Peritoneal endometriosis: validation of an in-vivo model

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BACKGROUND: The current medical treatment of endometriosis, a common gynaecological disease, is still associated with a high recurrence rate. To establish an appropriate in-vivo model to evaluate new therapeutic strategies we validated the nude mouse model for the intraperitoneal cultivation of human endometrial tissue. METHODS: Human endometrium of the proliferative phase was implanted into the peritoneal cavity of normal cycling and ovariectomized athymic mice and of cycling non-obese diabetic (NOD)-severe combined immunodeficiency (SCID) mice. Morphology, proliferation, differentiation, and angiogenesis in the ectopic endometrium at different time points after implantation was investigated. RESULTS: Adhesion of endometrial fragments was observed from day 2 onwards. The lesions persisted for up to 28 days revealing a well preserved glandular morphology. The glandular epithelium maintained cytokeratin expression even after 14 days of culture. With progressing culture, glands exhibited vimentin staining in combination with a decrease of surrounding stromal cells. Proliferation of glandular epithelium could be demonstrated throughout the investigated period of 28 days, whereas expression of oestrogen and progesterone receptors was maintained only in endometriotic lesions grown in cycling but not in ovariectomized mice. Neoangiogenesis occurred from day 4 onwards, independent from the intraperitoneal localization of the ectopic lesions. CONCLUSIONS: This in-vivo model is a promising tool to test the effect of compounds such as different hormone agonists/antagonists or anti-angiogenic factors to develop new therapeutic concepts in endometriosis.

Key words: angiogenesis/ectopic endometrium/endometriosis/NOD-SCID mouse/nude mouse

Introduction

Endometriosis is a common disorder often associated with pelvic pain, dysmenorrhoea and infertility. Reappearance of endometriotic lesions occurs with high incidence one year after apparently successful hormonal and/or surgical treatment (Wheeler et al., 1993). Since the reflux implantation theory due to retrograde menstruation is the most accepted theory for development of endometriotic lesions a non-appropriate differentiation of the eutopic endometrium of these patients could be a reason for this high recurrence rate. This idea is supported by the fact that endometriosis is accompanied by infertility in nearly 60% of the patients (Ledger, 1999). Though retrograde menstruation is a common event in women (Liu et al., 1986), it does not consequently lead to the development of lesions. It remains to be elucidated which cell biological mechanisms are responsible for adhesion and invasion of the shed endometrial fragments. In addition, mechanisms which keep the fragments alive and growing need to be investigated. It has been suggested that a non appropriate expression of integrin molecules in the eutopic endometrium of affected women could be responsible for the adhesion capacities of the peritoneal surface (Lessey and Young, 1997; Regidor et al., 1998) and a loss of the cell adhesion complex, E-cadherin and catenins, within these glandular cells (Gaetje et al., 1997; Scotti et al., 2000) could serve as a precondition for invasion properties of endometrial cells. However, the often contradictory cell biology characteristics of endometriotic lesions described in the literature makes it difficult to get more insight into the pathogenesis of this disorder (Guidic et al., 1998).

To design new strategies for therapy, the mechanisms for proliferation, neoangiogenesis and hormonal and immunological responsiveness need to be analysed. Since occurrence of spontaneous endometriosis is dependent on menstruation, the development of this disease is restricted to humans and subhuman primates. However, monkeys which could be used as a model (Sillel et al., 1996) have limited value. For this reason, animal models have been developed in recent years to investigate the pathomechanism and to validate therapeutic concepts of endometriosis. Several studies reported that human endometrial tissue transplanted into nude or severe combined immune deficiency (SCID) mice seems to be a valid and appropriate model to study the pathophysiology of endometriosis (Zamah et al., 1984; Bergqvist et al., 1985;
Aoki *et al.*, 1994; Bruner *et al.*, 1997; Awward *et al.*, 1999). These authors have shown that human endometrial tissue or cells grafted into the peritoneal cavity of immunodeficient mice have the ability to implant and to develop lesions with well preserved endometrial glandular tissue. Recently, it was reported (Tabibzadeh *et al.*, 1999) that adhesion and implantation of the endometrial cells is increased in this model when peritoneal fluid from endometriotic patients was used and was even more effective when human blood lymphocytes were injected into the peritoneal cavity. It could be shown that such ectopic endometrial tissues grown in nude mice developed a vascular network combined with a high vascular endothelial growth factor content within these ectopic fragments (Nisolle *et al.*, 2000).

The present study describes the temporal pattern of adhesion and development of the grafted endometrial fragments. We could show that fragments keep their hormonal receptors when transplanted into cycling mice indicating hormonal responsiveness and that neoangiogenesis seems to be a major event in early development of endometriotic lesions. These investigations confirm that implantation of human endometrium into the peritoneal cavity of athymic mice is a convincing model for the study of endometriosis.

**Materials and methods**

**Human endometrial tissue**

Endometrium of the proliferative phase of the menstrual cycle was obtained from premenopausal women (aged 21–44 years) undergoing endometrial biopsy or hysterectomy at the Department of Gynaecology, University Hospital Essen. Endometrial dating was performed using established criteria (Noyes *et al.*, 1950). Fresh endometrial tissue was collected in Moscona solution (Gibco) and cut into fragments of 1–2 mm in diameter under sterile conditions.

Some of the fragments were cultured in petri dishes on semi-solid agar medium [2% agar solution in 50% aqua dest., 25% Dulbecco’s modified Eagle medium (DMEM), Dulbecco, 25% Ham’s F12, Gibco] covered with culture medium [DMEM + Ham’s F12 (1:1) + 10% FCS] supplemented with oestradiol-17β (10^−9 mol/l) for 24 h prior to transplantation into nude mice.

**Transplantation of endometrial fragments into mice**

**Animals**

Athymic nude mice (Han:NMRI nu/nu) and non-obese diabetic (NOD)-SCID mice (Shultz *et al.*, 1995) were maintained in a barrier except that the primary antibody was omitted. To show the tissue unit in a well-controlled pathogen-free environment with regulated morphology and to confirm both orientation and localization parallel cycles of light/dark (12 h/12 h). All equipment and food entering the barrier was autoclaved. Mice had free access to food and water ad libitum.

**Transplantation of fragments and tissue processing**

Fragments of human endometrium were implanted into the peritoneal cavity of nude mice and NOD-SCID mice and cultivated up to 21 and 28 days, respectively. For each time point a minimum of three mice were transplanted with 10 fragments each. Endometrial fragments were placed by laparotomy into the endometrial cavity of nude and NOD-SCID mice in the region of gut, lateral abdominal wall, liver and mesenterium. In another set of experiments three fragments per mouse were fixed with surgical sutures (Ethicon 6/0, Johnson and Johnson, Belgium) to the outer gut wall, the mesenterium and the muscle layer of the lateral abdominal wall of nude mice, one fragment at each location.

At the end point of each experiment, mice were killed by cervical dislocation. Implanted endometrial lesions were dissected by laparotomy and were either frozen directly in liquid nitrogen or fixed in 10% formalin and routinely embedded in paraffin for morphological and immunohistochemical analysis.

**Hormonal treatment**

Nude mice were bilaterally ovariectomized and left untreated for at least 14 days. 17-β-oestradiol (oestradiol; Sigma, Germany), was dissolved in benzyl-benzoate and 0.2 µg/mouse were administered s.c. in a volume of 200 µl arachis oil. Each animal received one s.c. oestradiol injection per day from the time of transplantation onwards during the course of the experiment. Vehicle only was injected into animals of the control groups using the same experimental design.

**Immunohistochemistry**

Immunostaining for panendothelial antigen was performed on ethanol-fixed cryostat sections from freshly frozen tissues, all other immunohistochemical reactions were performed on paraffin sections. All sections were rinsed in phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) to reduce nonspecific antibody binding, endogenous peroxidase activity was blocked with 3% hydrogen peroxide. After washing in PBS, the sections were incubated for 1 h at room temperature with the following primary antibodies: Endothelia of mouse vessels were stained with a rat anti-mouse panendothelial cell antigen antibody (MECA-32; Pharmingen, Hamburg, 1:100), vessels of human origin with anti-von-Willebrand Factor (A082; Dako Denmark, 1:200). Staining of human endometrial epithelium and stroma was performed using anti-human cyto-keratin (M0821, Dako, Denmark, 1:100) and anti-human vimentin (M0725; Dako, Denmark, 1:100), respectively. Proliferation of endometrial glandular epithelial cells was evaluated using a monoclonal anti-Ki-67 antibody (MIB-1; DiaSorin, Dianova, Germany, 1:100) and human steroid hormone receptors by mouse monoclonal anti-human oestrogen alpha receptor (MU272-UC; Bio Genex, 1:50) and anti-human progesterone receptor (PR88; Bio Genex, undiluted). The following secondary antibodies were used: biotinylated rabbit anti-rat antibody (E0468; Dako, Denmark) for panendothelial cell antigen, biotinylated goat anti-rabbit immunoglobulin (E0432; Dako, Denmark) for anti-von-Willebrand Factor, LSAB plus kit (K0690; Dako, Denmark) for Ki-67, anti-cytokeratin and anti-vimentin, and a biotinylated anti-mouse IgG (HK330–5K; Bio Genex) for steroid hormone receptors.

The chromogenic reaction was carried out by incubating the sections with the peroxidase substrate 3,3′-diaminobenzidine for 5 min; sections were rinsed in PBS, dehydrated in ascending ethanol concentrations and coverslipped. To demonstrate specificity of the staining, consecutive sections were stained with the same protocol, except that the primary antibody was omitted. To show the tissue morphology and to confirm both orientation and localization parallel sections were stained with haematoxylin-eosin. Results were recorded with a Zeiss Axiophot photomicroscope.

**Proliferation studies**

For each experimental approach percentage of the Ki-67 positive fraction of 3–6 endometrial glands of three different endometrial fragments was analysed. Statistical analysis was performed using the ANOVA with Scheffe’s test. Differences were considered significant when P ≤ 0.05.

**Results**

**Localization of endometriotic lesions**

Peritoneal adhesion of endometrial fragments occurred 2 days after implantation (Table I). In nude mice as well as in NOD-
SCID mice 33–66% of transplanted non-fixed endometrial fragments could be rediscovered after a culture period of 21 days independent of hormonal treatment of mice, in NOD-SCID mice 37% were obtained even after 28 days. Sites of implantation of the endometrial fragments were preferentially the gut (28%) and the skeletal muscle of the abdominal side wall (36%), but attachment could also be observed to the liver (18%) and the adipose fat surrounding abdominal organs (18%). In-vitro culturing of human endometrial fragments for 24 h prior to transplantation into the peritoneal cavity of nude mice led to a clearly reduced rediscovery-rate of endometriotic lesions (16% on day 13, Table I). Fixation of the human endometrial fragments by suturing to the outer gut wall, the mesenteria, and the abdominal side wall of nude mice led to a rediscovery of 100% up to 9 days, and 66% of the fragments could still be detected 21 days after transplantation (Table I). Fixed as well as non-fixed fragments attached firmly to the host tissue and even revealed invasive behaviour since endometrial stromal cells could be observed growing between the cells of the host tissue e.g. between the muscle fibres of the abdominal side wall (not shown).

**Morphology of endometriotic lesions**

Endometriotic lesions showed a well preserved morphology throughout all stages investigated up to 21 days in nude mice and 28 days in NOD-SCID mice. The typical endometrial glands were lined by cylindrical or cuboidal epithelial cells and were surrounded by cellular stroma. In fragments grown in nude mice the glandular morphology changed from day 9 of culturing onwards, demonstrating dilated, alveolar structures of variable size, still lined by cuboidal to flat epithelial cells (Figure 1a,b). This development of a cyst-like appearance was independent of hormonal treatment or intraperitoneal localization of the fragments. The endometriotic lesions grown in NOD-SCID mice, however, showed a slightly better preserved morphology showing almost no large cysts but intact endometrial glands with cylindrical epithelial lining even after culturing for 28 days (Figure 1c).

No difference in morphology could be observed, either between endometrial fragments which had been fixed by a surgical suture and those which were unixed, or between fragments grown in untreated versus oestradiol supplemented ovariectomized mice. In contrast to these findings, morphology of endometriotic lesions which had been pre-cultured in-vitro for 24 h prior to transplantation was less well preserved showing flattened glandular epithelium, fewer stromal cells and more signs of necrosis (not shown).

Lymphocyte infiltration was found within the endometriotic lesions grown in nude mice to a different extent but was not greater in fragments which had been fixed by suturing and was not decreased by in-vitro pre-culturing of the fragments.

**Proliferation and differentiation markers**

**Expression of cytokeratin and vimentin**

Immunohistochemical staining using antibodies specific for human cytokeratin and vimentin led to a clear distinction between tissue of mouse and human origin, respectively. Intense cytokeratin staining of the glandular epithelial lining was observed exclusively in the human endometrial implants (Figure 1d,e) and maintained even after 14 days of culturing in nude mice (Figure 1f,g). Vimentin staining was found in the stromal but not the epithelial compartment of endometriotic fragments which had been cultured in nude mice for up to 9 days (Figure 1h). After 14 days of culturing, however, epithelial cells expressed vimentin in addition to cytokeratin (Figure 1i), indicating a dedifferentiation process of the epithelium. This expression of the additional intermediate filament vimentin in glandular epithelium was accompanied by a reorganisation of the stroma indicated by a decrease in the number of surrounding stromal cells and increase in intercellular matrix and fibrils. In those regions with a high amount of stromal cells indicated by a strong vimentin staining, glandular epithelial cells obviously maintained their differentiation status even after culturing for more than 14 days and revealed no vimentin staining (Figure 1k).

**Expression of steroid hormone receptors**

Oestrogen receptors were expressed in the glandular epithelia and in stromal cells of endometriotic lesions cultured for up to 14 and 28 days in cycling nude mice (Figure 2c) and NOD-SCID-mice (Figure 2a), respectively, whereas progesterone receptors could be detected mainly in glandular epithelial cells but only sparsely in the stromal compartment (Figure 2b). Steroid hormone receptors were preserved for a longer time period in endometrial tissue cultured in NOD-SCID mice compared to using nude mice as a host (Table II). Endometriotic lesions grown in untreated or oestrogen-treated ovariectomized nude mice revealed no staining for either oestrogen nor progesterone receptors (Table II).

**Proliferation of endometrial glandular cells**

Glandular epithelium of freshly explanted endometrial tissue showed an average Ki-67 positive fraction of 30.5%. Proliferation in cultured endometrial fragments showed a high variance within the experimental groups. The average percentage of Ki-67 positive cells, however, stayed in this range during the first week in NOD-SCID mice as well as in the different experimental groups of nude mice (Table II) showing no significant difference between the different experimental groups or to freshly explanted tissue. The amount of Ki-67 positive cells remained high even after 21 days of culture in untreated NOD-SCID and nude mice as well as in ovariectomized nude mice supplemented with oestrogen, whereas

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E2 = oestradiol; OVX = ovariectomized; n.d. = not done.
In-vivo model for peritoneal endometriosis

Figure 1. Histology of endometriotic lesions transplanted into the peritoneal cavity of nude and NOD-SCID mice, respectively. (a) Large cyst-like glands (EG) can be observed within an endometriotic lesion (E) attached to the musculature (M) of the abdominal side-wall of a nude mouse after 12 days. Whereas such cystic endometrial glands often are lined by flattened epithelium (b), cylindric glandular epithelia of endometriotic fragments are well preserved in NOD-SCID mice even 21 days after implantation (c). Endometrial glandular epithelium reveals a specific staining for human cytokeratin from day 2 (e) up to day 14 (g) after implantation. (d, f: parallel sections stained with haematoxylin-eosin). (b) Vimentin expression cannot be seen in the glandular epithelium after 2 days of culturing. (i) After 14 days, however, some glandular epithelial cells surrounded by cell-free stroma exhibit staining for vimentin in addition to cytokeratin. (k) Glandular epithelium surrounded by stroma containing numerous cells, as indicated by vimentin staining, does not express vimentin itself. (E = endometriotic lesion; EG = endometrial gland; S = endometrial stroma; F = mouse fat tissue; G = gut; M = muscle of the abdominal wall; * = surgical suture). Bars: a = 250 µm, b,i,k = 30 µm; c = 200 µm; e = 150 µm; h = 50 µm.
Figure 2. (a) Expression of oestrogen receptor is observed in the stroma (S) and in the glandular epithelial cells (EC) of endometriotic lesions after 28 days of culturing in NOD-SCID mice, whereas progesterone receptors are mainly expressed in the glandular epithelial cells (b). (c) Expression of oestrogen receptors in the glandular endometrial epithelium (EG) and stromal cells of endometriotic lesions grown in nude mice for 14 days. (d) Staining of the Ki-67 positive fraction in glandular epithelial cells of an endometriotic lesion grown in nude mice for 14 days. Bars: a,b,c = 100 µm; d = 50 µm.

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<th>Table II. Proliferation and expression of oestrogen receptors (ER) and progesterone receptors (PR) in endometriotic lesions</th>
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*Significance P ≤ 0.05.

endometrial glands of fragments grown in untreated ovariectomized mice showed a significant reduction in Ki-67 positive cells after 21 days of culture (Table II).

**Angiogenesis**

After 1 week of culturing in nude mice, numerous vessels could be observed in haematoxylin-eosin stained paraffin sections throughout the endometrial fragments (Figure 3a) as well as crossing the tunica muscularis of the gut in direction to the human endometrial tissue (Figure 3b). Staining of cryostat sections with an anti-mouse panendothelial antibody revealed that those vessels were of mouse origin. Whereas no staining for mouse endothelial cells could be detected in endometriotic lesions after 2 days of culturing (Figure 3c), neoangiogenesis into the endometrial tissue attached to the liver (Figure 3d), the adipose fat (Figure 3e), and the gut (Figure 3f) appeared from day 4 post-implantation onwards. The extent of angiogenesis, however, seemed not to be correlated to the localization of the endometriotic lesions within the peritoneal cavity.

In freshly explanted endometrium as well as in the ectopic lesions grown in nude mice the endothelial lining of vessels of endometrial origin was stained for von-Willebrand Factor (Figure 3g), however, with increasing time of culturing in
nude mice, the amount of vessels that were stained decreased (Figure 3h) and staining for von-Willebrand Factor was lost in the fragments from day 12 after implantation onwards.

**Discussion**

The present study validates an in-vivo model for the study of endometriosis. It has been demonstrated previously that the basic morphology of human endometrial fragments is maintained after successful transplantation either s.c. (Zamah et al., 1984; Bergqvist et al., 1985; Zaino et al., 1985; Aoki et al., 1994) or intraperitoneally (Zamah et al., 1984; Bruner et al., 1997; Awwad et al., 1999; Tabibzadeh et al., 1999) into nude and SCID mice. This study extends these observations by characterizing the development and maintenance of endometrial fragments. For this reason we focused on morphology, differentiation parameters and angiogenesis of endometrial fragments located at different peritoneal sites to validate the appropriate time span for experimental use. Human endometrial fragments were transplanted into the peritoneal cavity of athymic nude mice as well as of NOD-SCID mice which show a reduced immunological response as

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**Figure 3.** Angiogenesis in endometrial fragments grown in nude mice. (a) Numerous vessels (arrow) in an endometrial fragment attached to the gut 7 days after implantation. (b) Host vessels (V) can be observed crossing the tunica muscularis of the gut into the human endometrial tissue. (c) Staining with an anti-mouse panendothelial antibody reveals no vessels in the endometriotic lesion attached to liver after 2 days, but a clear ingrowth of vessels into fragments attached to the liver after 7 days (d), and to the adipose fat (e) and to the gut (f) after 4 days. (g) Vessels of endometrial origin stain for von-Willebrand Factor 2 days after transplantation into nude mice, while staining can hardly be detected after 9 days (h). (E = endometriotic lesion; EG = endometrial gland; L = mouse liver; F = mouse fat tissue; G = mouse gut; * = surgical suture). Bars: a = 150 µm; b,g = 75 µm; c,d,e = 250 µm; f,h = 200 µm.
they lack functional lymphoid cells and show a markedly reduced NK cell activity (Shultz et al., 1995), leading to a better preservation of implanted human tissue. In both animal groups adhesion of transplanted endometrial fragments took place from day 2 after transplantation onwards and persistence of ectopic lesions was observed up to 21 days in nude mice and up to 28 days in NOD-SCID mice showing a well preserved morphology. Thus, this model offers the opportunity to investigate very early interactions between the endometrial fragments and the host tissue and to perform investigations on the development of the endometriotic lesions.

In contrast to previous observations (Awad et al., 1999; Tabibzadeh et al., 1999; Nisolle et al., 2000) which describe adhesion of endometrial fragments mainly at the side wall and abdominal fat tissue, this study in addition observed implanted endometrial fragments at the gut and the liver. The difference in the implantation sites between the human (endometriotic lesions mainly in the region of the Pouch of Douglas attached for example to the peritoneum of the bladder, ovary, rectum, or oviduct) and the mouse (attachment of endometrial fragments mainly to the liver, intestine and abdominal side wall) can be explained by the different direction of gravity action between the quadruped mouse and the upright position of humans.

The comparable high survival rate of endometrial fragments described in this study may be due to the use of fresh, untreated human endometrial tissue without enzymatic pre-treatment in contrast to investigations performed previously, injecting endometrial tissue suspended with collagenase (Tabibzadeh et al., 1999). However, it has to be taken into account that the high adhesion rate of fragments could also be a result of the exposure of the mesothelial lining to air and surgical manipulations that lead to damage of the mesothelium and by this facilitate attachment of the endometrial tissue. A clear improvement in the adhesion rate could be achieved by fixing the fragments without impairment in morphology. Thus, this method may be very well suited for investigations of drug effects on progression of endometriosis. In contrast to the findings of other studies using precultured endometrial fragments (Bruner et al., 1997; Nisolle et al., 2000), short-time culture of endometrial tissue compromised the ability of the fragments to establish viable ectopic lesions in our study.

The implantation rate as well as preservation of morphology of endometrial lesions was not dependent on the availability of ovarian hormones. This corresponds to previous results describing that the growth of human endometrium was unaffected by the oestrous stage of the animal (Aoki et al., 1994) and that no difference could be observed in the development of necrosis of the transplants in unsupplemented versus oestradiol-supplemented mice (Zamah et al., 1984). Treatment of cycling mice with additional progesterone has been described to be associated with larger implants and the presence of fluid-filled endometrial glands referring to the preservation of secretory capacity of these implants (Awad et al., 1999), a phenomenon which we could observe in endometrial fragments grown in untreated cycling nude mice for the same time period. Substitution of oestrogen alone maintained proliferation of glandular epithelium in the ectopic human tissue for up to 28 days corresponding to observations in eutopic endometrial epithelium (Kimura et al., 1978; Gerschenson et al., 1984).

An influence of ovarian hormones could also be observed in correlation with the preservation of steroid hormone receptors. Expression of oestrogen receptor alpha and progestrone receptor was maintained for up to 28 days in endometriotic lesions grown in cycling but not in ovariectomized mice. Thus proliferation as well as expression of hormone receptors is reduced in untreated ovariectomized mice while rate of persistence of the lesions seems to be similar. This is in agreement with clinical observations that the extent of lesions in patients treated with GnRH analogue decreases while the maintenance of the lesions is not affected (Schindler et al., 1994). This survival of the ectopic endometrial lesions might explain the high recurrence rate in patients with endometriosis.

Expression of cytokeratin in the glandular epithelium indicated that the human endometrium conserved specific structural cellular patterns throughout the process of implantation. Interestingly, after 14 days of culture, some of the glands in nude mice exhibited vimentin in addition to cytokeratin staining. This phenomenon could be observed only in parallel with a decrease in surrounding stromal cells. Obviously, the preservation of specific endometrial stroma is necessary for the maintenance of the normal expression pattern of the glandular epithelium, confirming the importance of the interaction between the stromal and the epithelial compartment (Cooke et al., 1997). These results confirm previous observations that epithelial cells keep their original morphology only in areas where the stroma was highly developed whereas the glandular epithelium revealed a flattened morphology when surrounded by sparsely developed stroma (Nisolle et al., 2000).

The establishment of a new blood supply is essential for the survival of the endometrial implants and the development of endometriosis. Recent investigations in humans confirmed the importance of angiogenesis for the pathogenesis of endometriosis (Donnez et al., 1998; Abulafia and Scherer 1999; Fujishita et al., 1999). Angiogenesis of mouse vessels into the endometrial fragments occurred from day 4 onwards, independent of the localization of the ectopic lesions. It has been suggested that the expression of vascular endothelial growth factor (VEGF) might provoke an increase of the subperitoneal vascular network and facilitate implantation and viability of endometriotic lesions (Shifren et al., 1996; Fujimoto et al., 1999). A network of capillaries has been demonstrated in the stromal compartment of endometrial fragments grafted into the peritoneal pouch of nude mice after 3 weeks of cultivation in combination with VEGF positive stained cells in the glandular epithelium, and to a lesser extent in the stromal compartment of the endometrial grafts (Nisolle et al., 2000). Excessive angiogenesis suggests novel medical treatments for endometriosis aimed at the inhibition of angiogenesis, and further studies are needed to analyse the cell biological mechanisms and the role of angiogenesis, e.g. by inhibitors of angiogenesis for the development and persistence of endometriosis.

In conclusion, the nude mouse model is a model well suited to the study of endometriosis, as endometrial implants retain morphological characteristics and differentiation para-
meters for up to 14 days of intraperitoneal culture in nude mice, and for up to at least 28 days in NOD-SCID mice. This model allows investigation of the very early events of the cell biological mechanisms involved in the implantation of endometrial fragments, since attachment occurs 2 days after implantation and in-growth of numerous blood vessels can be observed after 4 days. For longer term experiments exceeding 3 weeks the NOD-SCID model provides advantages since endometriotic fragments reveal a better morphological preservation—possibly due to a reduced immunological response—as well as a good maintenance of proliferation rate and steroid hormone receptor status.

Thus, human endometrial tissue grown in the peritoneal cavity of nude mice is a very valuable tool to test the effect of compounds such as different (anti-) hormones or anti-angiogenic factors and to develop new therapeutic concepts in endometriosis.

Acknowledgements

The authors thank Gabriele Luhn, Georgia Rauter and Kathrin Ratajczak for excellent technical assistance, Johannes Husing for statistical analysis and Dave Kittel for preparation of illustrations.

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Zamah, N.M., Dodson, M.G., Stephens, L.C. et al. (1998) Vascular endothelial growth factor is a very valuable tool to test the effect of various compounds such as different (anti-) hormones or anti-angiogenic factors and to develop new therapeutic concepts in endometriosis.

Received on January 19, 2001; accepted on April 26, 2001