Size and spatial orientation of uterine tissue transplants on the peritoneum crucially determine the growth and cyst formation of endometriosis-like lesions in mice

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**BACKGROUND:** In many studies in rodents, intraperitoneal endometriosis-like lesions are surgically induced by syngeneic or autologous transplantation of uterine tissue samples, which are sutured to the abdominal wall. However, until now the surgical techniques have not been standardized, and we address this issue here.

**METHODS:** Uterine tissue samples were transplanted to the peritoneum of C57BL/6 mice (four study groups, n = 7 each). Using non-invasive high-resolution ultrasound imaging over a period of 4 weeks, we analyzed growth characteristics and cyst formation of the endometriosis-like lesions which developed, in relation to mode of transplantation (syngeneic versus autologous), type of tissue fixed adjacent to the peritoneum (endometrium versus perimetrium), and size of tissue transplanted (2 versus 3 mm). Immunohistochemical analysis was also performed.

**RESULTS:** When the perimetrium, with underlying myometrium, was sutured next to the host peritoneum the endometriosis-like lesions which developed exhibited a higher growth rate (P < 0.05 versus endometrium), and contained more proliferating cell nuclear antigen (PCNA)-positive cells and an increased microvessel density (both P < 0.05 versus endometrium). In the group with 3 mm uterine tissue grafts, lesion growth was significantly decreased when compared with 2 mm samples (P < 0.05). However, the larger grafts developed more cysts throughout the observation period than the smaller ones. There was no difference between syngeneic and autologous endometriosis-like lesions.

**CONCLUSIONS:** Our study demonstrates that size and spatial orientation of peritoneally fixed uterine tissue samples crucially determine growth and cyst formation of endometriotic lesions in mice. These findings should improve the standardization and reliability of future studies, performed in the frequently used mouse model of surgically induced endometriosis.

**Key words:** Endometriosis / ultrasound / animal model / transplantation / perimetrium

**Introduction**

Endometriosis, i.e. the presence and proliferation of endometrial glands and stroma outside the uterine cavity, represents one of the most common benign gynecological disorders which affects ~10% of women of reproductive age (Wheeler, 1989; Cramer and Missmer, 2002). The most widely accepted theory for the development of peritoneal endometriotic lesions is the implantation theory of Sampson (1927), postulating that endometrial fragments reach the peritoneal cavity by retrograde menstruation. Thus, endometriosis occurs naturally only in menstruating humans and some non-human primates, and not in rodents (Grümmner, 2006). Genetically well defined rodent models, however, are important tools in endometriosis research because they allow for a standardized analysis of new molecular targets for future medical therapies. Therefore, endometriosis-like lesions are induced iatrogenically in rodents by transplanting endometrial tissue to ectopic sites (Laschke and Menger, 2007; Laschke et al., 2008).

Endometrial tissue transplantation is either syngeneic, when using a donor and recipient animal of identical genotype (Somigliana et al., 1999; Bacci et al., 2009), or autologous, when transplantation involves only one animal, and is from, for example, the uterus to the peritoneal
cavity (Fainaru et al., 2008). Both approaches may affect the nature of the establishment of endometriosis-like lesions. In the case of syngeneic transplantation, differences in estrogen levels between individual mice may influence the growth of the lesions. On the other hand, resection of a uterine horn in the autologous model is associated with surgically induced inflammation and wound healing, which may result in a non-physiological composition of peritoneal fluid that subsequently affects the engraftment of endometrial tissue inside the peritoneal cavity.

In addition, various techniques for endometrial tissue transplantation have been described in the past. For example, endometrial tissue fragments may be gently peeled from the myometrium and subsequently injected as a suspension into the abdomen (Somigliana et al., 1999; Hirata et al., 2005). However, this approach bears the major disadvantage that it is quite difficult and time consuming to detect and analyze the randomly distributed endometriosis-like lesions in the peritoneal cavity, especially when non-invasive techniques, such as bioluminescence or high-resolution ultrasound imaging, are applied (Becker et al., 2006; Laschke et al., 2010). To overcome this problem, endometriosis-like lesions are often surgically induced by suturing uterine tissue samples to the peritoneal wall (Becker et al., 2006, 2008; Laschke et al., 2010): an isolated uterine horn is opened longitudinally and tissue samples of comparable size are removed by means of a dermal biopsy punch. Accordingly, these tissue samples not only consist of endometrium, but also contain the myometrium and perimetrium of the uterus wall. Thus, the establishment of endometriosis-like lesions may be affected by the spatial orientation of the transplant when sutured to the abdominal wall i.e. depending on whether the endometrium or perimetrium is in direct contact with the peritoneum. Moreover, the size of transplanted uterine tissue samples may be a crucial determinant of endometriosis-like lesion growth.

On the basis of these considerations, the aim of the present study was to compare the growth and cyst formation of intraperitoneal (i.p.) endometriosis-like lesions which were surgically induced either by syngeneic or autologous uterine tissue transplantation in mice. Furthermore, we analyzed the influence of (i) size of the uterine tissue samples transplanted, and (ii) spatial orientation of tissue fixation, on the establishment of endometriosis-like lesions. For this purpose, we used the technique of high-resolution ultrasound imaging, which allows for the repetitive and non-invasive in vivo analysis of the development of i.p. endometriosis-like lesions in mice (Laschke et al., 2010).

Materials and Methods

Animals

For the study, 12- to 16-week-old female C57BL/6 mice with a body weight of 20–25 g were used. The mice were housed one per cage and had free access to tap water and standard pellet food (Altromin, Lage, Germany). To exclude differences between individual animals related to different sex hormone levels, estrous cycles were evaluated by cytological analysis of vaginal lavage samples. For this purpose, 15 μL of 0.9% saline were carefully pipetted into the vagina and subsequently transferred to a glass slide for examination under a phase contrast microscope (CH-2; Olympus, Hamburg, Germany). Only those mice which were in the estrus stage were used as donor or recipient animals.

All experiments were approved by the local governmental animal care committee and were conducted in accordance with the German legislation on protection of animals and the NIH Guidelines for the Care and Use of Laboratory Animals (NIH Publication #85-23 Rev. 1985).

Model of i.p. endometriosis

i.p. endometriosis-like lesions were induced surgically by suturing uterine tissue samples to the abdominal wall. For syngeneic transplantation, donor mice were anesthetized by i.p. injection of ketamine (75 mg/kg body weight; Pharmacia GmbH, Erlangen, Germany) and xylazine 2% (15 mg/kg body weight; Rompun, Bayer, Leverkusen, Germany). Both uterine horns were removed and transferred to a Petri dish containing 37°C warm Dulbecco’s modified Eagle’s medium (DMEM; 10% fetal calf serum, 100 1U/mL penicillin, 0.1 mg/mL streptomycin; PAA, Colbe, Germany). In the case of autologous transplantation, only the left uterine horn of the recipient animal was transferred to DMEM. Subsequently, the uterine horns were opened longitudinally with microscissors under a stereo-microscope (M651; Leica Microsystems GmbH, Wetzlar, Germany) and tissue samples of a comparable size, either 2 or 3 mm, were removed using a dermal biopsy punch (Stiefel Laboratorium GmbH, Offenbach am Main, Germany) (Fig. 1). Then, two tissue samples were fixed with a 7-0 Prolene suture (Ethicon Products, Germany). To exclude differences between individual animals related to different sex hormone levels, estrous cycles were evaluated by cytological analysis of vaginal lavage samples. For this purpose, 15 μL of 0.9% saline were carefully pipetted into the vagina and subsequently transferred to a glass slide for examination under a phase contrast microscope (CH-2; Olympus, Hamburg, Germany). Only those mice which were in the estrus stage were used as donor or recipient animals.

Figure 1 Macroscopic appearance (A) and immunohistochemical sections (B, C) of a uterine tissue sample, isolated from a C57BL/6 mouse by means of a 2 mm dermal biopsy punch. Note that even macroscopically the endometrium (A, asterisks) can clearly be distinguished from the myometrial layer (A, arrows) with the underlying perimetrium of the uterine wall. (B) and (C) display the direction of puncture with the surgical needle in relation to the myometrial layer (stained for α-SMA), in order to fix the sample with the endometrium (B) or the perimetrium (C) adjacent to the peritoneum of the abdominal wall. Scale bars: A = 900 μm; B, C = 430 μm.
Norderstedt, Germany), one to the right and one to the left abdominal wall of the anesthetized recipient animals, through a midline incision. In all mice, the tissue samples were sutured at identical positions of the abdominal wall to help ensure that the host tissue sites exhibited a comparable vascularization. Finally, the laparotomy was closed with a 5-0 Prolene muscle and skin suture and the establishment of endometriosis-like lesions was observed over a period of 4 weeks.

**High-resolution ultrasound image acquisition and analysis**

Developing endometriosis-like lesions in mice were analyzed by means of a Vevo 770TM high-resolution in vivo micro-imaging system (VisualSonics, Toronto, ON, Canada), as described previously in detail (Laschke et al., 2010). Quantitative analysis of the ultrasound images included the determination of the overall volume of endometriosis-like lesions, their stromal tissue and their cysts (in mm³) by manual image segmentation (Laschke et al., 2010). Moreover, we calculated the growth of lesions and stromal tissue (as a percentage of the original size). Finally, for each experimental group we assessed the number of mice with lesions which contained cysts, the overall number of lesions which contained cysts and the cyst volume (mm³).

**Experimental protocol**

The mice were randomly divided into four groups, which differed in terms of the induction of endometriosis-like lesions (Table I). In Group 1, uterine tissue samples (2 mm) were transplanted (syngeneic) into seven animals, with the endometrium adjacent to the peritoneum. In Group 2 (n = 7), an autologous transplantation of 2 mm tissue samples was performed. In Groups 3 and 4 (each n = 7), 2 and 3 mm tissue samples, respectively, were transplanted (syngeneic) with the perimetreum adjacent to the peritoneum. In all groups, two tissue samples were sutured to the abdominal wall in each animal. Ultrasound image analysis of the volume of developing endometriosis-like lesions, the cysts and stroma was performed directly after tissue transplantation (day 0), as well as on days 7, 14, 21 and 28. At the end of the experiment, the mice were sacrificed with an overdose of pentobarbital and the endometriosis-like lesions were excised for further histological examinations.

In additional experiments, we analyzed the early vascularization of developing endometriosis-like lesions in relation to the spatial orientation of the transplant. For this purpose, 12 uterine tissue samples (all 2 mm) were transplanted (syngeneic) into 6 mice, with either the endometrium (n = 6) or the perimetreum (n = 6) adjacent to the peritoneum. After 7 days, the mice were sacrificed with an overdose of pentobarbital and the endometriosis-like lesions were excised for the quantitative analysis of the microvessel density within the lesions by means of immunohistochemical detection of the vessel marker CD31.

**Histology and immunohistochemistry**

For light microscopy, formalin-fixed specimens of endometriosis-like lesions were embedded in paraffin. Five-micrometer-thick sections were cut and stained with hematoxylin and eosin according to standard procedures.

Sections were immunohistochemically stained for α-smooth muscle actin (α-SMA) using a rabbit anti-α-SMA antibody as primary antibody (1:100; Sigma-Aldrich, Taufkirchen, Germany) to identify the myometrium within the transplanted uterine tissue samples. The uterine wall consists of endometrium (mucosa layer), myometrium (smooth muscle cell layer) and perimetreum (serosa layer), allowing us to confirm the fixation of perimetreum or endometrium adjacent to the peritoneum of the abdominal wall. Proliferating cells within the endometriosis-like lesions were stained for proliferating cell nuclear antigen (PCNA) using a mouse monoclonal anti-PCNA antibody as primary antibody (1:50; Daco Cytomation, Hamburg, Germany). To detect apoptotic cell death, a rabbit polyclonal anti-cleaved caspase-3 antibody (1:100; Cell Signaling Technology, Frankfurt, Germany) was used as primary antibody. Subsequently, the tissue sections were incubated with the corresponding secondary antibodies. 3,3′-diaminobenzidine (α-SMA-staining) and 3,3′-diaminobenzidine tetrahydrochloride (PCNA- and caspase-3-staining) were used as chromogens. The sections were counterstained with hemalaun (α-SMA-staining) or 1% methyl green (PCNA- and caspase-3-staining) and examined by light microscopy (BX60; Olympus, Hamburg, Germany). Numbers of caspase-3- and PCNA-positive endometrial stromal cells were assessed in seven lesions per group and are given as a percentage of the total.

To analyze the microvessel density of developing endometriosis-like lesions at day 7 after uterine tissue transplantation, paraffin-embedded 5-μm-thick sections were stained with a rat-anti-mouse CD31 antibody (1:30; dianova GmbH, Hamburg, Germany) coupled with a goat-anti-rat Cy3 antibody (1:50; dianova GmbH). On each section, cell nuclei were additionally stained with Hoeschst (1:500; Sigma-Aldrich). Sections were examined using a BZ-8000 microscope (Keyence, Osaka, Japan) for the quantitative analysis of microvessel density within endometriosis-like lesions, given in mm⁻².

**Statistics**

A two-way analysis of variance (ANOVA) was performed to determine the effect of the two major factors ‘localization of uterine tissue samples’ (right versus left abdominal wall within individual animals) and ‘group characteristics’ (mode of transplantation, spatial tissue orientation and tissue size) and their effect on the growth characteristics of surgically induced endometriosis-like lesions. The Bonferroni posthoc test, compensating for multiple comparisons, was then used to detect pairwise differences among experimental groups. To test for time effects within each experimental group, ANOVA for repeated measurements was applied. This was followed by the appropriate posthoc test (parametric data:

### Table I Overview of the experimental groups used in a study of the development of endometriosis-like lesions in uterine tissue which was transplanted to the peritoneum of C57BL/6 mice.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Mode of transplantation</th>
<th>Tissue adjacent to the peritoneum</th>
<th>Size of tissue samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>n = 7 mice syngeneic</td>
<td>endometrium</td>
<td>2 mm</td>
</tr>
<tr>
<td>Group 2</td>
<td>n = 7 mice autologous</td>
<td>endometrium</td>
<td>2 mm</td>
</tr>
<tr>
<td>Group 3</td>
<td>n = 7 mice syngeneic</td>
<td>perimetreum</td>
<td>2 mm</td>
</tr>
<tr>
<td>Group 4</td>
<td>n = 7 mice syngeneic</td>
<td>perimetreum</td>
<td>3 mm</td>
</tr>
</tbody>
</table>
Bonferroni; non-parametric data: Tukey) including the correction of the α-error according to Bonferroni probabilities to compensate for multiple comparisons (SigmaStat; Jandel Corporation, San Rafael, CA, USA). Differences between numbers of PCNA- and caspase-3-positive endometrial stromal cells, and microvessel density within the two experimental groups which differed in terms of spatial orientation of fixed uterine tissue samples, were assessed by the unpaired Student’s t-test. Parametric data are given as mean ± SEM. Non-parametric data are given as median, 1st/3rd quartiles, 10th/90th percentiles. Statistical significance was accepted for a value of $P < 0.05$.

**Results**

**Influence of localization of uterine tissue samples on growth characteristics and cyst formation of endometriosis-like lesions**

In the present study, uterine tissue samples were fixed to the right and left abdominal wall of each animal. Therefore we first performed a two-way ANOVA to analyze whether the localization of uterine tissue samples influenced the growth of endometriosis-like lesions. We found that the factor ‘localization of uterine tissue samples’ did not significantly influence the growth of endometriosis-like lesions in all four experimental groups.

Uterine tissue samples were capable of developing endometriosis-like lesions with typical sonographic features, such as anechoic cyst-like dilated endometrial glands (referred to as cysts). From a total of 28 mice, 11 mice had no cysts at day 28 after implantation of tissue into the peritoneal cavity. In another 11 mice, only one cyst could be detected in a lesion on the right ($n = 5$) or left ($n = 6$) abdominal wall. Finally, six mice at day 28 exhibited a cyst in each of the two lesions. Throughout the observation period of 4 weeks, the number of mice exhibiting cyst-containing lesions did not differ between groups (Table II).

**Syngeneic versus autologous uterine tissue transplantation**

Transplanted uterine tissue samples developed endometriosis-like lesions, independent of whether the transplant into the peritoneal cavity was syngeneic or autologous (Fig. 2A and B). Quantitative analysis of ultrasound images demonstrated that the lesions of both groups exhibited a comparable initial volume of $\approx 1.0 \text{ mm}^3$, which increased throughout the observation period of 28 days without significant differences between the groups (Fig. 2C and D). This increase was related to the growth of stromal tissue (Fig. 2E and F) and the development of cysts inside the lesions (Table II). Of interest, the volume of the cysts tended to increase in the autologous transplant group (Group 2) between days 7 and 28 when compared with Group 1, the syngeneic group (Table II). Groups 1 and 2 exhibited a comparable number of lesions which contained cysts (Table II).

**Spatial orientation of fixed uterine tissue samples**

Uterine tissue samples were sutured with either the endometrium or the perimetrium adjacent to the abdominal wall (Fig. 3A and B). We found that developing endometriosis-like lesions exhibited a higher

| Table II | Results obtained in the experimental groups 1–4 (as defined in Table I) after transplantation of uterine tissue samples into the peritoneal cavity of C57BL/6 mice, as assessed by high-resolution ultrasound imaging over a period of 28 days. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Number of mice with lesions which contained cysts | d7 | d14 | d21 | d28 |
| Group 1 | 6 | 3 | 4 | 2 |
| Group 2 | 4 | 4 | 6 | 4 |
| Group 3 | 4 | 4 | 5 | 4 |
| Group 4 | 3 | 4 | 6 | 7 |
| Overall number of lesions which contained cysts | d7 | d14 | d21 | d28 |
| Group 1 | 8 | 5 | 5 | 3 |
| Group 2 | 5 | 5 | 6 | 4 |
| Group 3 | 6 | 7 | 6 | 5 |
| Group 4 | 5 | 6 | 10 | 11 |
| Cyst volume (Median, first/third quartiles; mm³) | d7 | d14 | d21 | d28 |
| Group 1 | 0.05 (0.30/0.12) | 0.14 (0.04/0.24) | 0.17 (0.02/0.26) | 0.14 (0.08/0.40) |
| Group 2 | 0.08 (0.02/1.62) | 0.45 (0.06/0.58) | 0.18 (0.04/0.32) | 0.35 (0.10/1.14) |
| Group 3 | 0.08 (0.01/0.18) | 0.15 (0.10/0.19) | 0.32 (0.21/1.11) | 0.60 (0.34/2.41) |
| Group 4 | 0.39 (0.22/1.07) | 0.48 (0.14/3.20) | 1.42 (0.39/3.35) | 2.60 (0.43/10.47) |

$^{a}P < 0.05$ versus Day 7 (d7) (one-way ANOVA for repeated measurements; Bonferroni posthoc test).

$^{b}P < 0.05$ versus d7 (repeated measures ANOVA on ranks; Tukey posthoc test).
lesion volume when the perimetrium, with the underlying myome-
trium, was in direct contact with the peritoneum (Fig. 3C–F). More
detailed ultrasound analysis of stromal tissue and cyst volumes
revealed that this was related to a markedly increased stromal tissue
growth between days 7 and 28 (Fig. 3G and H), whereas the cyst
volumes and the number of cyst-containing lesions did not differ
between the two groups (Table II).

Histological examination of endometriosis-like lesions at day 28
confirmed the results of our ultrasound image analysis. Uterine
tissue samples which had been sutured with the perimetrium next
to the abdominal wall developed large endometriosis-like lesions
with endometrial glands and a considerably increased stromal tissue
size, when compared with tissue samples which were fixed with the
endometrium next to the peritoneum (Fig. 4A and B). Immunohisto-
chemical detection of α-SMA staining clearly indicates the different spatial
orientations of the tissue samples in relation to the peritoneal wall.
Scale bars: 400 μm. (C, D) High-resolution ultrasound imaging of a
developing endometriosis-like lesion (borders marked by red broken line; cyst borders marked by yellow broken line) 28 days
after fixation of uterine tissue samples to the peritoneal wall of C57BL/6 mice with the endometrium (C) or perimetrium (D) in
direct contact with the peritoneum. Scale bars: 1 mm. (E–H)
Overall lesion volume (E), lesion growth (F), stromal tissue volume
(G) and stromal tissue growth (H) of endometriosis-like lesions
with either the endometrium (grey bars) or the perimetrium (white
bars) in direct contact to the peritoneum. Mean ± SEM; *P < 0.05 versus endometrium (two-way ANOVA; Bonferroni posthoc test);
**P < 0.05 versus d0 (one-way ANOVA for repeated measurements; Bonferroni posthoc test).

Figure 2 (A, B) High-resolution ultrasound imaging of developing endometriosis-like lesions (borders marked by red broken line) 28
days after syngeneic (A) and autologous (B) transplantation of uterine tissue samples into the peritoneal cavity of C57BL/6 mice. Note
that the formation of anechoic cysts (borders marked by yellow broken line) can easily be visualized with this non-invasive technique. Scale bars: 1 mm. (C–F) Overall lesion volume (C), lesion growth (D, percentage of original sample), stromal tissue volume (E) and stromal tissue growth (F, percentage of original sample) of endometriosis-like lesions which were surgically induced by syngeneic (grey box plots) or autologous transplantation (white box plots). Median, 1st/3rd quartiles, 10th/90th percentiles; 

Figure 3 (A, B) Immunohistochemical detection of α-SMA in uterine tissue samples (borders marked by black broken line) at day 7 after fixation to the peritoneal wall of a C57BL/6 mouse with either the endometrium (A) or the perimetrium (B) in direct contact with the peritoneum. Identification of the myometrium (asterisks) by α-SMA staining clearly indicates the different spatial orientations of the tissue samples in relation to the peritoneal wall. Scale bars: 400 μm. (C, D) High-resolution ultrasound imaging of a developing endometriosis-like lesion (borders marked by red broken line; cyst borders marked by yellow broken line) 28 days
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bars) in direct contact to the peritoneum. Mean ± SEM; *P < 0.05 versus endometrium (two-way ANOVA; Bonferroni posthoc test);
**P < 0.05 versus d0 (one-way ANOVA for repeated measurements; Bonferroni posthoc test).

contrast, analysis of PCNA revealed that tissue samples sutured
with the perimetrium adjacent to the peritoneal wall exhibited an
increased proliferating activity of endometrial stromal cells, as indicated
by a significantly increased fraction of PCNA-positive cells (10.0 ±
1.2%), when compared with tissue samples sutured with the endometrium adjacent to the peritoneal wall (1.6 \pm 0.4%; \( P, 0.05 \)) (Fig. 4E and F). Moreover, our immunofluorescent microscopic analysis of early lesion (day 7) vascularization revealed that suturing tissue samples with the perimetrium adjacent to the peritoneal wall resulted in a significantly increased microvessel density compared with endometrium (226 \pm 18 versus 162 \pm 6 mm\(^2\); \( P, 0.05 \)) at day 7 (Fig. 4G and H).

**Initial size differences of isolated uterine tissue samples**

We compared uterine tissue samples which were isolated using a 2 or 3 mm dermal biopsy punch. We found that endometriosis-like lesions developing from 3 mm samples exhibited an increased overall lesion volume and stromal tissue volume throughout the observation period of 28 days when compared with lesions developing from
2 mm samples (Fig. 5A–C and E). Lesion and stromal tissue growth, however, were decreased at days 7–14 in the group with 3 mm samples, compared with the group with 2 mm samples (Fig. 5D and F). In contrast, lesions from mice with the 3 mm samples (Group 4) presented with larger cyst volumes and an increased number of cyst-containing lesions at day 28 when compared with lesions from mice with 2 mm samples (Group 3) (Table II).

Discussion

In the present study, we could demonstrate that the size and spatial orientation of peritoneally fixed uterine tissue samples crucially determine growth and cyst formation of surgically induced endometriosis-like lesions in mice. For our in vivo analyses, we used high-resolution ultrasound imaging. Using a scan head with a high frequency of 40 MHz, this recently introduced technology allows for an easy visualization and three-dimensional reconstruction of i.p. endometriosis-like lesions located at the lateral abdominal wall of mice (Laschke et al., 2010). Accordingly, it is possible to monitor repetitively and non-invasively the development of individual endometriosis-like lesions over time without the need for repeated laparotomies.

Because the development of spontaneous endometriosis is dependent on menstruation and thus restricted to humans and subhuman primates (Gürnberg, 2006), endometriosis-like lesions in rodents are usually induced by syngeneic or autologous transplantation of uterine tissue samples into the peritoneal cavity. Importantly, we herein show that the mode of transplantation does not affect the engraftment and development of endometriosis-like lesions.

Our findings are quite surprising, considering the fact that the resection of an uterine horn in the autologous model is associated with surgically induced inflammation. i.p. inflammation with increased levels of pro-inflammatory peritoneal factors such as tumor necrosis factor-α and interleukin-6, in turn, has been proposed to be an important trigger for i.p. endometriosis (Oral et al., 1996; Weiss et al., 2009). On the other hand, our results are in line with a recent study of Nowak et al. (2008), reporting that inflammation induced by thioglycolate medium does not increase disease burden in a mouse model of i.p. endometriosis. Thus, Nowak et al. (2008) concluded that pre-existing inflammation may not be the main factor supporting the development of endometriosis, but rather other factors, such as primary macrophage defects, are involved. On the basis of these considerations, we suggest that endometriosis-like lesions can be induced by both autologous and syngeneic uterine tissue transplantation in mice without major consequences for lesion growth and cyst formation. However, syngeneic transplantation of uterine tissue samples from a donor animal may have the advantage that the surgery for the recipient animal is shorter and less traumatizing, maintaining a more physiological peritoneal environment. Besides, syngeneic transplantation has to be performed in cases of target identification, where uterine tissue samples from wild-type mice are transplanted into knockout mice of identical genetic background and vice versa.

In another set of experiments, we analyzed whether the establishment of endometriosis-like lesions differs in relation to spatial orientation of the transplant i.e. endometrium or perimetrium in direct contact with the peritoneum. Interestingly, we found an enhanced lesion growth with increased numbers of proliferating stromal and glandular cells in the endometrial layer, when the perimetrium with the underlying myometrium was sutured to the peritoneum. This observation indicates that the tissue type at the interface of the peritoneal wall and the uterine graft is an important determinant for the development of surgically induced endometriosis-like lesions. This may be related to the fact that the endometrium of uterine tissue samples used in this model is covered by an epithelial cell layer, which under physiological conditions lines the uterus lumen. Outside the receptive phase, this luminal epithelium exhibits anti-adhesive properties and, thus, acts as a physical barrier for trophoblast invasion (Denker, 1994; Gipson et al., 2008). Moreover, the luminal epithelium presents an important immunological barrier, which confers protection against various pathogens (Ochiel et al., 2008). If this epithelial cell layer is located in direct contact with the peritoneum of the abdominal wall, it may also act as a natural cellular barrier, which inhibits the ingrowth of new blood vessels and granulation tissue from the surrounding host tissue into the implanted uterine tissue samples. In contrast, the perimetrium and myometrium may not exert this barrier function on the adjacent peritoneum, supporting a faster vascularization and proliferating activity of the uterine graft. In line with this interpretation, we could demonstrate that tissue samples sutured with the perimetrium adjacent to the peritoneal wall exhibited a significantly increased microvessel density at day 7 when compared with tissue samples sutured with the endometrium adjacent to the peritoneal wall. Hence, uterine tissue samples should always be sutured in the same spatial orientation with respect to the abdominal wall in the model of surgically induced endometriosis to prevent variability in growth characteristics of individual endometriosis-like lesions linked to differing interactions between graft and host tissue.

Finally, we could demonstrate that endometriosis-like lesions which develop from 2 mm uterine tissue samples exhibit a higher growth rate when compared with lesions originating from 3 mm tissue samples. This finding is in line with a former study from our group, where endometriosis-like lesions were induced by transplanting endometrial tissue fragments of different sizes into the dorsal skinfold chambers of Syrian golden hamsters and subsequently analyzed by means of intravital fluorescence microscopy (Laschke et al., 2005): in this study, sequential analysis of graft size over a 14-day observation period revealed an increase of ~60–70% in large grafts and ~80–90% in small grafts. Our results may be explained by the fact that the uterine tissue grafts lack a functional vascular supply during the initial days after transplantation into the peritoneal cavity and thus are solely dependent on oxygen diffusion. Accordingly, the larger grafts may be more susceptible to ischemic damage and cell death during this critical time period owing to longer diffusion distances for oxygen to reach cells in the graft centre and a higher metabolic demand because of the tissue mass. This may result in a decreased growth rate, especially during the first 7–14 days after transplantation, until the lesions are fully vascularized. On the other hand, we found that cyst-containing lesions were increased in the mice with 3 mm transplants (Group 4) on day 28, which may be attributed to a higher number of glands in these samples, increasing the probability for the development of cysts. Nonetheless, considering the practical aspect that, compared with a 3 mm biopsy punch, many more uterine tissue samples can be harvested from a mouse uterine horn if a 2 mm biopsy punch is used, we propose to use the 2 mm sample size for future studies in this mouse model of i.p. endometriosis.
Taken together, our study indicates that uterine tissue samples can be transplanted in either a syngeneical or autologous manner, resulting in the growth of surgically induced endometriosis-like lesions in mice. In addition, the size and spatial orientation of uterine tissue samples fixed to the peritoneum are crucial determinants for lesion growth and cyst formation. Accordingly, for comparative studies, transplanted uterine tissue samples should always be of comparable size and should be sutured in the same spatial orientation to the abdominal wall. This may limit the variability in growth characteristics of individual endometriosis-like lesions and, thus, increase the standardization and reliability of future studies performed in the frequently used mouse model of surgically induced endometriosis.

Authors’ roles

C.K.: Acquisition of data, analysis and interpretation of data; drafting the article; final approval of the article. M.D.M.: Interpretation of data; revising the article; final approval of the article. M.W.L.: Interpretation of data; revising the article; final approval of the article.

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