Animal models in endometriosis research

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Endometriosis is a common gynaecological disease, defined as the presence of endometrial tissue outside the uterus, causing pelvic pain and subfertility in ~10% of women of reproductive age. Current therapies lead to pain relief, however, do not address the causes and entail severe side effects. Still little is known about the pathogenic processes leading to the development and maintenance of endometriosis. Because endometriosis occurs spontaneously only in humans and some non-human primates, animal models of induced endometriosis have been developed and are of high value for the evaluation of pathophysiological mechanisms underlying the development of this disease. These experimental models include the autotransplantation of uterine fragments into the peritoneal cavity of rodents and non-human primates or the heterotransplantation of human endometrial or endometriotic tissue to immunodeficient mice or onto the chicken chorioallantoic membrane (CAM). This review describes the animal models for endometriosis and assesses their different potentials and limitations in regard to endometriosis research, with the aim of developing novel non-invasive diagnostic tools and improved strategies for the treatment of endometriosis in women.

Key words: animal model/CAM/endometriosis/non-human primates/rodents

The need for animal models in endometriosis research

Endometriosis is one of the most frequent benign gynaecological diseases; it is thought to occur in 7–10% of women (Wheeler, 1989) but may vary up to 60% of women of reproductive age with pelvic symptoms or disturbance of fertility (Pellicer et al., 2001; Giudice and Kao, 2004). Yet, little is known about the pathogenic processes leading to the development and maintenance of this disease, and currently available medical therapies are unsatisfactory, because they focus on treating the symptoms rather than curing the causes. In addition, they cannot be used for prolonged duration because of severe secondary side effects (Rice, 2002). Thus, there is a definite need to develop new drugs to provide specific and more efficient therapeutic alternatives that eliminate endometriotic lesions, prevent recurrences and do not interfere with the fertility potential.

One widely accepted mechanism for the development of peritoneal endometriotic lesions is the adhesion and growth of endometrial fragments deposited into the peritoneal cavity via retrograde menstruation (Sampson, 1927). However, because retrograde menstruation occurs in nearly all women of reproductive age (Halme et al., 1984), impairment of additional factors is needed for the establishment and maintenance of these ectopic lesions. Because, at the time of clinical presentation, most women already have established endometriosis, it is hardly possible to give experimental evidence for physiological roles in the pathogenesis of this disease in humans. In addition, ethical considerations limit the performance of controlled experiments, and it is not possible to monitor the disease progression without performing repeated laparoscopies. Thus, research into the fundamental mechanisms by which menstrual endometrium adheres, invades and establishes a functional vasculature to persist in an ectopic site, as well as the development of new therapeutic approaches, is best performed in experimental animal models.

Because menstrual shedding is a requirement for the spontaneous development of this disease, endometriosis occurs naturally only in humans and some non-human primates. Non-human primates have been extensively used for the investigation of endometriosis; however, the very high costs of animal handling limit the use of monkeys as an experimental model. For this reason, the establishment of small laboratory animals, especially rodents, as models for endometriosis by the transplantation of pieces of endometrial tissue to ectopic sites has greatly extended in recent years. This review provides a survey of commonly used animal models and their application for different challenges.

CAM assay

Characterization of the CAM assay

The chicken chorioallantoic membrane (CAM) model can be considered as an animal model in the broader sense. Originally, it had been developed to study the invasive, metastatic and angiogenic potential of neoplastic cells (Scher et al., 1976; Armstrong et al., 1982). It has been established as a model for endometriosis by culturing fragments of human endometrial tissue on the basal layer of the CAM of fertilized chicken eggs after prior incubation for 7–10 days (Malik et al., 2000; Maas et al., 2001). Endometrial fragments
from the proliferative and secretory phase of the menstrual cycle as well as the menstrual endometrium invade across the epithelium into the mesenchymal layer and develop endometriosis-like lesions in this layer of the CAM within 3 days after grafting of the human tissue (Maas et al., 2001; Nap et al., 2003, 2004). It was shown that these endometrial fragments needed to contain intact glandular structures as well as stromal components.

Application of the CAM assay

Angiogenesis could be documented by ingrowth of vessels from the CAM into the human endometrial fragments (Maas et al., 2001). The anti-angiogenic factors endostatin, TNP-470 and anti-vascular endothelial growth factor (VEGF) antibodies all significantly decreased the angiogenic response to endometrial transplants (Nap et al., 2005). Endometrial fragments of women with and without endometriosis showed no significant differences in VEGF expression or angiogenic induction in this assay (Gescher et al., 2005). In contrast, Oosterlynck et al. (1993) demonstrated that peritoneal fluid of women with endometriosis induced significantly higher angiogenic responses in the CAM than peritoneal fluid of women without endometriosis.

Because the CAM contains extracellular matrix components similar to the ECM of the human peritoneum (Giannopoulou et al., 2001), it can be used to investigate the invasive potential of endometriotic tissue. Nap et al. (2004) demonstrated that the expression profiles of matrix metalloproteinases (MMPs) were similar in experimentally induced endometriosis in CAMs compared with human endometriosis and that the development of early endometriotic lesions in the CAM model was prevented by inhibiting MMP-1, -2, -3, -7 and -13 activity. According to Wolber et al. (2003), relative concentrations of MMP-1 mRNA, but not MMP-2 mRNA, increased strongly after culture on the CAM.

Evaluation of the CAM assay

The CAM assay is well suited for the investigation of mechanisms involved in invasion and angiogenesis during the first steps in the establishment of endometriotic lesions, for example, by the inhibition of factors known to participate in these processes. The ectopic lesions are easily accessible and can be simply monitored during the course of an experiment. This endometriosis model is hardly applicable for the investigation of immunological or inflammatory responses or to analyse long-term drug effects in the course of the disease.

Rodent models

In contrast to humans and non-human primates, estrous animals do not shed their endometrial tissue and therefore do not develop endometriosis spontaneously. However, endometriosis can be induced by transplanting endometrial tissue to ectopic sites. These models are classified into two types, homologous and heterologous models. Homologous models have been employed utilizing the surgical transplantation of endometrium of the same or syngeneic animals in immunocompetent animals, whereas in heterologous models, human endometrial fragments are transferred either intraperitoneally or subcutaneously to immunodeficient mice.

Application of endometrium to immunocompetent mice and rats

Characterization of the homologous model

Autotransplantation of uterine tissue to ectopic sites of small laboratory animals has been established not only in rats (Golan et al., 1984; Jones, 1984; Vernon and Wilson, 1985; Rajkumar et al., 1990; Sharpe et al., 1991) and mice (Cummings and Metcalf, 1995; Somigliana et al., 1999; Rossi et al., 2000) but also in hampsters (Steinleitner et al., 1991b) and rabbits (Schenken and Asch, 1980; Dunselman et al., 1989; Homm et al., 1989; Rock et al., 1993). Of those non-primate models, mainly rat and mouse models have been further developed in recent years. In these models of endometriosis, uteri are removed and cut into small pieces which are then reintroduced, mostly by suturing, into the peritoneal cavity. In most of these studies, endometrium was not separated from myometrium; thus, both compartments were implanted (Vernon and Wilson, 1985; Cummings and Metcalf, 1995). In the rat, the uterine tissue develops into fluid-filled, ovoid, cystic structures composed of myometrial and endometrial tissue. The cysts grow but stabilize their size by ~2 months and remain viable for at least 10 months (Vernon and Wilson, 1985).

In the mouse, the ectopic uterine fragments show histological characteristics of the human disease, including the formation of multiple, highly vascularized lesions containing endometrial glands, stroma and cysts, independent of their peritoneal localization in the abdomen (Cummings and Metcalf, 1995). A limited number of experiments separating the endometrium from the myometrium, only inoculating the endometrium to ectopic sites, have been performed in rats (Katsuki et al., 1998) and mice (Somigliana et al., 1999; Hirata et al., 2005; Yao et al., 2005). In the study of Somigliana et al. (1999), isolated endometrial tissue was minced and reintroduced into the peritoneal cavity of recipient, syngeneic animals. Both donor and recipient mice had been ovarioctomized and received estrogen treatment. In this study, all recipient animals revealed evidence of peritoneal endometriosis after 3 weeks, and neovascularization was observed at the surface of the lesions. However, the ‘take-rate’, that is the number of lesions recovered after a known number of endometrial fragments have been randomly inoculated, was on average 30% of the inoculated tissue.

For better identification of size and localization of ectopic endometriotic lesions after transplantation, Hirata et al. (2005) developed a homologous mouse model using ‘green mice’ that ubiquitously express green fluorescent protein (GFP) as donors and transplanted the fluorescent endometrial tissue into recipient mice. They could show that the weight of endometriotic lesions significantly correlated with the measured fluorescence intensity. This fluorescence was significantly higher in the estrogen-supplemented mice as compared with control animals, supporting the estrogen dependency of these ectopic endometrial lesions.

Application of the homologous model

Endometriosis is known to be an estrogen-dependent disease in women, and the suppression of the serum estrogen level supports the regression of those ectopic lesions (Lapp, 2000; Rice, 2002). Autotransplanted uterine tissue in the rodent model shows steroid hormone dependency, and thus, this model has been widely used for determining the responsiveness of ectopic lesions to steroid
hormones as well as to drugs interfering with steroid action. Corresponding to the situation in humans, the growth of the ectopic endometrial tissue in both rodent species was shown to be estrogen dependent (Vernon and Wilson, 1985; Rajkumar et al., 1990; Rossi et al., 2000). In rats which had been ovariotomized 3 weeks after engrafts of uterine tissue, the ectopic fragments recovered much better in animals treated with estrogen alone after ovariotomy than in those with combined estrogen and progesterone treatment (Schor et al., 1999). Fang et al. (2004) confirmed the important role for estrogen as a factor determining the size of the implants in mice and demonstrated that the antiproliferative effect of progesterone on the estrogen-dependent growth of endometriotic tissue is mediated by intact progesterone receptor, because this estrogen-dependent growth could be suppressed by progesterone in uterine tissues of wild-type but not of progesterone-receptor-deficient mice.

According to the situation in women, the regression of uterine ectopic implants could be induced in rats by generating a hypoestrogenic state, for example, by ovariotomy (Kudoh et al., 1997), by the application of GnRH agonists (Sakata et al., 1990; Wright and Sharpe-Timms, 1995; Kudoh et al., 1997), by synthetic progestational compounds such as levonorgestrel or dienogest (Jones, 1984; Katsuki et al., 1998) or by danazol treatment (Sakata et al., 1990). Corresponding effects could be achieved in this model by reducing the estrogen concentration by the application of anti-estrogens (Saito et al., 2003), by using the selective estrogen receptor modulator raloxifen (Yao et al., 2005) or by treatment with aromatase inhibitors which interfere with estrogen synthesis (Yano et al., 1996; Kudoh et al., 1997).

Moreover, the autologous rat model in particular has been extensively used for studies of immune-modulating drugs and anti-inflammatory agents in endometriosis. Uchiide et al. (2002) demonstrated that uterine autotransplantation in rats induces the infiltration of allergic inflammatory-related cells in peritoneal stroma attached to the ectopic uterine tissue. Immunomodulation by intraperitoneal or subcutaneous treatment with interferon-α-2b in the rat reduced the size of induced lesions as monitored by consecutive laparotomies over a period of up to 4 months (Ingelmo et al., 1999) as did the application of the immunomodulator lexoribine in rats (Keenan et al., 1999) and the intraperitoneal injection of interleukin-12 in a syngeneic mouse model (Somigliana et al., 1999).

In addition, the development of experimentally induced endometriosis in rats was inhibited by recombinant human tumour necrosis factor (TNF)-binding protein-1 (r-hTBP-1), which is the soluble form of TNF receptor type I (D’Antonio et al., 2000). This effect was also seen after treatments of rats with pentoxyfilline, an anti-inflammatory substance reported to reduce the production of inflammatory cytokines without inducing a hypoestrogenic state (Nothnick et al., 1994), as well as with cigitazone, a ligand for proliferator-activated receptor-γ (PPAR-γ) (Lebovic et al., 2004). Moreover, the initial development of ectopic implants in rats has been reduced by cyclooxygenase-2 (COX-2) inhibitors (Matsuzaki et al., 2004) and also by the application of various non-steroidal anti-inflammatory drugs such as celecoxib, indomethacin, sulinac and ibuprofen (but not aspirin) in a mouse model for endometriosis (Efstathiou et al., 2005).

Furthermore, this rodent model provides the opportunity to investigate the effect of environmental contaminants on the establishment of ectopic uterine implants. It has been demonstrated that exposure to dioxin preceding the surgical induction of endometriosis produces a dose-dependent increase in endometriotic site diameter in rats and mice, which was much stronger in mice (Cummings et al., 1996).

Recently, in an interesting approach, Dinulescu et al. (2005) described the first mouse model of de-novo endometriosis. Activation of the oncogene K-ras in ovarian surface epithelial cells, but not in cells of the peritoneal lining, resulted in the development of benign epithelial lesions with histomorphological features of human endometriosis. In addition to developing ovarian lesions, nearly half the mice also developed peritoneal endometriosis 8 months after the K-ras activation of ovarian surface epithelial cells. In combination with a conditional deletion of Pten, which has been implicated in the progression of ovarian endometriosis to endometrioid ovarian carcinoma in humans (Sato et al., 2000), K-ras expression even induced metastatic endometrioid ovarian adenocarcinomas. However, to date, no mutations of K-ras have been found in human endometriosis (Vercellini et al., 1994; Otsuka et al., 2004).

The hallmark symptoms of endometriosis in women include severe pelvic pain and significantly reduced fertility (Elshiekh et al., 2003). The influence of ectopic lesions on fertility has been investigated in laboratory rodents. Although Cummings and Metcalf (1996) did not observe a decrease in fertility in mice, a reduced fecundity could be observed in rats with artificially induced endometriosis (Vernon and Wilson, 1985), which could be due in part to an increased number of luteinized unruptured ovarian follicles (Moon et al., 1993). In addition, a role of pelvic adhesions has not be excluded. Moreover, peritoneal inflammatory cell hyperactivation could have an effect on fertility in endometriosis. This was supported by the work of Steinleitner et al. (1991a), demonstrating that macrophage-mediated subfertility in the mouse model could be compensated by pentoxyfilline, a drug that reverses the effect of macrophage hyperactivation, and that the drug-mediated inhibition of macrophage activation enhanced fertility in a hamster endometriosis model (Steinleitner et al., 1991b).

Recently, studies have been published investigating the effect of ectopic endometrial lesions on pain behaviour in the rat model of endometriosis. Here, the autotransplanted ectopic endometrial cystic fragments develop their own innervation including sympathetic efferent as well as sensory fibres (Berkley et al., 2004), which may have a general influence on the nervous system. This is supported by the finding that vaginal nociception was increased in rats with endometrial cysts similar to the situation in women with endometriosis. In addition, those rats also displayed vaginal hyperalgesia (Berkley et al., 2001), one of the symptoms of increased pelvic pain in women. It is speculated that neuroactive agents contained in the endometrial cysts may activate nociceptive afferents influencing central neural mechanisms associated with vaginal nociception as well as with reproduction via visceral–visceral interactions (Cason et al., 2003; Berkley et al., 2004). It remains to be elucidated whether the rats exhibit chronic pelvic pain symptoms other than vaginal hyperalgesia in this model of endometriosis.
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Evaluation of the homologous model

There are relevant differences between rodent and human reproductive physiology; consequently, these models possess limitations. Because rodents do not menstruate, they do not develop spontaneous endometriosis, and the disease has to be induced artificially by the autotransplantation of uterine tissue. In addition, in most of these rodent studies, the uterine fragments which were transplanted included the myometrial layer which could affect the development of the lesions. When evaluating the weight and size of the ectopic lesions, the myometrium could make up a considerable proportion, not representing the situation of human endometriotic lesions.

Despite these limitations, the rodent model offers significant advantages. Apart from the limited costs and the opportunity to perform studies in large groups of genetically similar animals, long-term studies can be performed because there is no rejection of the autotransplanted ectopic tissue. It is well suited for the investigation of mechanisms involved in the peritoneal attachment of endometrial cells as well as effects of therapeutic drugs and chemicals, and it allows the evaluation of ectopic lesions at different time intervals. Moreover, the homologous rodent model of surgically induced endometriosis provides a well-characterized immune system and is appropriate and extensively used to study the effect of immune-modulating drugs and anti-inflammatory agents on endometriosis. Furthermore, in regard to new insights in the aetiology of endometriosis obtained by genomic and proteomic approaches, transgenic mice models may gain importance as models of endometriosis to examine functions driven by single genes.

Xenotransplantation of human endometrium to immunodeficient mice

Characterization of the heterologous model

Because causal factors for the development and maintenance of endometriosis could originate in the endometrium itself and may not be present in the rodent endometrium, models have been developed by implanting human endometrial tissue instead of autologous endometrium into immunodeficient mouse strains. Most widely used for this purpose is the athymic nude mouse, which lacks mature T-lymphocytes. Human endometrial fragments from the proliferative or secretory phase of the menstrual cycle (Zamah et al., 1984; Bergqvist et al., 1985; Zaino et al., 1985; Bruner et al., 1997; Nisolle et al., 2000; Grummer et al., 2001; Beliard et al., 2002) as well as the menstrual endometrium (Nisolle et al., 2000; van Langendonckt et al., 2004; Eggermont et al., 2005) have been successfully implanted either subcutaneously or intraperitoneally into nude mice. These fragments implant and form endometriotic-like lesions which resemble those found in patients in terms of macroscopic and histological appearance, whereas the stage of the menstrual cycle at the time of collecting the human tissue seems to have no impact on the development of those ectopic lesions (Nisolle et al., 2000). Preservation of estrogen and progesterone receptors and steroid responsiveness have been demonstrated in the ectopic human tissue (Zamah et al., 1984; Bruner et al., 1997, 1999; Grummer et al., 2001). Angiogenesis guarantees the maintenance of the transplants and the transport of systemically applied drugs to the human endometrial tissue. The establishment of blood vessels of mouse origin occurred from just 4 days after transplantation onwards, independent of the localization of the ectopic lesions (Grummer et al., 2001; Hull et al., 2003).

When endometrial tissue was randomly inoculated into the peritoneal cavity, peritoneal adhesion occurred from 2 days after implantation onwards. The sites of implantation of the endometrial fragments were preferentially the gut, the muscles of the abdominal wall, the liver and the adipose fat surrounding abdominal organs (Grummer et al., 2001; Fortin et al., 2003). However, studies from our group as well as others revealed that the recovery rate is highly variable from one animal to another and in average does not exceed 30% (Bruner et al., 1997; Grummer et al., 2001). This ‘take-rate’ is further reduced after in vitro culturing of endometrial tissue for 24 h before transplantation but could be enhanced to 100% by suturing the human endometrial fragments to different intraperitoneal sites (Grummer et al., 2001). This is favourable, in regard to drug testing, because a difference in the number of lesions in the two groups of animals could be due to different ‘take-rates’ at the beginning of the experiment rather than to the effect of a drug.

For non-invasive detection and quantification of implanted endometrial tissue, Tabibzadeh et al. (1999) loaded dissociated glands and stromal cells with a lipophylic fluorescent dye (DiO) before in vivo transplantation. However, an effect of the solvent [dimethylsulphoxide (DMSO) and absolute ethanol] on the endometrial tissue cannot be excluded. An interesting approach that is better for monitoring of subcutaneously or intraperitoneally transplanted endometrial fragments was recently described by Fortin et al. (2003). They transduced endometrial fragments of the proliferative phase with GFP-cDNA before implantation, resulting in a transient fluorescence of the endometrial tissue allowing optical in vivo imaging of the implantation and development of ectopic lesions. Because adenoviruses are transient expression vectors, they observed that fluorescence of endometrial tissue fades out around the third or fourth week, limiting the non-invasive follow-up to this time frame. In addition, when using GFP as a reporter gene, in vivo quantification would probably be limited to subcutaneous lesions, which can be directly visualized through the skin of living mice, because the wavelength of GFP-emitted light from intraperitoneal fragments is absorbed by surrounding tissues. Thus, this model allows dynamic studies of lesion by monitoring implantation and development. However, a yet unknown interaction between GFP and lesions or applied drugs might interfere with the studies.

Because, in the heterologous mouse model, human tissue is transplanted, the time frame for maintenance of intact and well-preserved tissue is limited. In most of the studies performed, culture of human endometrium in nude mice does not exceed a period of 4 weeks. From 3 weeks after inoculation onwards, dedifferentiation parameters and lymphocyte infiltration can be observed (Grummer et al., 2001).

Because longer maintenance of human tissue in this animal model would be advantageous for special experimental approaches, mice with an even more defective immune system have been established as heterologous models for endometriosis. Human endometrial tissue has successfully been transplanted to severe combined immunodeficient (SCID) mice (Aoki et al., 1994; Kaufmann et al., 1995; Awwad et al., 1999) as well as to non-obese diabetic (NOD)-SCID mice (Grummer et al., 2001),
both of which show a combined congenital deficiency in T- and B-lymphocyte function. Compared with the nude mouse model, human endometrium engrafted in SCID/NOD-SCID mice revealed a higher ‘take-rate’ of fragments (Aoki et al., 1994) and a better preserved morphology and expression of steroid hormone receptors over a period of 4 weeks (Gruemmer et al., 2001). Although these mice lack B and T lymphocytes, they do have some natural killer (NK) cell activity. Recently, Matsuura-Sawada et al. (2005) cultured human endometrial tissue subcutaneously in NOD/SCIDγcnull (NOD) immunodeficient mice which are additionally defective in NK-cell activity. In this model, they reproduced one human menstrual cycle of 28 days by hormone application in ovarioctomized animals. At the level of histology, they showed cyclic changes also observed in eutopic human endometrium but without signs of decidualization.

Greenberg and Slayden (2004) subcutaneously engrafted human endometriotic tissue in transgenic recombinase-activating gene-2 knockout [RAG-2/γcKO] mice, which are not only devoid of B and T lymphocytes but also lack NK cells. Only endometriotic biopsy specimens that contained endometrium-like glands developed visible lesions. Red peritoneal lesions, but not biopsy specimens of ovarian endometrioma, established grafts at a high rate. In this animal model for endometriosis, they mimicked four consecutive human menstrual cycles by applying estrogen and progesterone via hormone-releasing capsules. The established grafts were well-vascularized and maintained steroid hormone receptors.

**Application of the heterologous model**

Animal models of endometriosis based on the xenotransplantation of human endometrium permit the elucidation of mechanisms involved in the establishment of endometriosis as well as testing the effects of potential therapeutical compounds on human tissue. One approach to prevent the development of peritoneal endometriosis could target the adherence of endometrial tissue to and the invasion of the peritoneal lining. MMPs are involved in such invasion processes (Hudelist et al., 2005; Zhou and Nothnick, 2005). It could be shown that MMPs are regulated by steroid hormones and cytokines in endometrial tissue and that the suppression of MMPs inhibits the establishment of ectopic lesions by human endometrium in nude mice (Bruner et al., 1997, 1999; Bruner-Tran et al., 2002).

Another therapeutic target discussed is the development of new blood vessels, which is essential for the persistence of ectopic endometriotic lesions. Endometrial xenografts in nude mice have been shown to produce high levels of VEGF (Nisolle et al., 2000), and anti-angiogenic agents such as soluble VEGF receptor (sflt-1) and VEGF-A-antibodies significantly inhibited the growth of endometriotic lesions in nude mice (Hull et al., 2003).

Because endometriosis is an estrogen-dependent disease, the responsiveness of human ectic lesions to steroid hormones has been investigated in this mouse model. Pretreatment of human endometrial cells with estrogen alone or in combination with a progestin, but not with progestin alone, before injection into the peritoneal cavity of nude mice resulted in a higher percentage of animals developing endometriotic-like lesions (Beliard et al., 2002). Using optical in vivo imaging, Fortin et al. (2004) demonstrated a significantly reduced lesion size in estrogen-untreated versus estrogen-treated ovarioctomized nude mice.

Recent studies have identified aberrations in the expression of aromatase and 17β-hydroxysteroid reductase-2 in human endometriotic lesions (Zeitoun et al., 1998; Bulun et al., 1999; Zeitoun and Bulun, 1999), suggesting a role for steroid-metabolizing enzymes in the pathogenesis of endometriosis. We could demonstrate that the transcription of estrogen-metabolizing enzymes is maintained in human endometrial tissue cultured in nude mice and is regulated by the exogenously applied drugs danazol, dydrogesterone and medroxyprogesterone acetate, which are used in the treatment of endometriosis and are known to interact with the estrogen metabolism, as well as by an aromatase inhibitor (Gruemmer et al., 2005).

In contrast to the homologous rodent model, mice used in the heterologous models have a deficient immune system and thus are hardly used in studies evaluating the effect of immune-modulating drugs. Application of an inhibitor of COX-2, which is known to be up-regulated at sites of inflammation, had no influence on the number or size of ectopic endometrial lesions in nude mice (Hull et al., 2005). However, anti-inflammatory components, contained for example in peritoneal fluid of women, could be investigated in this model. Tabibzadeh et al. (1999) demonstrated that endometrial cells suspended in peritoneal fluid of women without endometriosis established significantly less ectopic lesions than those suspended in peritoneal fluid of women with endometriosis after intraperitoneal injection into nude mice.

**Evaluation of the heterologous model**

This model shares the advantages described for the homologous model, such as easy availability and low costs, as well as some of the limitations such as differences between rodent and human physiology. However, it does not provide an intact immune system. Yet, the heterologous mouse model for endometriosis represents a promising tool for experimental approaches evaluating the aetiology of this disease and for therapeutic testing of pharmacological and hormonal modulations because it is based on human endometrium. For these investigations, human endometrial tissue can be taken from women with and without endometriosis as well as from endometriotic lesions. Regulatory mechanisms, for example, after drug administration, can be evaluated in the human ectopic tissue in an in vivo situation. Long-term studies that are not feasible in women can be performed, and the hormonal status of the human menstrual cycle can be mimicked in ovarioctomized animals.

Recently established methods using fluorescence allow in vivo imaging and may improve the quantification of both size and number of the ectopic lesions. In addition, techniques using magnetic resonance imaging (MRI) for in vivo monitoring of the lesions are in progress.

**Primate models**

**Characterization of the primate model**

Menstruating primates develop spontaneous endometriosis, establishing ectopic lesions histologically identical and at similar sites compared with the human disease (MacKenzie and Casey, 1975; D’Hooghe et al., 1991; Dick et al., 2003). Spontaneous endometriosis does not develop with a high frequency in all monkeys but could be shown to increase with time in capture possibly because of less pregnancies leading to a higher number of consecutive
menstrual cycles with increased exposure to retrograde menstruation (D’Hooghe et al., 1996a). Although spontaneous endometriosis has been reported for 11 species of non-human primates (for review, see Story and Kennedy, 2004), most studies have been performed in rhesus macaques and baboons.

Because spontaneous endometriosis in monkeys develops slowly over a period of several years, methods have been employed for the artificial induction of this disease. In the first studies, menstrual flow was diverted into the abdomen by repositioning the cervix of rhesus monkeys (Te Linde and Scott, 1950) and later by occluding the cervix to increase the amount of retrograde menstruation in baboons (D’Hooghe et al., 1994). In addition, surgical induction of the disease can be performed by suturing fragments of endometrial tissue at ectopic sites or by seeding the peritoneal cavity with fragments of endometrial tissue (D’Hooghe et al., 1995b; Yang et al., 2000; Fazleabas et al., 2002). D’Hooghe et al. (1995b) induced endometriosis in a baboon model by intraperitoneal injection of endometrial tissue, demonstrating that endometriosis developed similarly to that observed in the spontaneous disease and that ectopic lesions were induced more efficiently by inoculating menstrual endometrium compared with luteal phase endometrium. Although, in this study, menstrual endometrium was extracted from each baboon by uterine curettage, thus also containing endometrial cell layers not physiologically shed during menstruation, other studies used shed menstrual endometrium obtained using a pipelle (Fazleabas et al., 2002, 2003). By this means, induced ectopic endometrial lesions in the baboon could be shown to express estrogen receptor-β, MMP-7, VEGF and aromatase (Fazleabas et al., 2002, 2003). Ongoing efforts focus on the non-invasive diagnosis for the identification of intraperitoneal lesions using MRI (Zondervan et al., 2004).

These methods of induced endometriosis in non-human primates have been extensively reviewed previously (D’Hooghe, 1997; D’Hooghe and Debrock, 2002; Story and Kennedy, 2004; Fazleabas, 2005).

Application of the primate model

Many studies have been performed evaluating spontaneous endometriosis on the one hand and using artificially induced endometriosis on the other hand.

It could be shown that elevated estrogen levels as well as frequent hysteroscopies, but not laparoscopies, lead to an increased risk for the development of spontaneous endometriosis (Hadfield et al., 1997) and that genetic predisposition also seems to contribute to a higher risk for this disease (Zondervan et al., 2004).

Regression of peritoneal endometriotic lesions could be demonstrated after the application of progesterone receptor modulators, either alone or in combination with a GnRH analogue, in induced endometriosis in cynomolgous monkeys (Grow et al., 1996). The influence of environmental compounds on the development of spontaneous endometriosis was proven by exposure of rhesus monkeys to dioxin or polychlorinated biphenyls (PCBs), which resulted in a dose-dependent increase in incidence and severity of endometriosis (Rier et al., 1993; Vanden Heuvel et al., 1994; DeVito et al., 1995).

The primate model is well suited to investigate the role of the immune system on the development and progression of endometriosis. Subclinical peritoneal inflammation occurs in baboons during menstruation as well as after the intrapelvic injection of endometrium, as demonstrated by an increase in peritoneal fluid leukocyte concentration and the amount of peritoneal fluid cells positive for TNF-α, transforming growth factor (TGF-β1) and certain leukocyte markers (D’Hooghe et al., 2001). It was shown that immunosuppression led to an increase in the number and size of spontaneously developed endometriotic lesions (D’Hooghe et al., 1995a). Neutralization of TNF-α with r-hTBP-1 inhibits the development of endometriotic lesions and adhesions after the artificial induction of the disease (D’Hooghe et al., 2006), and a decrease in active red lesion number and surface area was observed in female baboons with spontaneous peritoneal endometriosis after neutralizing TNF activity by treatment with etanercept (Barrier et al., 2004).

With regard to the impact of endometriosis on fertility, it was proven that mild-to-severe endometriosis leads to lower pregnancy rates in baboons (D’Hooghe et al., 1996b) as well as to a reduction in chemical and term pregnancy rates in another monkey species, Macaca fascicularis (Schenken et al., 1984). Whether these effects on fertility are due to peritoneal adhesions and/or to immunological responses is still to be investigated. In addition, a feedback effect of the endometriotic lesions on the eutopic endometrium is discussed, and it has to be evaluated whether the differences in fecundity result in, or are the result of, endometriosis. Whether the peritoneal presence of ectopic implantation sites influences the physiology of the eutopic endometrium can effectively be investigated in the primate model. Recently, an increase in the angiogenic factor Cyr61, which is known to be deregulated in the endometrium of women with endometriosis (Absenger et al., 2004), could be demonstrated in the eutopic endometrium of baboons following peritoneal inoculation with menstrual endometrium (Gashaw et al., 2006). This study provides evidence for a feedback mechanism from established lesions to induce a shift in gene expression patterns in the eutopic endometrium in the baboon.

Evaluation of the primate model

There is no doubt that primate models for endometriosis are most closely related to the situation in humans and thus are most appropriate to investigate the numerous aspects of this disease, including the initiation and progression of endometriosis, immunological parameters, inheritance patterns as well as feedback mechanisms on the eutopic endometrium. They reveal many similarities to women with respect to pelvic anatomy, reproductive physiological characteristics and immunological features. Primates develop endometriosis spontaneously, and the disease can also be induced for research purposes. They represent the only experimental model, allowing the comparison of parameters in spontaneous endometriosis with parameters before and after disease induction, thus gaining information about cause and effect of the disease. However, ethical considerations and particularly the high costs of performing experiments using primates limit the use of this model in endometriosis research.

Conclusions

Animal models of endometriosis are of extreme value and indispensable for the evaluation of pathophysiological mechanisms
underlying the development of this prevalent gynaecological disease. This review summarizes different commonly used animal models, their potentials limitations. These have to be weighted up when designing experimental approaches with regard to their applicability, for example, considering species-specific characteristics of metabolism or reproductive physiology, as well as to their cost–effect ratio. New insights into the aetiology of endometriosis will be obtained using genomic and proteomic approaches. In this regard, transgenic mice models might gain importance as models of endometriosis to examine functions driven by single genes. In general, animal models will help to develop novel non-invasive diagnostic tools and improved therapeutical approaches for a better treatment of endometriosis in women.

References


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