

Characteristics of histologically confirmed endometriosis in cynomolgus monkeys

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STUDY QUESTION: What are the characteristics of spontaneous endometriosis in cynomolgus monkeys?

SUMMARY ANSWER: Spontaneous endometriosis in cynomolgus monkeys exhibited similar characteristics to the human disease.

WHAT IS KNOWN ALREADY: One previous report described the prevalence and the basic histopathology of spontaneous endometriosis in cynomolgus monkeys.

STUDY DESIGN, SIZE, DURATION: Endometriotic lesions that had been histologically confirmed in 8 female cynomolgus monkeys between 5 and 21 years old were subjected to study.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The monkeys died of, or were sacrificed because of, sickness consequent on endometriosis. Specimens were evaluated histopathologically with haematoxylin and eosin staining, iron staining and immunohistochemistry (CD10, CD31, α -SMA and PGP9.5), and by observing them under a microscope.

MAIN RESULTS AND THE ROLE OF CHANCE: Endometriotic and stromal cells (CD10-positive) with haemorrhage and inflammation were observed. Smooth muscle metaplasia and nerve fibres were also noted in the endometriotic lesions. Endometriotic lesions in lymph nodes were incidentally found.

LIMITATIONS AND REASONS FOR CAUTION: Since laparoscopic analysis for monitoring the disease state was not set as a parameter of the current study, time course changes (progression) of the disease were not assessed.

WIDER IMPLICATIONS OF THE FINDINGS: Further investigation of spontaneous endometriosis in cynomolgus monkeys may contribute to better understanding of the disease pathobiology.

STUDY FUNDING/COMPETING INTEREST(S): No external funds were used for this study. A.N.K., S.M., S.H., T.I., O.K., A.K. and M.S. are full-time employees of Chugai Pharmaceutical Co., Ltd. R.K. received lecture fees from Chugai Pharmaceutical Co., Ltd., unrelated to the submitted work. S.N., S.O., L.Y., K.Y. and T.S. have nothing to declare.

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Introduction

Endometriosis is defined as the presence of endometriotic glands and stroma outside the uterine cavity, predominantly but not exclusively in the pelvic compartment. It is an oestrogen-dependent chronic inflammatory condition which affects 2–10% of women in their reproductive period and is associated with pelvic pain and infertility (Dunselman *et al.*, 2014; Vercellini *et al.*, 2014). The pathogenic hypothesis supported by the most robust evidence is retrograde menstruation, which hypothesizes that when endometrial fragments become attached to and invade the mesothelium, a suboptimal immune response does not adequately clear the implants and this results in their continued survival, growth and cyclical bleeding, which in turn causes fibrosis and then adhesion of several organs (Giudice and Koo, 2004; Khan *et al.*, 2008; Asante and Taylor, 2011; Burney and Giudice, 2012; Jiang and Wu, 2012).

Invasion, immune response, localized bleeding and fibrosis are considered to be related to the pain, which is the main symptom of the disease and a leading cause of the deterioration in the patient's quality of life (De Graaff *et al.*, 2015). However, there is evidence that more elusive mechanisms are also likely to contribute to the pain associated with endometriosis and these mechanisms may explain why the extent and morphological characteristics of the disease correlate poorly with the intensity and character of the pain symptoms (Asante and Taylor, 2011). Mechanisms so far proposed to explain the pain are the involvement of components such as smooth muscle metaplasia and innervation of the diseased tissue (Odagiri *et al.*, 2009; Barcena de Arellano *et al.*, 2011; Barcena de Arellano and Mechsner, 2014; Morotti *et al.*, 2014).

Despite intensive research into endometriosis in the past, the underlying pathobiology of the disease is far from being well understood, which means that animal models of the disease are crucial for further research in this area. Because of the anatomical similarity with human reproductive organs, endometriosis of non-human primates (NHP) is considered to be a possible candidate for researching the disease (Story and Kennedy, 2004; Braundmeier and Fazleabas, 2009; Yamanaka *et al.*, 2012), and reports have described the spontaneous endometriosis of the baboon (Cornillie *et al.*, 1992; Dick *et al.*, 2003; Dehoux *et al.*, 2011), the rhesus monkey (Ito *et al.*, 2001) and the cynomolgus monkey (*Macaca fascicularis*) (Fanton and Hubbard, 1983; Ami *et al.*, 1993; Cline *et al.*, 2008). The most intensively investigated of these is endometriosis in the baboon, because baboons are larger, which makes it easier to obtain tissue samples with laparoscopies and to perform complex surgery (Braundmeier and Fazleabas, 2009; D'Hooghe *et al.*, 2009). However, because baboons are scarce and research facilities that can handle animals of their size are limited, more widely used experimental animal species, such as the rhesus or cynomolgus monkey, are considered to have their benefits (Yamanaka *et al.*, 2012). From the viewpoint of clinical relevance, since the menstrual cycle of cynomolgus monkeys is continuous over 4 weeks like the human cycle, while that of the rhesus is seasonal, cynomolgus monkeys are suitable for large-scale research of the disease (Yamanaka *et al.*, 2012).

A previous report on endometriosis in the cynomolgus monkeys of our breeding colony (Ami *et al.*, 1993) showed that the prevalence and basic histopathological characteristics were largely similar to other reports (Fanton and Hubbard, 1983; Cline *et al.*, 2008). However, information on more recent clinical findings, such as smooth muscle

metaplasia and innervation, is lacking. In addition to these components, several recent clinical reports have described endometriotic lesions in the lymph nodes and discussed their clinical importance as a potential source of the disease recurrence in women undergoing surgical therapy of endometriosis (Mechsner *et al.*, 2010; Tempfer *et al.*, 2011). With these clinical updates in mind, we report the detailed pathological characteristics of histologically confirmed spontaneous endometriosis in cynomolgus monkeys of a large breeding colony.

Materials and methods

Animals

The study was conducted on cynomolgus monkeys in the breeding colony of Tsukuba Primate Research Center (TPRC) at the National Institute of Biomedical Innovation, Health and Nutrition (Ibaraki, Japan). The facility has always kept ~1500–2000 cynomolgus monkeys and has nearly 200 neonates annually. Based on the observation of general condition, abdominal examination and ultrasound sonography scanning (ProSound $\alpha 7$, Hitachi-Aloka Medical, Ltd., Tokyo, Japan) at this facility, eight animals were suspected of having symptoms of endometriosis. While continuing to monitor the animals, five were sacrificed as moribund and three were found dead between 2005 and 2011 and all eight were finally diagnosed at histopathology as having endometriosis, which was considered the cause of death or deterioration of the general condition of the animals. Five animals were sacrificed by exsanguination under deep anaesthesia with hydrochloric ketamine (Ketalar, Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan) and xylazine hydrochloride (Seractal, Bayer Yakuhin, Ltd., Osaka, Japan) followed by a barbiturate (Somnopenyl, Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan).

All animals were reared at the primate centre and were housed indoors in individual cages. The animals were between 5 and 21 years of age, four of them had experienced pregnancy and giving birth (of which two experienced caesarean section (c-section)) and the other four had never experienced pregnancy. Menstruation of all animals had been noted periodically (Table I).

The environment of the animal room was set at 25°C \pm 2°C room temperature, 60% \pm 5% relative humidity and a 12-h light-and-dark cycle. Animals were given water *ad libitum* and fed daily with 70–100 g of commercially prepared monkey chow (35 pieces of type AS, Oriental Yeast, Tokyo, Japan) and 100 g of apple.

In addition to the above, embryo transfer and oocytes collection by aspiration of the ovarian follicles using a needle by laparotomy were conducted in two animals (00180–94014) and some animals received abdominal paracentesis in order to reduce cyst fluids with the guide of ultrasound sonography scanning.

Ethical approval

All experimental monkeys in this study were cared for using procedures approved by the Animal Care and Use Committee of the National Institutes of Biomedical Innovation, Health and Nutrition. Besides, protocols for all experiments involving animals were in compliance with the guidelines set by the Institute for the care, use and biological hazard countermeasures of laboratory animals.

Histopathology

After a gross observation, two or three lesions that were considered to represent the character of the abnormality in each animal were fixed in 4% (v/v) neutral buffered formalin and a paraffin-embedded thin section was stained with hematoxylin and eosin (HE) by the conventional method.

Adenomyosis was diagnosed by histopathological criteria (the presence of endometriotic gland and stromal cells in the inner 90% of the myometrium of the uterine wall) (Bergeron et al., 2006; Sato et al., 2012).

To confirm the origin of tissue components, a few representative sections of representative lesion were subjected to iron staining (one slide with Berlin blue; 2% potassium ferrocyanide solution (Muto Pure Chemicals, Tokyo, Japan)) or immunohistochemistry against CD10 (four slides with a marker for stromal cells (McCluggage et al., 2001)), CD31 (two slides with a marker for blood vessels (Witmer et al., 2002)). To know the character and its prevalence within the lesions, the sections which contained enough tissue elements of each animal were subjected for the immunohistochemistry against α -smooth muscle actin (15 slides with SMA) and PGP9.5 (14 slides with markers for nerve fibre (Morotti et al., 2014)).

Briefly, for immunohistochemistry, after antigen retrieval by microwave, the primary antibodies of CD10 ($\times 80$; clone: 56C6, AbD Serotec, Oxford, UK), CD31 (2.0 $\mu\text{g}/\text{mL}$; clone: JC70A, Dako, Glostrup, Denmark), α -SMA (1 $\mu\text{g}/\text{mL}$; clone: 1A4, Dako) and PGP9.5 (2.0 $\mu\text{g}/\text{mL}$; rabbit polyclonal, Dako) were applied at 4°C overnight. Each primary antibody was detected with EnVision™+ (Dako), visualized with diaminobenzidine (Wako Pure Chemical, Osaka, Japan) and counterstained with hematoxylin (Muto Pure Chemicals). As a negative control, immunoglobulin corresponding to each species (CD31, α -SMA and PGP9.5) or buf-

fer (CD10) was applied as a substitute for each primary antibody. These tissue sections were read under a light microscope (Optiphot-2, Nikon, Tokyo, Japan).

Results

General features of the endometriotic lesions in cynomolgus monkeys

Table II shows the in-life findings on general condition, abdominal examination and ultrasound sonography scanning. The data show that abnormality of stool and decrease in food consumption especially in the period of menstruation were most frequently observed while monitoring the general condition. From the abdominal examination, abnormality of the uterus was frequently detected in these animals. From the ultrasound sonography scanning, cysts could be detected, and it was also possible to perform abdominal paracentesis in order to reduce cyst fluid with ultrasound sonography scanning as a guide.

Table III shows the macroscopic findings of each cynomolgus monkey. In four out of eight animals, a large cystic lesion that often covered

Table I Reproductive histories of the cynomolgus monkeys with histologically confirmed endometriosis.

Animal no.	Age at necropsy (years)	Total pregnancies (times mated)	Vaginal delivery	Caesarean sections	Abortions	Years since previous pregnancy at diagnosis	Menstruations during previous 6 months
00178	5.1	NA (0)	NA	NA	NA	NA	2
00180	5.8	NA (0)	NA	NA	NA	NA	5
93164	12.4	2 (14)	1	0	1	2.5	5
93144	13.9	1 (10)	1	0	0	4.5	3
94043	15.7	0 (11)	NA	NA	NA	NA	6
94014	16.6	NA (0)	NA	NA	NA	NA	3
89093	20.6	6 (32)	5	1	0	4.3	6
85005	21.1	12 (22)	9	3	0	1.7	7

NA, not applicable.

Table II In-life symptoms and clinical observations of the cynomolgus monkeys with histologically confirmed endometriosis.

Animal no.	Pre-existing symptoms	Findings of abdominal examination	Ultrasound findings
00178	Decreased food consumption; no faeces; loose faeces	Abdominal distension	None
00180	Decreased food consumption; no faeces; loose faeces; vomiting	Enlargement, induration and irregular shape in uterus	Cyst
93164	Decreased food consumption; loose faeces with blood	Enlargement, induration and irregular shape in uterus; abdominal distension	Cyst
93144	Decreased food consumption; no faeces; loose faeces	Enlargement, induration and irregular shape in uterus	Cyst; nodule
94043	Decreased food consumption; no faeces; loose faeces	Enlargement and induration in uterus; abdominal distension	Cyst; nodule
94014	Decreased food consumption; loose faeces	Enlargement and induration in uterus; abdominal distension	Cyst; enlargement in uterus
89093	Decreased food consumption; no faeces; loose faeces	None	Cyst
85005	Decreased food consumption; no faeces; loose faeces	None	None

Table III Macroscopic findings in cynomolgus monkeys with histologically confirmed endometriosis.^a

Animal no.	Nodule	Cyst	Adhesion
00178	Co ¹ , Om ² , Ub, Ut		Co-Om, Om-Ub, Ub-Ut
00180	Co ¹ , Si, Ut ² , Om	Co ¹ , Ut ² , Si, Om	Co-Ut ³ , Si-Ut, Ub-Ut ² , Pt
93164	Dp ¹	Ut ²	Nodule-Pt, Ub-Ut
93144		Ch ¹ , Dp ² , Co, Om, Ut ²	Co-Ut, Om-Ut
94043		Ch ¹	Om-Ut ² , Ub-Ut, Co-Ut, Si-Ut, St-Pc ³
94014		Ch ¹ , Pt ²	Ch-Pt
89093	Ut	Ch ¹ , Dp, Ut ² ,	Om-Ub, Om-Ut, Ub-Ut
85005	Pt	Co ¹ , Ub, Ut ²	Co-Pt, Pt-Ub, Pt-Ut

^aThe slide number (1, 2 or 3) corresponding to the histopathological findings is shown in Table IV. Ch, chocolate cyst (large cyst that sometimes includes ovary, uterus and several pelvic organs); Dp, Pouch of Douglas; Co, colon; Om, omentum; Ov, ovary; Pt, peritoneal wall; Si, small intestine; Ub, urinary bladder; Ut, uterus; St, stomach; Pc, pancreas; empty columns, no finding.

the ovary, uterus and/or some other pelvic organs was observed and was confirmed by histopathology as a so-called chocolate cyst (~3–10 cm in diameter; Fig. 1). Regardless of the existence of this large lesion, smaller cysts (blueberry, black, brown or red in colour and ~0.3–2 cm in diameter) or nodular lesions (red or brown in colour and ~0.5–2 cm in diameter) were also observed on the surface of several organs and the peritoneal wall (Fig. 1). In addition, severe adhesions that covered several abdominal organs were consistently observed in all eight animals (Fig. 1, Table III).

Basic histopathological components of the endometriotic lesions in cynomolgus monkeys

Table IV lists the microscopically observed findings in the representative macroscopic lesions of each animal. Within the lesion tissues, endometriotic and stromal cells were observed, and the latter were confirmed by CD10 immunohistochemistry (Fig. 2A and B). In addition, haemorrhage and/or inflammatory cells were often accompanied with haemosiderin-laden macrophages (Fig. 2C and D) that also showed positive in iron staining (Berlin blue, Fig. 2E). Copious blood vessels were observed (Fig. 2F).

In three cases, adenomyosis, defined as the existence of endometriotic gland in the inner 90% of the myometrium, was observed (Fig. 3A). The lesions were composed of endometriotic glands and stromal cells surrounded by reactive hypertrophic myometrium (Fig. 3B). All of those lesions were accompanied with similar lesions in the outer 10% of the myometrium or parametrium (Fig. 3C). In addition, some of the lesions in the tissue blocks from the Douglas pouch and colon invaded the colonic muscle layer deeply and reached the submucosa (Fig. 3D–F).

Interstitial smooth muscle cells and nerve fibres of endometriotic lesions, and the lesions in lymph nodes of cynomolgus monkeys

Cells stained with α -SMA were observed in all cases (Table IV). These α -SMA-positive interstitial smooth muscle cells surrounded the endometriotic epithelial and stromal cells (Fig. 4A). In addition to these

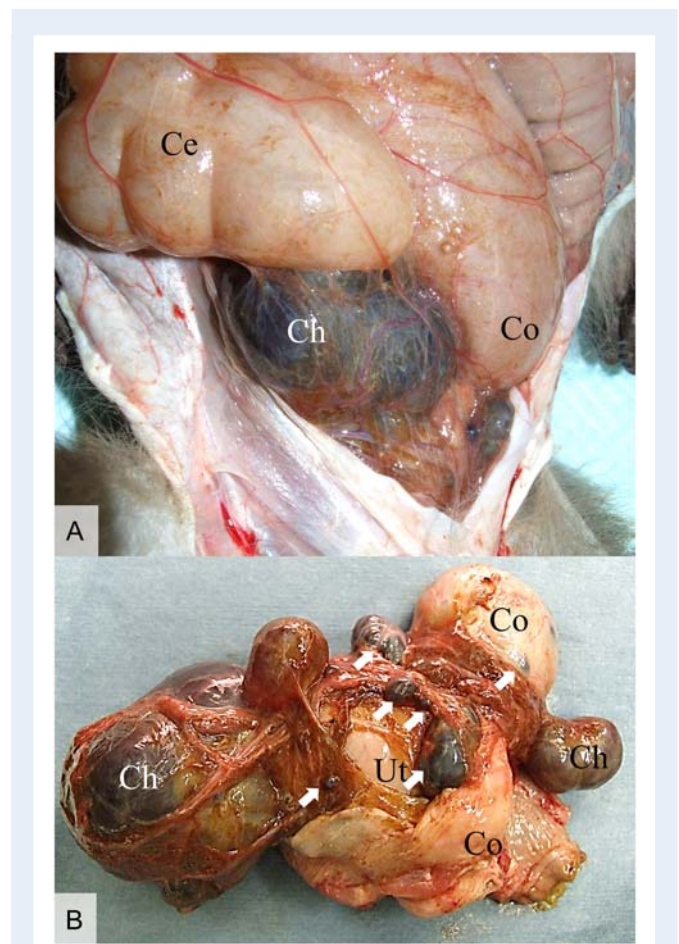


Figure 1 Gross appearance of spontaneous endometriosis in cynomolgus monkey. (A) Ventral aspect of the lesion of 93144. A large chocolate cyst (Ch) was observed, and the caecum (Ce) and colon (Co) were inflated with gas. (B) The same dissected lesion from the same animal. Uterus (Ut) was entangled with several organs by severe fibrosis. Arrows indicate small cysts or nodular lesions.

findings, PGP9.5 immunohistochemistry revealed sporadic distribution of fine nerve fibres in 9 out of 14 of PGP9.5 immunostaining slides and in 6 out of 8 animals (Fig. 4B, Table IV).

Table IV Histopathological findings in cynomolgus monkeys with histologically confirmed endometriosis.

Animal no.	Slide no. (organs) ^a	Findings ^b
00178	1 (Co)	Ep, Sc, Fi, He, Ic, Hm, Sm, Nf
	2 (Om)	Ep, Sc, Fi, He, Ic, Hm, Sm, Nf
00180	1 (Co)	Ep, Sc, Fi, He, Ic, Hm, Ly, Nf
	2 (Ut, Ub-Ut)	Ep, Sc, Fi, He, Ic, Hm, Sm, Nf
	3 (Co-Ut)	Ep, Sc, Fi, He, Ic, Hm, Sm, Nf
93164	1 (Dp)	Ep, Sc, Fi, He, Ic, Hm, Sm
	2 (Ut)	Ep, Sc, Fi, He, Ic, Hm, Ad, Sm
93144	1 (Ch)	Ep, Sc, Fi, He, Ic, Hm, Sm
	2 (Dp, Ut)	Ep, Sc, Fi, He, Ic, Hm, Sm, Nf
94043	1 (Ch)	Ep, Sc, Fi, He, Ic, Hm, Sm, Nf
	2 (Om-Ut)	Ep, Fi, He, Ic, Hm, Ad, Sm
	3 (St-Pc)	Ly
94014	1 (Ch)	Ep, Sc, Fi, He, Ic, Hm, Sm
	2 (Pt)	Ep, Sc, Fi, Ic, Hm, Sm
89093	1 (Ch)	Ep, Sc, Fi, He, Ic, Hm, Sm, Nf
	2 (Ut)	Ep, Sc, Fi, He, Ic, Hm, Sm
85005	1 (Co)	Ep, Sc, Fi, He, Ic, Hm, Sm, Nf
	2 (Ut)	Ep, Sc, Fi, He, Ic, Ad

^aThe slide number and organs correspond with those in macroscopic findings. Ch, chocolate cyst; Dp, Douglas pouch; Co, colon; Om, omentum; Pt, peritoneal wall; Ub, urinary bladder; Ut, uterus; St, stomach; Pc, pancreas.

^bMicroscopic findings: Ep, endometriotic epithelium; Sc, stromal cell; Fi, fibrotic interstitium; He, haemorrhage; Ic, inflammatory cells; Hm, hemosiderin-laden macrophages; Ad, adenomyosis; Ly, lymph node endometriosis; Sm, smooth muscle metaplasia; Nf, nerve fibre.

Endometriotic lesions within the lymph nodes were incidentally noted in two cases (Table IV) and both were from tissue blocks that included the mesocolic border of the colon. Endometriotic gland (Fig. 4C) and stromal cells (which were confirmed with CD10 immunohistochemistry) (Fig. 4D and E) were observed, but no interstitial components, such as connective tissues or smooth muscle, were observed.

Discussion

The information gained in this study is consistent with a previous study of the same breeding colony and on other earlier reports of endometriosis in cynomolgus monkeys. The prevalence of endometriosis in this breeding colony was previously reported (Ami et al., 1993) as 28.7% (ranging from 11 to 23 years of age with an average of 15.3 years) which is similar to that reported for the prevalence in rhesus monkey (31.4%; Zondervan et al., 2004). Ami et al. (1993) also reported that surgical procedures on the abdominal cavity did not affect the incidence of the disease in this cynomolgus monkey colony, which was confirmed in this study, because many animals other than those studied received c-section, embryo transfer and/or oocytes collection without any sign of endometriosis (data not shown). Surgical procedures did not appear to be a risk factor for the lesions, and other background and in-live information did not suggest any other factors that correlated with the prevalence or disease state.

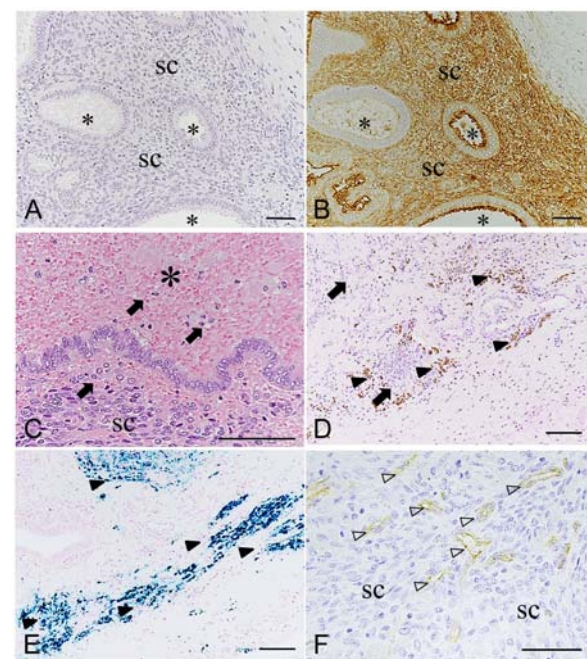


Figure 2 Basic components of endometriotic lesions in cynomolgus monkey. (A) The lumen of a cyst (asterisks) was covered with endometriotic epithelium and surrounded with stromal cells (sc). HE staining. Bar = 10 μ m. (B) The stromal cells were positive for CD10. CD10 immunostaining of a serial section of (A). Bar = 10 μ m. (C) The lumen of the cyst (asterisk) was filled with haemorrhage and inflammatory cells (arrows). HE staining. Bar = 10 μ m. (D) Haemosiderin-laden macrophages (closed triangles) and inflammatory cells (arrows) were observed in the cyst wall. HE staining. Bar = 10 μ m. (E) The macrophages were positive to iron staining (closed triangles). Berlin blue. Bar = 10 μ m. (F) Ample CD31-positive blood vessels (open triangles) were observed, mainly in the stromal (sc) and interstitial areas. CD31 immunostaining. Bar = 5 μ m.

Moreover, the frequently observed macroscopic (including so-called chocolate cysts) and histopathological (ectopic endometriotic and stromal cells, along with haemorrhage and/or inflammation) findings in this study were consistent with previous reports (Fantan and Hubbard, 1983; Ami et al., 1993; Cline et al., 2008) and with the findings in a severe case of surgically induced endometriosis in cynomolgus monkey (Schenken et al., 1987). Adenomyosis and deep infiltrating lesions of the colon were observed in this study, as in previous reports (Ami et al., 1993; Cline et al., 2008; Wilkinson et al., 2008; Sato et al., 2012), although some of the earlier reports noted spontaneous adenomyosis in cynomolgus monkeys without the accompanying endometriosis (Ami et al., 1993; Wilkinson et al., 2008; Sato et al., 2012).

We also compared the common features of endometriosis found in cynomolgus monkeys with those found in baboon, since lesions have been most intensively investigated in that species (Braundmeier and Fazleabas, 2009; D'Hooghe et al., 2009). Although major macroscopical and microscopical findings were commonly shared with the cynomolgus monkey, there are inter-facility differences in some aspects of the baboon cases. These inter-facility differences include prevalence,

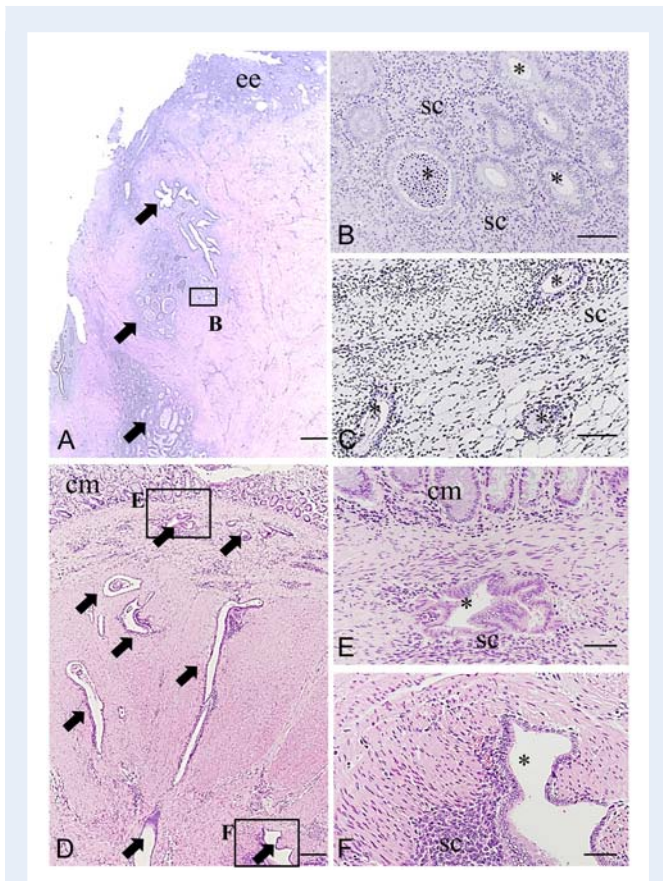


Figure 3 Adenomyosis and deep infiltrating endometriosis of the colon. **(A)** Lesions caused by adenomyosis (arrows) were distributed within the myometrium of the uterine wall. Eutopic endometrium (ee). HE staining. Bar = 1 mm. **(B)** Higher magnification of adenomyosis (box area in A). The lumen of the glands (asterisks) was covered with endometriotic epithelium and surrounded with stromal cells (sc). HE staining. Bar = 10 μ m. **(C)** Similar lesions to those in (B) were also observed in the parametrium. HE staining. Bar = 10 μ m. **(D)** The endometriotic lesions (arrows) invaded the colon muscle layer and reached through to the submucosa. Colonic mucosa (cm). HE staining. Bar = 50 μ m. **(E and F)** Higher magnifications of boxes in D. The lumen of the glands (asterisks) was covered with endometriotic epithelium and surrounded with stromal cells (sc). Colonic mucosa (cm). HE staining. Bar = 10 μ m.

which varied from a high of 25% to a low of 1.7% or 4.8%; abdominal operation (surgery or laparoscopy) as a risk factor, which was reported for two facilities but not in others; and ovarian endometriosis (the equivalent of a chocolate cyst) and deep infiltrating endometriosis, which were frequently observed at one facility but not recorded at others (Cornillie *et al.*, 1992; Dick *et al.*, 2003; Dehoux *et al.*, 2011). Since endometriosis in a certain sample size of cynomolgus monkeys has only been investigated at the facility of this study, it is crucial to accumulate information from other facilities in order to understand the total picture of the disease in the cynomolgus monkey.

Other than the above basic findings in NHP, the current report is the first to describe smooth muscle metaplasia and innervation in the disease tissue in NHP. Studies in a rat model and also of human

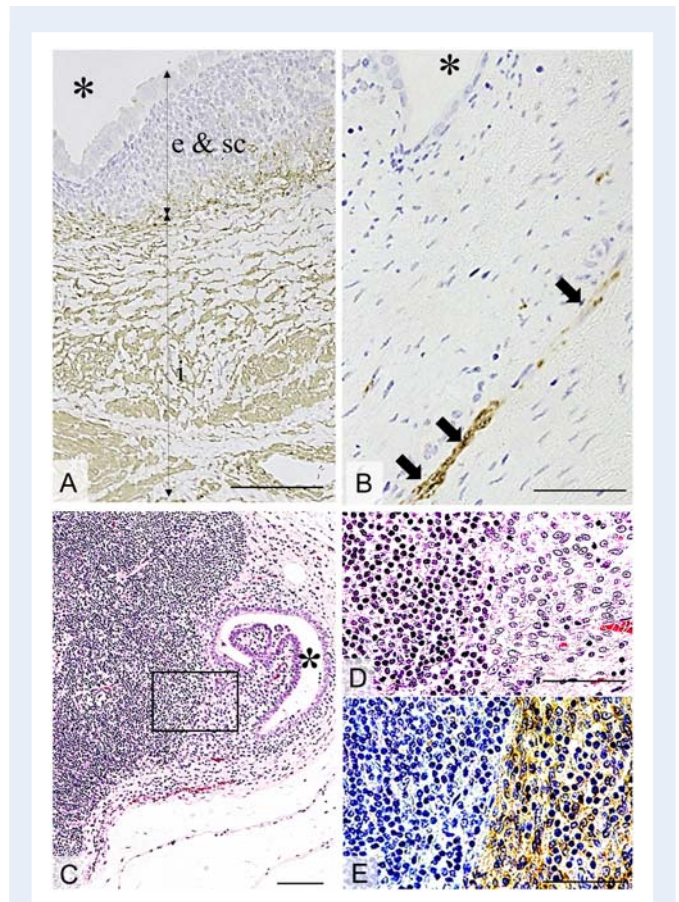


Figure 4 Smooth muscle metaplasia, nerve fibres and an endometriotic lesion in the lymph node. **(A)** The endometriotic epithelium and stromal cells (e & sc) were surrounded by metaplastic smooth muscle that was observed in the interstitium (i). α -SMA immunostaining. Bar = 10 μ m. **(B)** PGP9.5-positive nerve fibres were observed (arrows). PGP9.5 immunostaining. Bar = 10 μ m. **(C)** An endometriotic gland (asterisk) was observed in the lymph node. HE staining. Bar = 10 μ m. **(D)** High magnification of the boxed area of C. HE staining. Bar = 5 μ m. **(E)** CD10-positive stromal cells were observed in the serial sections of C and D. CD10 immunostaining. Bar = 5 μ m.

endometriotic lesions demonstrated that sympathetic and sensory nerves developed within the diseased tissue, and in the rat model, the fibres were found to be directly connected to the central nervous system via the splanchnic and vagus nerves (Berkley *et al.*, 2005). It has been suggested that sympathetic nerve fibres are distributed in metaplastic smooth muscle, which may cause pelvic pain when the muscle contracts (Odagiri *et al.*, 2009; Barcena de Arellano *et al.*, 2011; Barcena de Arellano and Mechsner, 2014). Additionally, the sensory nerves may also cause endometriosis-associated pain because several immune mediators of the diseased tissues directly stimulate these nerves (Odagiri *et al.*, 2009; Morotti *et al.*, 2014; Laux-Biehlmann *et al.*, 2015). Since this report describes these findings for the first time in NHP, further research on their aspects is needed, not only in cynomolgus monkeys but in other NHPs, including baboon.

In addition, this study incidentally found two cases of endometriosis within the lymph nodes for the first time in NHP. The lesions

were composed of an endometriotic gland and a CD10-positive stromal cells, which accords with clinical cases (Mechsner et al., 2010; Tempfer et al., 2011). These clinical cases were recently intensively discussed, and many researchers speculate that endometriosis in the lymph nodes may be related to recurrence after surgery or to a lymphatic dissemination of the disease (Mechsner et al., 2010; Tempfer et al., 2011). Since the presence of CD10-positive stromal cells in the lymph nodes of baboons was also reported previously (Hey-Cunningham et al., 2011), thorough investigation of abdominal lymph nodes in the spontaneous cases of endometriosis in NHPs may provide further information regarding the clinical implication of this finding.

The current investigation of spontaneous endometriosis in cynomolgus monkeys has revealed that many disease aspects are commonly shared with those found in other reports of the species, other NHPs and even with clinical cases. It is particularly noteworthy that there were several disease components in the cynomolgus monkey that might contribute to endometriosis-related pain and recurrence of the disease.

Authors' roles

A.N.K. and S.N. designed, performed and supervised experiments, analysed and interpreted data and wrote the manuscript. S.M., S.H. and T.I. acquired the data and prepared the figures. S.O., L.Y. and K.Y. acquired the data. O.K., A.K. and M.S. analysed and interpreted the data and gave critical discussions. R.K. and T.S. designed, performed and supervised experiments, analysed and interpreted data, held critical discussions and gave final approval of the manuscript.

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Conflict of interest

A.N.K., S.M., S.H., T.I., O.K., A.K. and M.S. are full-time employees of Chugai Pharmaceutical Co., Ltd. R.K. received lecture fees from Chugai Pharmaceutical Co., Ltd., unrelated to the submitted work. S.N., S.O., L.Y., K.Y. and T.S. have nothing to declare.

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