Lesion kinetics in a non-human primate model of endometriosis

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BACKGROUND: Endometriosis is a common cause of pelvic pain and infertility in women of reproductive age. It is characterized by the presence of endometrial tissue outside the normal location, predominantly in the pelvic peritoneum causing severe abdominal pain. However, the severity of the symptoms of endometriosis does not always correlate with the anatomic severity of the disease. This lack of correlation may be due to morphological lesion variation during disease progression. This study examined lesion kinetics in a non-human primate model of endometriosis to better understand lesion dynamics.

METHODS: Endometriosis was experimentally induced in nine normal cycling female adult olive baboons (Papio anubis) by i.p. inoculation of autologous menstrual endometrium on Day 2 of menses for two consecutive menstrual cycles. Diagnostic laparoscopies were performed between Day 8–12 post-ovulation at 1, 3, 6, 9 and 12 months, followed by a necropsy at 15 months, after the second inoculation. In two animals, lesions were excised/ablated at 6 months and they were monitored for lesion recurrence and morphological changes by serial laparoscopy. Furthermore, five control animals underwent surgeries conducted at the same time points but without inoculation.

RESULTS: A total of 542 endometriotic lesions were observed. The location, macroscopic (different colours) and microscopic appearance confirmed distinct endometriosis pathology in line with human disease. The majority of the lesions found 1 month after tissue inoculation were red lesions, which frequently changed colour during the disease progression. In contrast, blue lesions remained consistently blue while white lesions were evident at the later stages of the disease process and often regressed. There were significantly lower numbers of powder burn, blister and multicoloured lesions observed per animal in comparison to black and blue lesions (P-value ≤ 0.05). New lesions were continually arising and persisted up to 15 months post-inoculation. Lesions reoccurred as early as 3 months after removal and 69% of lesions excised/ablated had reoccurred 9 months later. Interestingly, endometriotic lesions were also found in the non-inoculated animals, starting at the 6-month time point following multiple surgeries.

CONCLUSIONS: Documentation of lesion turnover in baboons indicated that lesions changed their colour from red to white over time. Different lesion types underwent metamorphosis at different rates. A classification of lesions based on morphological appearance may help disease prognosis and examination of the effect of the lesion on disease symptoms, and provide new opportunities for targeted therapies in order to prevent or treat endometriosis. Surgical removal of endometriotic lesions resulted in a high incidence of recurrence. Spontaneous endometriosis developed in control baboons in the absence of inoculation suggesting that repetitive surgical procedures alone can induce the spontaneous evolution of the chronic disease. Although lesion excision/ablation may have short-term benefits (e.g. prior to an IVF cycle in subfertile women), for long-term relief of symptoms perhaps medical therapy is more effective than surgical therapy.

Key words: endometriosis / diagnostic laparoscopy / lesion development / lesion ablation / lesion recurrence

Introduction

Endometriosis is a chronic, endocrine-dependent gynaecological disorder affecting 6–10% of women of reproductive age, in particular 50–60% of women and teenage girls with pelvic pain and up to 50% of women with infertility (Eskenazi and Warner, 1997). It is characterized by the presence of endometrial tissue outside the normal location, predominantly in the pelvic peritoneum (Giudice and Kao, 2004). The most common clinical presentations of endometriosis are chronic pelvic pain, dyspareunia and subfertility (Giudice, 2010).
The activity of endometriotic lesions or implants formed by the misplaced endometrial tissue, and the development of subsequent adhesions, are the likely causes of the described disease symptoms. Hence inhibition of lesion progression and establishment of new lesions is thought to alleviate clinical symptoms and to mediate endometriosis regression.

Observational studies suggest that endometriosis is a dynamic disorder. Follow-up studies of women with endometriosis showed that 17–29% of the lesions resolved spontaneously, 24–64% progressed and 9–59% were stable over a 12-month period (Sutton et al., 1997). Another study following 14 women 6 months after initial laparoscopy demonstrated dynamic progression and regression of disease burden, as assigned by the revised American Society for Reproductive Medicine (ASRM, formerly known as the American Fertility Society) guidelines (AFS, 1985), independent of endocrine treatment (Telimaa et al., 1987). Therefore, using the quantitative scoring system as a metric, the literature suggests that endometriosis has a variable, if not largely progressive, clinical course (Konincx et al., 1991).

Lesion dynamics describe spontaneous lesion progression and regression, the development of new lesions and changes in lesion appearance over time. Lesion turnover is a consequence of these dynamics, which are likely induced by hormonal and inflammatory fluctuations. Hormonal fluctuation has been implicated in a study by Konincx et al. (1996) where palpation during menstruation increased the detection rate of endometriosis by 5-fold versus routine examination not timed to menstruation. Studying lesion turnover may provide clues regarding the biological activity of the endometrial implants, increase our understanding of the natural history of endometriosis and possibly reveal new insights into its clinical management and prognosis.

Lesion turnover is difficult to study in women with endometriosis because there are significant delays in diagnosis as well as numerous variations in symptomatology and disease progression. Thus the olive baboon (Papio anubis) has been developed as an appropriate model to better examine the establishment and progression of endometriotic lesions. Dynamic progression and regression of lesions has been demonstrated in this induced endometriosis model (Hastings et al., 1996a). There is evidence of lesion turnover in baboons with spontaneous endometriosis. In a 32-month kinetic analysis, there was oscillation of the total number, as well as remodelling and transformation, of lesions in animals with spontaneous endometriosis (D’Hooghe et al., 1992, 1996a).

The severity of the symptoms of endometriosis has not always correlated with the anatomic severity of the disease (Crosignani et al., 1996; Garry et al., 2000; Abbott et al., 2003). This lack of correlation may be due, in part, to variations in the activity of the endometriotic lesions present at different episodes of the disease. Furthermore, indirect and distant effects of established lesions might be a major precondition for new lesion development and disease progression. Therefore, understanding the macroscopic appearance of lesions may afford additional assistance in the prognosis of the disease and determining the appropriate treatment method. Here, we examine lesion kinetics in a non-human primate model of endometriosis with the goal of providing a better understanding of lesion dynamics, which may lead to new opportunities for targeted therapies to prevent and/or treat endometriosis.

### Materials and Methods

#### Animals and induction of endometriosis

All animal procedures were conducted according to protocols established and approved by the Animal Care Committee of the University of Illinois at Chicago, IL, USA. Endometriosis was experimentally induced in nine adult female olive baboons (Papio anubis) with documented regular menstrual cycles by i.p. inoculation of autologous menstrual endometrium on Day 2 of menses for two consecutive menstrual cycles, as previously described (Fazleabas et al., 2002). Menstrual endometrium was harvested on Day 1 or 2 of menses using a Unimar Pipelle (Cooper Surgical Inc., Shelton, CT, USA) immediately prior to laparoscopy. The peritoneal cavity and reproductive organs were visualized and the absence of any lesions or adhesions was documented by laparoscopic video recording. Under laparoscopic guidance, ~1 g of menstrual tissue and fluid was deposited from the Pipelles at four sites: the pouch of Douglas, the uterine fundus, the cul de sac and the ovaries (Hastings and Fazleabas, 2006).

Diagnostic laparoscopies accompanied by endometrectomies to harvest eutopic tissue were performed during the window of uterine receptivity (Days 8–12 post-ovulation) at 1, 3, 6, 9 and 12 months following the second inoculation (Fig. 1). After laparoscopic entry a complete survey of the abdomen and pelvic cavity was performed in a systematic fashion. The same survey protocol was strictly applied for each laparoscopy. To avoid inter-surgeon variability, the same experienced gynaecologic laparoscopist was involved in performing all laparoscopies. The number, colour

![Figure 1](image-url)
Lesion kinetics in experimental endometriosis

and position of each visible endometriotic lesion were documented by videography along with a comprehensive script documentation prepared by the surgeon after the laparoscopy. As a control, five animals did not undergo inoculation, but underwent diagnostic laparoscopies accompanied by endometrectomies at identical time points. At 15 months following the second inoculation a necropsy was carried out including a comprehensive investigation of the abdominal cavity.

Lesion excision/ablation
In two animals, lesions were removed at 6 months. For excision of lesions the abnormal peritoneal surface was grasped and elevated away from underlying tissues. An elliptical incision was made around the lesion with 0.5–1 cm margins. Any deeper foci that were felt to be firm or fibrotic were also excised. Alternatively, lesions were ablated using monopolar energy at 25 W. Adequate treatment was assessed by visible desiccation of the lesions and surrounding tissues with a similar 0.5–1 cm margin. Excision and ablation were used interchangeably, as determined by the surgeon.

Analyses of lesion dynamics
The video and script documentation of each laparoscopy was analysed with the number, location and colour of individual lesions recorded.

Statistical analysis
When applicable the results are presented as mean ± SD. Statistical analysis was conducted using GraphPad Prism 5.0 software (La Jolla, CA, USA). The one-way analysis of variance (ANOVA) along with Tukey’s multiple comparison post hoc test was utilized for the comparison of three or more unpaired groups with a P-value ≤ 0.05 being considered as significant.

Results
Baboon endometrial lesion location and morphology
A total of 542 endometriotic lesions were observed in the baboons investigated. Lesions were commonly found on the peritoneal surfaces involving the pouch of Douglas, bladder and the perimetrium (Fig. 2A). Lesions were characterized as red, black, blue, powder burn, blister-like, multicoloured and white. Morphologically, Gomori trichrome staining of baboon lesions revealed distinct endometrial glands and stroma that was observed in all lesion types (Fig. 2B). A comparison of lesions characterized by different colours exhibited visible haemorrhaging in the red lesions and the presence of more connective tissue in the white lesions.

Lesion development in baboons inoculated with menstrual tissue
Early lesion occurrence and persistence
At each diagnostic laparoscopy a total of 9 (± 5) lesions per animal were detected. This average excludes the 15-month necropsy where 22 (± 6) lesions per animal were detected. During the 15 months following induction of endometriosis, 330 new lesions were detected in the nine baboons. There was no significant variation in the number of new lesions observed between laparoscopies and 15-month necropsy (Fig. 3A, one-way ANOVA Tukey’s multiple comparison post hoc test). Interestingly, at 12 months post-inoculation 19 of 37 (51%) lesions present were newly detected lesions indicating that new lesions were continuously evolving throughout the disease model. With regard to lesion persistence, there was no significant difference between the number of lesions persisting over time between each time point (Fig. 3B). Twenty-four of 59 (41%) lesions initially observed at second inoculation were still present at the 15-month necropsy.

Lesions characterized by different colours
The colour and location of the endometriotic lesions observed at each laparoscopy in the inoculated group was recorded. The most frequent colours observed in each baboon were blue (15 ± 8, 138 total), followed by black (15 ± 6, 135 total), white (10 ± 6, 86 total) and red lesions (8 ± 5, 74 total, Fig. 4A). Multicoloured lesions did not allow a clear classification into certain lesion type but were significantly less than the above-mentioned lesion types. Figure 4B summarizes the frequency of lesion types according to colour at each surgical intervention. Heterogeneity in colour was noted across all time points.

As each lesion was recorded, the site of the lesion was specifically assessed at subsequent laparoscopies and 15-month necropsy. When the site of the lesion was accessible and no lesion was observed, the lesion was categorized as regressed (not present). When the site of the lesion was not surgically accessible the lesion was categorized as not found/not visible. Figure 5A, C, E and G show the total number of red, black, blue and white lesions at each laparoscopy. Figure 5B, D, F and H describe changes in lesion colour over time. The majority (31/59) of lesions found at the second inoculation were red lesions (Fig. 5A). Red lesions subsequently transformed into endometriotic foci characterized by several different colours over the 15-month period (Fig. 5B). The highest occurrence of black lesions, as determined by laparoscopy, was at 6 months with 24 total. At necropsy, there were 74 black lesions present (Fig. 5C). Black lesions most often remained black, or turned blue, white or regressed (Fig. 5D). The highest occurrence of blue lesions was observed at 3 months post-inoculation (Fig. 5E) and in contrast to the red lesions, blue lesions remained consistently blue (Fig. 5F). White lesions were present at each time point, the lowest frequency being at 3 months (Fig. 5G). White lesions often disappeared (not present) or became scar tissue at a subsequent surgery (Fig. 5H). The infrequent lesions, such as powder burn, blister and multicoloured, turned into different lesion types at subsequent surgeries or disappeared (data not shown).

Recurrence of excised/ablated lesions in baboons inoculated with menstrual tissue
All visible lesions (26 total) from two animals with induced endometriosis were removed 6 months after inoculation. Subsequent laparoscopies were conducted at 9 and 12 months followed by necropsy at 15 months post-inoculation and recurrence of lesions was recorded. In one animal, 3 out of 16 visible lesions reappeared as early as 3 months later. In the two animals, 18 out of 26 (69%) lesions removed at 6 month returned by necropsy (Fig. 6A). Excision and ablation were used interchangeably as determined by the surgeon. Fifteen out of 20 (75%) excised lesions returned while 3 out of 6 (50%) ablated lesions returned (Fig. 6B).
Figure 2 Endometriotic lesion location and morphology in an experimental model of endometriosis in baboons. Nine baboons were experimentally induced with endometriosis by i.p. inoculation with autologous menstrual endometrium, as described in Materials and Methods. (A) Common locations of baboon endometriotic lesions in the abdomen were the perimetrium, the bladder and the peritoneum at the Pouch of Douglas. (B) Gomori trichrome staining revealed distinct endometrial glands and stroma. Haemorrhaging was observed in the red lesions (arrow), while more connective tissue was present in the white lesions (arrow). Scale bar: 100 μm.

Figure 3 Number of new lesions and their persistence over time in an experimental model of endometriosis in baboons. Nine baboons underwent diagnostic laparoscopies at 1, 3, 6, 9 and 12 months as well as a necropsy at 15 months following two i.p. inoculations with autologous menstrual endometrium. Each lesion was recorded upon initial sighting and the site was specifically assessed at subsequent laparoscopies. (A) Mean number of new lesions per inoculated animal at each laparoscopy and 15-month necropsy. (B) The mean number of lesions first seen at each laparoscopy in the inoculated animals that are still present at 15-month necropsy. Columns represent the mean number of lesions per animal (n = 9). Error bars depict SD. ns, not significant (one-way ANOVA Tukey’s multiple comparison post hoc test).
The excised/ablated lesions included 1 red, 11 black, 6 blue, 1 powder burn, 3 blisters and 4 white lesions. The single red lesion recurred as blue at subsequent laparoscopy and remained blue until 15-month necropsy. Of the 11 excised/ablated black lesions, 2 returned as black, 2 as blisters and 7 were not found/not visible at subsequent laparoscopy. At 15-month necropsy, four were black, one was blue, three were blisters and three had regressed (not present). All six of the removed blue lesions were not found/not visible at subsequent laparoscopy while at necropsy one appeared as black, three were blue, one was a blister and one had regressed. Figure 7 shows recurrence of the excised/ablated black and blue lesion at subsequent laparoscopy (Figure 7A) and at 15-month necropsy (Figure 7B). One removed blister lesion recurred as blue and two were not found/not visible at subsequent laparoscopy. At necropsy, two were blue and one had regressed. Of the four removed white lesions, one recurred as white, one as a blister and two were not found/not visible at subsequent laparoscopy while at necropsy one was a blister and three had regressed (data not shown).

Lesion occurrence in baboons not inoculated with menstrual tissue

Five control baboons did not undergo inoculations but were subjected to diagnostic laparoscopies followed by endometrectomy to harvest eutopic tissue at each surgical time point. All five animals developed endometriotic lesions with one animal having as many as 13 lesions (Fig. 8A). In this animal, the two earliest lesions appeared at 6-month laparoscopy and were characterized as red and blue. These two lesions were not found/not visible at subsequent laparoscopies and by 15-month necropsy had regressed. A total of 27 peritoneal lesions were observed in the five control animals with the majority being detected at 12-month laparoscopy (n = 11) and 15-month necropsy (n = 14; Fig. 8B).

Five out of 11 lesions discovered at 12-month laparoscopy were present at necropsy and had progressed to different colours. They included one red lesion turning to white, two black lesions, one remaining black and one turning to blue, one blue lesion staying blue and one multicoloured lesion that turned to black (data not shown). The location of lesions, as with the induced endometriosis group, was on both visceral and parietal peritoneum. Similarly, a heterogeneous mixture of endometriotic lesion colours was observed with the majority being black and blue coloured lesions (Fig. 8C).

Discussion

Lesion turnover in baboons inoculated with menstrual tissue

Experimental endometriosis was induced by mimicking retrograde menstruation using the well-established baboon model of endometriosis (Braundmeier and Fazleabas, 2009). The experiments were performed in a controlled setting where all animals were normally cycling and negative for endometriosis and adhesions prior to inoculation. Upon induction of endometriosis, the lesion colour, location and turnover were assessed at precise and consistent time points. This enabled, for the first time, kinetic analysis of how quickly lesions can turnover (not just that they change colour). Additionally, once lesions were found, they were followed at subsequent laparoscopies to determine their progression.

A high number of lesions persisted for up to 15 months post-inoculation. At each laparoscopy, on average 9 (±5) lesions per animal was observed. These data compared favourably with a human study that focused on the number of superficial endometrial implants in women with endometriosis, in which an average of 10 implants per patient (43 total) was observed (Muzii et al., 2000).
were found in the peritoneum, especially in the pouch of Douglas, bladder and the perimetrium. Interestingly, in contrast to the human disease, no ovarian cysts (endometrioma) or deeply infiltrating recto-vaginal lesions were detected. This absence could be related to the early termination point of the baboon experiment (15 months), whereas women can suffer with endometriosis for 8–12 years even before diagnosis (Hadfield et al., 1996). Conversely, this could be explained by pathophysiological differences in the aetiology of endometriosis: it has been proposed that the development of the three endometrial implants—peritoneal, ovarian cysts and deep infiltrating

Figure 5 Turnover of endometriotic lesions characterized by different colours in an experimental model of endometriosis in baboons. At each surgery the locations of previously identified endometriotic lesions were analyzed and the evolution of each lesion tracked. When the site of the lesion was not accessible, the lesion was categorized as not found/not visible. When the site of the lesion was accessible and no lesion was observed, the lesion was categorized as regressed (not present). (A, C, E and G) Show the number of red, black, blue and white lesions at each laparoscopy. (B, D, F and H) Demonstrate the evolution of red, black, blue and white lesions at the subsequent laparoscopy. The bars correspond to the total number of lesions per time point or lesion type, respectively.
lesions—can be caused by different mechanisms (Brosens, 2004; Garry, 2004). The baboon model primarily reflects peritoneal disease.

Five hundred and forty-two endometriotic lesions were documented and analyzed in this study. Menstrual tissue inoculation was performed only twice at the initiation of the study, however new lesions continued to develop throughout the duration of the model. There are three plausible explanations for this: implantation of new eutopic endometrium from subsequent retrograde menstruation, shedding from existing lesions or visualization of lesions previously unseen at prior laparoscopies. While all visible lesions were removed in two animals by surgical excision, a high frequency returned within 6 months (see the following section); supporting the hypothesis that subsequent retrograde menstruation promotes further evolution of new endometriotic lesions. Moreover, the presence of blood in the baboon peritoneal cavity during menses has been reported, which can act as seeding for new lesions (D’Hooghe et al., 1996b). As for shedding from existing lesions, patients treated with amenorrhea-inducing GnRH agonists also developed new lesions (Fedele et al., 2000). Furthermore, pro-inflammatory signalling molecules released by the existing lesions may create a highly inflammatory environment in the peritoneum where transdifferentiation of the peritoneal mesothelium into endometriotic lesions can occur (Suginami, 1991; Vinatier et al., 1997).

**Figure 6** Recurrence of excised/ablated endometriotic lesions in an experimental model of endometriosis in baboons. In two animals that were inoculated with autologous menstrual tissue, lesions were excised or ablated 6 months after the second inoculation. Laparoscopies were performed at 9 and 12 months and a necropsy at 15 months to control for lesion recurrence. (A) Of the removed lesions at 6 months post-inoculation, 3 of 16 returned 3 months after excision, 7 of 26 returned 6 months after excision and at 15-month necropsy 18 of 26 (69%) lesions had returned. (B) Excision and ablation were used interchangeably as determined by the surgeon. Fifteen of 20 (75%) excised lesions returned by 15-month necropsy while three of six (50%) ablated lesions returned by necropsy.

**Figure 7** Turnover of reoccurring excised/ablated endometriotic lesions in an experimental model of endometriosis in baboons. Excision/ablation of all visible endometriotic lesions was performed in two baboons 6 months after experimental induction of endometriosis, as described in Materials and Methods (Fig. 1). Laparoscopies were conducted at 9 and 12 months and a necropsy at 15 months post-inoculation to determine the incidence of lesion recurrence and macroscopic changes within the peritoneal cavity. When the site of the lesion was not accessible, the lesion was categorized as not found/not visible. When the site of the lesion was accessible and no lesion was observed, the lesion was categorized as regressed (not present). Black and blue bars represent the status of excised/ablated black and blue endometriotic lesions after recurrence. (A) The reoccurring excised/ablated black and blue lesions at subsequent laparoscopy. Each bar represents the number of each type of lesion manifested after reoccurrence. (B) The reoccurring excised/ablated black and blue lesions at 15-month necropsy. Each bar represents the number of each type of lesion manifested at necropsy.
types require further attention, as they are potentially hyperactive due to their fast turnover. These previously neglected lesion types frequently transformed into lesions of different colours. Indeed, thereafter there was a heterogeneous mixture of lesions characterized by different colours, consistent with the dynamic character of the pathology. Most of the lesions observed in this model were black and blue. Black lesions most often remained black, or turned blue, white or regressed. Blue lesions had a slower turnover cycle and often remained blue at subsequent laparoscopies. Powder burn, blister and multicoloured lesions are postulated to have the shortest turnover cycle or to represent an intermediate appearance, as these lesions were present in significantly lower numbers than others. White lesions were evident at the later laparoscopies and often disappeared or became scar tissue, indicating that they may occur at late stages of the lesion life cycle.

These results confirm in a more precise manner a previous finding where a high number of red lesions were observed immediately after inoculation and, over time, decreased in parallel with an increase in white lesions (D’Hooghe et al., 1995). In the current study, detailed monitoring of each lesion type also allowed for continuous observation of powder burn lesions and blisters that seem to be highly active due to their fast turnover. These previously neglected lesion types require further attention, as they are potentially hyperactive endometriotic tissues, which often get overlooked during routine diagnostic laparoscopies.

Changes in lesion morphology over time have been observed in women with endometriosis. Jansen and Russell (1986) documented changes in lesion colour between non-pigmented lesions and pigmented lesions at 6–24 months after initial laparoscopies in patients with endometriosis. There is evidence that lesions characterized by different colours possess different biochemical activity (Vernon et al., 1986). Immunohistochemical staining for proliferating cell nuclear antigen demonstrated that red lesions possess a higher proliferation index compared with black lesions and continue to proliferate, more so than white lesions (Fujishita et al., 1999). Additionally, the number of endothelial cells and the number of blood vessels were higher in red lesions when compared with black and white lesions. Kuroda et al. (2009) confirmed increased vessel density in red lesions versus black and white lesions using narrow-band imaging system. Furthermore, our previous studies in the baboon model revealed higher levels of the angiogenic factors, vascular endothelial growth factor A and CYR61 in red lesions (Gashaw et al., 2006). Moreover, in patients with endometriosis the increased vascularization and morphological appearance of red lesions suggest that red lesions may be recently implanted refluxed endometrial cells (Nisolle and Donnez, 1997). Hence, increased cell proliferation and angiogenesis could be critical processes in the formation of new ectopic implants, which may explain why red lesions are observed in the early stages of the lesion life cycle.

In the revised ASRM classification of endometriosis (ASRM, 1997), peritoneal and ovarian implants are categorized into three subgroups: red (including red, red–pink and clear lesions), white (including white, white–brown, yellow–brown and peritoneal defects) and black (including black and blue lesions). Accordingly, blue and black lesions belong to one entity. However, in our model we clearly differentiated between blue and black lesions and their turnover is evidently different, as blue lesions remain blue for several months. Blue lesions appear to have been derived from black lesions or the change in the colour may simply reflect the amount of hemosiderin contained within the lesions.
Recurrence of excised/ablated lesions in baboons inoculated with menstrual tissue

For the first time, lesion excision/ablation recurrence was analyzed in the baboon model for endometriosis. In two animals with experimentally induced endometriosis all of the lesions (26 total) were excised/ablated at 6 months. Animals were subsequently subjected to laparoscopies at 9 and 12 months followed by necropsy at 15 months. The majority (18/26) of lesions recurred by the 15-month necropsy. However, it has been shown that pelvic inflammation related to manipulation and microtrauma to the pelvic organs can occur during laparoscopy in baboons (D’Hooghe et al., 1999). It remains unclear whether this lesion recurrence was caused by partial removal of the lesion or implantation of new eutopic endometriotic lesions from subsequent retrograde menstruation preferentially at these sites of surgical microtrauma. Additionally, it has been hypothesized that the mesothelial lining of the peritoneum acts as a defensive barrier, which may prevent the adhesion of ectopic tissues (van der Linden et al., 1994; Groothuis et al., 1998; Groothuis et al., 1999). Therefore, disrupting the peritoneal integrity through excision and ablation together with retrograde menstruation may positively promote the development of disease.

These findings, although preliminary in nature and only including two animals, indicate that surgical management may not be the best approach for permanently eliminating endometriotic lesions. Indeed, women with endometriosis who undergo local excision of lesions experience a short-term relief from the symptoms. Shakiba et al. (2008) demonstrated that of 109 women who underwent lesion excision, as many as 55% had to have a repeat operation within 7 years. Furthermore, Candiani et al. (1991) reported that in a sample size of 42 patients with endometriosis who underwent lesion excision, 75% had to have repeat surgeries within 2 years owing to the return of pain. It must be noted that the number of lesions does not necessarily correlate with the severity of pain, although in the Candiani et al. (1991) study, the removal of lesions did lead to a short-term relief from the pain. Lesion excision/ablation may have short-term benefits (i.e., prior to an IVF cycle in subfertile women), but for long-term relief of symptoms perhaps medical therapy is more effective than surgical therapy. The baboon model primarily reflects the peritoneal disease and due to the limitations of the experimental model, we were unable to evaluate fertility before and after lesion excision/ablation.

Lesion occurrence in baboons not inoculated with menstrual tissue

The rate of spontaneous endometriosis for baboons in captivity, as reported in the literature, is 1–25% (D’Hooghe et al., 1991, 1996c; Dick et al., 2003; Dehoux et al., 2011). In our study, spontaneous endometriosis resulting from consecutive surgeries was demonstrated using animals with no previous surgeries. Indeed, the control group underwent the same schedule of surgeries as that of the experimental group allowing precise assessment of the effects of consecutive surgical procedures. All five animals developed endometriotic lesions, albeit at a much lower rate and at a later time point than the inoculated animals. A total of 27 lesions were observed in the control group; the earliest lesion development was observed at the 6-month laparoscopy. This incidence of non-induced endometriosis may be attributed, in part, to the repeat endometrectomies these animals underwent at the time of diagnostic laparoscopies. D’Hooghe et al. (1991) have shown that history of previous hysterectomy greatly increased the rate of spontaneous endometriosis in baboons. However, the more likely cause could be repeat laparoscopies. It has been demonstrated that the incidence of spontaneous endometriosis in baboons increased over time owing to repeat laparoscopies (D’Hooghe et al., 1996d). Therefore, as with lesion excision/ablation, the inflammation induced by repeated surgeries could leave these animals more susceptible to endometriosis.

Currently, the gold standard for diagnosis of pelvic disease is surgical assessment, via laparoscopy. Additionally, surgical approaches to relieve endometriosis-related pain are frequently used as first-line therapy or after brief and failed medical therapies. However, laparoscopic removal of lesions leads to short-lived relief from symptoms (Jacobson et al., 2009, Yeung et al., 2009). Therefore, a focused emphasis on non-surgical diagnosis and therapies to prevent or control endometriosis is critical. Understanding the kinetics of lesion development in an appropriate non-human primate model could be an initial step towards this goal.

Conclusions

The lack of correlation between endometriosis symptoms and anatomic severity may be related to variations in the activity of the endometriotic lesions present at different stages of the lesion cycle. Lesion turnover was documented in this non-human primate model of endometriosis. The lesions changed their morphology from red to white over time. Different lesion types underwent metamorphosis at different rates. Thus, classification of lesions based on morphological appearance may help in determining the prognosis of the disease and in the examination of the effects of the lesions on symptoms. This understanding may provide new opportunities for targeted therapies to prevent or control endometriosis.

Surgical excision/ablation of endometriotic lesions resulted in a high incidence of recurrence. Spontaneous endometriosis developed in control baboons in the absence of deposited autologous menstrual endometrium suggesting that repetitive surgical procedures alone can induce the spontaneous evolution of the chronic disease.

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Authors’ roles

P.H.: Acquisition, analysis and interpretation of data as well as drafting of the manuscript. I.G.: Design of the study, discussion and interpretation of data as well as revising the manuscript critically for important intellectual content. S.T.L.: Baboon surgeries, lesion excision, acquisition of data and revising the manuscript critically for important intellectual content. A.G.B., J.M.H., M.R.O.: Baboon surgeries, acquisition of data and revising the manuscript critically for important intellectual content. A.T.F.: Conception and design of the study, discussion and interpretation of the data as well as revising the manuscript critically for important intellectual content.
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Conflict of interest


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