downstream targets, during the window of receptivity, affects not only the ability of the eutopic endometrium to respond to ovarian hormonal priming but also the response of the endometrium to the implanting embryo, resulting in implantation failure. This is clearly evident in our baboon model of simulated pregnancy. Following the induction of endometriosis, the eutopic endometrium is unresponsive to chorionic gonadotrophin (CG) stimulation, and the expression of a number of genes thought to be critical for implantation and decidualization are markedly altered (Fazleabas *et al.*, 2003; Hastings *et al.*, 2008). The examination of steroid hormone responsiveness can elucidate the effectiveness of hormonal treatment of endometriosis and corroborate the finding of progesterone therapy resistance in some cases of endometriosis (Bulun *et al.*, 2006; Burney *et al.*, 2007).

Ultrastructural abnormalities

The endometrium is one of the most dynamic tissues in the entire body. At the beginning of each menstrual cycle, this unique tissue

regenerates following the shedding of endometrial tissue known as menstruation. Because the endometrium goes through histological changes each menstrual cycle, pathologists have created a dating system to classify the different phases of the menstrual cycle (Noyes *et al.*, 1975). Recently, we have reported changes in the ultrastructure of the endometrium in each phase of the menstrual cycle (Jones *et al.*, 2006). Control eutopic endometrial tissues from the late proliferative, mid-secretory and late secretory phases of the menstrual cycle were analyzed using electron microscopy. During the window of uterine receptivity, the endometrium was characterized by columnar cells containing glycogen-filled vesicles which were associated with Golgi bodies, small and narrow mitochondria, lateral membranes with few interdigitations and apical junctional complexes (Jones *et al.*, 2006). Following the induction of endometriosis, eutopic endometrium isolated during the window of receptivity (mid-secretory) did not resemble that of control mid-secretory endometrium. Instead, we observed a shift in the ultrastructure of the endometrium to that of late proliferative control endometrium at early stages of disease. At later stages of disease, the ultrastructure of the eutopic endometrium resembled that of control late secretory endometrium (Jones *et al.*, 2006). These findings have since been validated in the eutopic and ectopic endometrium of women (Jones *et al.*, 2009a, b). Thus, in addition to the changes in gene expression, changes in the ultrastructural morphology of the eutopic endometrium during the window of uterine receptivity are also clearly documented. Thus, it is evident that the presence of peritoneal disease is associated with an inherent endometrial effect that is manifested in multiple etiologies, all of which may be detrimental to successful embryo implantation.

The studies summarized here indicate that the sub-infertility that is associated with endometriosis is the result of several factors. The use of the baboon model of inducible endometriosis has clearly established that ectopic endometrial lesions distinctly influence the eutopic endometrium, and these changes sequentially result in an overall resistance to progesterone. These types of studies are not feasible when evaluating the consequences of spontaneous disease. Thus, on the basis of these studies, the effectiveness of treatment modalities can be evaluated to determine whether these therapies can re-establish normal fecundity rates in women with endometriosis.

Translational research

The baboon model of endometriosis, either inducible or spontaneous, provides an excellent tool to evaluate the effectiveness of therapeutic targets on reducing disease development and also on improving pregnancy success rates. The inflammatory cytokine TNF- α is a potent stimulator of the inflammatory process and has been shown to be elevated in the peritoneal fluid of women with endometriosis (Halme, 1989; Arici *et al.*, 1998). For this reason, it has been the primary target for therapeutic design and development.

Anti-TNF therapy has shown to be a potent, non-hormonal therapeutic option for the treatment of endometriosis. Pretreatment of the pelvic cavity with recombinant human TNF-binding protein-1 (r-hTBP-1) prior to disease induction resulted in a reduction in the lesion surface area and revised American Fertility Society (rAFS) scoring of disease (D'Hooghe *et al.*, 2006). When r-hTBP-1 was given after disease induction, they found that r-hTBP-1 treatment could reduce the surface area of lesions, improve rAFS scoring and

eliminate the formation of adhesions involving the reproductive organs and cul-de-sac. However, neither pre- nor post-treatment with r-hTBP-1 affected inflammatory cytokine mRNA levels for TNF- α , ICAM-1, MMP-1, IL-8, IL-6 or RANTES (regulated upon activation, normal T-cell expressed, and secreted) in either the ectopic or the eutopic endometrium (Kyama *et al.*, 2008). The authors did report that post-disease induction treatment with r-hTBP-1 did reduce TGF- β mRNA levels in the ectopic endometrium but not in the eutopic endometrium.

Another compound, etanercept, currently used for rheumatoid arthritis, juvenile rheumatoid arthritis and psoriatic arthritis has been investigated for its effectiveness as therapy for endometriosis (Barrier *et al.*, 2004). Etanercept, a fusion protein consisting of human recombinant soluble TNF receptor 2 which neutralizes TNF activity, reduced the formation of red lesions in baboons with established endometriosis. This treatment did not have an effect on blue or white lesions.

Because anti-TNF- α therapy has had beneficial results for the reduction of endometriosis development, it has recently been used to examine the potential benefits on pregnancy rate and outcome in endometriotic animals. Treatment of the anti-TNF- α agent c5N following disease induction has been shown to reduce the formation of ectopic lesions (Falconer *et al.*, 2006). Subsequent to this study, these same animals were then evaluated for pregnancy success and outcome (Falconer *et al.*, 2008). The treatment of animals with c5N was not able to increase pregnancy success or pregnancy outcome. The authors believe that the failure of c5N to improve fertility was due to the short time of disease induction, 2 months, and this short time period of disease was not adequate for the induction of subfertility. The treatment of animals with spontaneous proven infertility with c5N would be indicative of the effectiveness of c5N for the improvement of pregnancy parameters.

Another family of immunomodulators that have been investigated as a potential therapeutic treatment for endometriosis are peroxisome proliferator-activated receptors (PPARs). The ligand for PPAR- γ induces the regression of endometriotic explants in the rat model and also in the induced baboon model (Lebovic *et al.*, 2004, 2007). The reduction of lesion surface area was not due to a decrease in serum E2 or P4 levels, but instead they believe that PPAR- γ actions may be through its inhibition of nuclear factor- κ B (NF- κ B) in macrophages (Chinetti *et al.*, 1998; Jiang *et al.*, 1998; Wang *et al.*, 2001; Chen *et al.*, 2002; Liu *et al.*, 2005). The constitutive expression of NF- κ B in endometriotic lesions may play a role in the development of endometriosis (Guo, 2007), and targeting this transcription factor is a potential therapy for reducing the pathogenesis of this disease.

Summary

The use of the NHP (baboon) model of endometriosis allows for the investigation of molecules involved not only in the pathogenesis of disease but also in the genetic dysregulation of the eutopic endometrium and immune system. It is heavily debated whether defects of the endometrium cause ectopic lesion development or whether the presence of ectopic lesions causes defects of the eutopic endometrium. Because the baboon can develop endometriosis spontaneously, as well as in response to induction with menstrual tissue, investigators can begin to elucidate factors that are dysregulated due to the

presence of ectopic lesions and compare these factors to the spontaneous model. This information could identify markers early in disease development that could be used as diagnostic tools eliminating the need for surgical diagnosis. Additionally, the baboon is a powerful model for evaluating and testing therapeutic targets for the treatment of disease. The use of the baboon allows for repetitive surgical evaluations of disease, which provides a well-controlled model for the study of the effectiveness of drug therapies and reduces the ethical and medical concerns that arise in human studies requiring surgical evaluation and intervention. The baboon model of endometriosis is critical for the discovery of a future treatment or possible prevention of endometriosis.

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