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Invasion process of induced deep nodular endometriosis in an experimental baboon model: similarities with collective cell migration?

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Objective: To determine the implications of collective cell migration in the invasion phenomenon observed in deep endometriotic lesions induced in a baboon model.
Design: Study of morphology and collective cell migration markers in invasive and noninvasive deep endometriotic lesions induced in a baboon model. Invasive lesions were defined as the presence of endometrial glands and stroma in surrounding organs, and a distinction was made between the center of the lesion (glands present in the main lesion) and the invasion front (glands present in surrounding organs).
Setting: Academic research unit.
Animal(s): Ten female baboons (*Papio anubis*).
Intervention(s): Recovery of induced deep nodular endometriotic nodules.
Main Outcome Measure(s): Evaluation of the morphology of glands by analysis of noninvasive and invasive lesions (center of the lesion and invasion front); staining with specific antibodies (Ki67, E-cadherin, β-catenin) for immunohistochemical study of mitotic activity and cell-cell junctions.
Result(s): Glands from invasive lesions, particularly from the invasion front, showed a significantly lower thickness coefficient, higher mitotic activity, and lower expression of E-cadherin and β-catenin than glands from noninvasive lesions and the center of invasive lesions.
Conclusion(s): We report altered morphology, increased mitotic activity, and fewer adhesion molecules in invasive glands present in induced nodular endometriosis, particularly along the invasion front, suggesting that collective cell migration is involved in the invasion process of deep endometriotic lesions induced in a baboon model. (Fertil Steril® 2015;104:491–7. ©2015 by American Society for Reproductive Medicine.)
Key Words: Induced endometriosis, deep nodular endometriosis, baboon model, collective cell migration, cell-cell junction, invasion

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Endometriosis, one of the most frequently observed benign gynecologic diseases, is known to occur in 7%–10% of women of reproductive age (1, 2). It is now widely recognized that the three different forms of endometriosis affecting the pelvis, namely peritoneal endometriosis, ovarian endometriosis, and deep endometriotic nodules, are separate entities (3, 4), although they may coexist.

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The first experimental model of deep nodular endometriosis, mimicking deep lesions found in humans, was recently developed, allowing us to investigate local invasion phenomena associated with these lesions (5, 6). In this model, morphologic similarities can be found with the invasion behavior of deep nodular endometriosis, which is known to invade the anterior rectal muscularis. Moreover, sigmoid wall invasion in our model was morphologically suggestive of collective invasion, with native nodular tumors and secondary groups of cells able to invade surrounding organs and even colonize the sigmoid mucosa. This suggests that nodular lesions induced in our model have a high potential for local invasion of surrounding tissue.

Even if endometriosis is regarded as a benign condition, some investigators have pointed out certain parallels with malignancy, such as distant foci and invasion of other tissue, with subsequent damage (7–9). According to Leighton et al., invasion in histopathologic samples is traditionally defined as penetration of normal tissue by single cells, compact groups of cells, or elongated strands of connected cells (10). Collective cell migration occurs in many physiologic and pathologic processes, including morphogenesis, tissue repair, and cancer (11), and strongly depends on chemokine and growth factor signaling to establish and maintain collective cell polarity and migration (12, 13). Collective cell invasion occurs when groups of cells that retain their cell-cell junctions move together to invade the peritumoral stroma (12, 13), and the process is stratified by a combination of different parameters, including multicellular morphology, degree of cell-cell adhesion and, of course, cell proliferation (12, 13).

The aim of the present study was to investigate the invasion process involved in deep nodular endometriosis induced in a baboon experimental model, and consider the hypothesis that collective cell migration may well be implicated in this process.

MATERIALS AND METHODS

Induction of Deep Nodular Endometriosis in Baboons

The experimental baboon model was previously described in our recent publications (5, 6). Ten female baboons (*Papio anubis*) were studied at the Institute of Primate Research (IPR), Nairobi, Kenya. Approval was obtained from the Institutional Scientific Evaluation and Review Committee and the Animal Care and Use Committee of the IPR.

The baboons initially underwent median laparotomy. After complete pelvic and abdominal exploration, no spontaneous endometriosis was identified in any of the ten animals. Bilateral salpingo-oophorectomy was performed to avoid individual variations in hormone secretion, and hormone replacement therapy was initiated on the same day (E_2 valerate, 2 mg/d orally; Progynova; Bayer Schering Pharma). Hormone replacement therapy was given daily with food under the supervision of a designated technician and controlled by a veterinarian. Large uterine biopsies were obtained with the use of a cold knife. Anterior hysterotomy was performed to prevent iatrogenic adhesions in the Douglas pouch (5, 6).

Fragments (±10 mm in size) to be grafted were immediately prepared and divided as follows: endometrium alone, endometrium plus the junctional zone (JZ), total uterine thickness, and deep myometrium without endometrium. Endometrium alone was grafted to the Douglas pouch by means of one simple suture with the use of absorbable Vicryl 3/0 FS2 (Johnson & Johnson). Deep myometrium alone was grafted to the peritoneum covering the right paravesical fossa. Total uterine thickness was grafted to the anterior wall of the rectum. Two fragments of endometrium containing the JZ were respectively grafted to the left iliac fossa and the right iliac fossa in front of the ureter. Between 20 and 24 weeks after grafting, laparotomy was performed to recover induced lesions. One of the animals was excluded owing to protocol violation (5, 6).

In accordance with previous publications (5, 6), invasion was defined as the presence of glands and stroma inside surrounding organs, confirmed by consecutive serial sections (50–200 sections). Invasion of the grafting site was not considered to be true invasion, because we could not exclude the influence of the grafting technique with suture. Invasion of surrounding organs was observed in fatty tissue, as well as the cervix, uterus, and sigmoid wall (5). Lesions showing invasion of surrounding organs (Supplemental Fig. 1A) were classified as invasive lesions (n = 11), and a morphologic distinction was made between the center of the lesion (training edge) and the invasion front (leading edge; Supplemental Fig. 1A). Well circumscribed lesions were classified as noninvasive lesions (n = 9; Supplemental Fig. 1B, available online at www.fertstert.org). Fifty to 200 consecutive serial sections were cut and stained with hematoxylin-eosin for histologic evaluation (morphology) and with specific antibodies for immunohistochemical study. All sections were scanned with the use of the Leica SCN400 scanner (Leica Biosystems), and image acquisition was performed with the use of the Tissue IA system (Leica Biosystems). Image J software was used for immunohistochemical quantification and morphologic analysis, with the use of the color deconvolution plugin to isolate the diaminobenzidine (DAB) channel.

Morphologic Study

Gland thickness was calculated by measuring the distance between the external (basal) and internal (apical) parts of the gland. To avoid artifacts due to sectioning, we excluded glands without the lumen and areas with multilayer cells.

Immunohistochemical Analysis

Mitotic activity was assessed by means of Ki67 immunohistochemical staining and counting of Ki67-positive nuclei per gland (proliferation index) in the defined phenotypes of whole lesions and areas. Briefly, deparaffinization was performed with the use of X-Solv (Yvsolab 53-65-66), followed by a permeabilization step with the use of Tris-buffered saline solution/Triton 0.1%. Endogenous peroxidase activity quenching, heat epitope retrieval, and blocking of nonspecific staining were all carried out. Thereafter, Ki67 monoclonal

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