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Pelvic inflammation induced by diagnostic laparoscopy in baboons

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Objective: To test the hypothesis that diagnostic laparoscopy can cause pelviperitoneal inflammation.

Design: Retrospective analysis of data collected during a prospective controlled study in baboons.

Setting: An academic research environment.

Animal(s): Samples were collected during laparoscopies in female baboons at the Institute of Primate Research, Kenya.

Intervention(s): In the first part of the study, 44 laparoscopies were performed in 16 baboons (5 with a normal pelvis, 11 with endometriosis) during the luteal phase, with a time interval of 1 month. In the second part of the study, 53 laparoscopies were performed in 15 baboons (6 with a normal pelvis, 9 with endometriosis) during the late follicular and luteal phases of one cycle, with a median time of 3–4 days between each laparoscopy.

Main Outcome Measure(s): Peritoneal fluid (PF) was measured and analyzed for white blood cell (WBC) concentrations and, in the second part of the study, for the distribution of lymphocyte subsets (CD3, CD4, CD8, and CD20) and for the presence of cytokines transforming growth factor-β1, interleukin (IL)-6, and IL-10.

Result(s): In the first part of the study, PF volumes and WBC concentrations were comparable at the baseline and follow-up laparoscopies. In the second part of the study, PF obtained at the second laparoscopy showed a 10-fold increase in volume, a 3-fold increase in WBC concentration, a 10-fold increase in IL-6 concentration, and a 2-fold increase in transforming growth factor-β1 concentration when compared with PF obtained at the first laparoscopy. The PF subset of granulocytes and CD3-positive cells was higher and the PF subset of macrophages was lower at follow-up laparoscopies than at the baseline laparoscopy.

Conclusion(s): Diagnostic laparoscopy can cause peritoneal inflammation in baboons. (Fertil Steril® 1999; 72:1134–41. ©1999 by American Society for Reproductive Medicine.)

Key Words: Baboon, laparoscopy, inflammation, peritoneal fluid, cytokine, white blood cells

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Diagnostic laparoscopy is often performed in gynecologic practice for infertility and pelvic pain and is generally considered a safe procedure, although surgical complications (e.g., accidental puncture of the bowel and blood vessels) and anesthesia-related complications can occur. We hypothesized that local pelvic inflammation occurs after diagnostic laparoscopy, which may be due to manipulation and microtrauma of the pelvic organs, together with toxic effects induced by the CO_2 used for intraabdominal insufflation. Such iatrogenically induced inflammation could have adverse effects on female fertility and may be involved in facilitating the establishment and spontaneous evolution of endometriosis, as hypothesized previously (1–3).

To test this hypothesis would require serial laparoscopies with assessment of indices of pelvic inflammation within a few days after the initial diagnostic laparoscopy. Obviously, such testing is impossible in women because of ethical considerations. Baboons are very similar to women with respect to their pelvic anatomy, reproductive physiology, and immunology, and are well-established models for the study of endometriosis (4). The laparoscopic techniques needed for baboons have been described extensively (5). An opportunity to test our hypothesis that laparoscopy is associated with inflam-

TABLE 1

Peritoneal fluid volume obtained at 53 laparoscopies performed during the late follicular phase (n = 7) or luteal phase (n = 46) in 15 baboons.

Animal no.	PF volume (mL) at indicated cycle phase					
	1–5 d before ovulation	1–2 d after ovulation	4–5 d after ovulation	8–9 d after ovulation	12–13 d after ovulation	16–17 d after ovulation
Controls						
Pan 1435	—	7	NA	NA	8	—
Pcy 71	—	—	2	15	22	8
Pan 1706	—	—	—	1.2	—	NA
Pan 1655	—	—	1	NA	3	—
Pan 1526	—	—	1.5	—	1.5	0.5
Pan 1668	—	—	0.5	NA	4	9
Baboons with endometriosis						
Pan 1587	—	1.5	—	14	8	NA
Pan 1443	—	—	2	NA	8	8
Pan 867	1	—	18	—	—	—
Pan 873	7.5	—	45	9	35	8
Pan 1651	0.3	—	5	—	1.5	—
Pan 1422	3	—	15	9	NA	5.5
Pan 833	2	—	1.5	NA	3	—
Pan 865	4	—	3	11	—	34
Pan 1574	0.3	—	8.5	—	—	—

Note: Pan = *Papio anubis*; — = no laparoscopy performed; NA = PF volume not assessed; Pcy = *Papio cynocephalus*.

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mation arose when baboons underwent serial laparoscopies during the luteal phase of the same cycle (6) or during the luteal phase of consecutive cycles (7).

MATERIALS AND METHODS

Animals

In the first part of this study (1991–1992), 16 adult female baboons (12 *Papio anubis*, 4 *Papio cynocephalus*) of proved fertility were housed in single cages at the Institute of Primate Research, Nairobi, Kenya. They had been in the colony for a mean (±SD) of 43 ± 29 months (median, 50; range, 6–84 months), and their mean (±SD) weight was 12.2 ± 2.3 kg (median, 12.8; range, 7–16.8 kg). Study animals consisted of primates with a normal pelvis (n = 5) and animals with spontaneous endometriosis (n = 11). Endometriosis was staged according to the adapted classification of the American Fertility Society (AFS) (8) after modification for baboon size. The 11 baboons with endometriosis included animals with adapted revised AFS stage I (n = 6) or stage II (n = 5) disease. All animals with endometriosis had histologically proved disease (presence of both endometrial glands and stroma).

In the second part of this study (1993), 15 adult female baboons (14 *Papio anubis*, 1 *Papio cynocephalus*) of proved fertility were housed in single cages at the Institute of Primate Research, Nairobi, Kenya. They had been in the colony for a mean (±SD) of 53 ± 39 months (range, 18–128 months), and their mean (±SD) weight was 12.3 ± 2.0 kg (range, 8.3–15.9 kg). This group included 6 animals that had also participated in the first part of this study (2 with a normal pelvis and 4 with spontaneous endometriosis, adapted revised AFS stage I [n = 1] or stage II [n = 3]). Overall, the 15 animals included primates with a normal pelvis (n = 6) and animals with both spontaneous (n = 6) and induced (n = 3) endometriosis (Table 1). The 3 baboons with adapted revised AFS stage III–IV endometriosis had advanced disease based primarily on the adhesion score and not on the presence of cystic ovarian endometriosis. Induction of endometriosis had been performed by intraperitoneal injection of menstrual endometrium in the pelvic cavity, as described previously (9). All animals with endometriosis had histologically proved disease (presence of both endometrial glands and stroma).

The study protocol was reviewed and approved by the Institute of Primate Research Scientific Resources Evaluation and Research Committee, which assesses the need for primate studies, the health care of the baboons involved, and the experimental design and methods of proposed studies.

Laparoscopy

Perineal staging was used to detect ovulation because perineal inflation and deflation correspond well with the follicular and luteal phases, respectively, with ovulation occurring 3 days before perineal deflation in the baboon (10). Techniques of anesthesia and diagnostic laparoscopy were

performed as described previously (5). The induction of anesthesia, actual surgical procedure, and baboon recovery lasted about 15, 15, and 10 minutes, respectively. The CO_2 insufflation rate was 1–2 L/min, resulting in a total intraperitoneal CO_2 volume of 15–30 L. Peritoneal fluid (PF) was aspirated with a laparoscopic needle from the posterior cul-de-sac, vesicouterine fold, and pararectal gutters immediately after insertion of the laparoscope and before organ manipulation at the beginning of the laparoscopy, before the baboon was placed in the Trendelenburg position. Three laparoscopies performed in two baboons were excluded from further analysis because of iatrogenic bleeding caused by manipulation of adhesions. The PF was collected in sterile heparinized tubes and kept at 4°C until processing, which occurred within 2 hours of collection.

In the first part of this study (1991–1992), 44 laparoscopies were performed in 16 baboons. The first laparoscopy was performed in the early luteal phase, followed by a second laparoscopy in the early luteal phase of the next cycle in all 16 baboons, and by a third laparoscopy in the early luteal phase of the subsequent cycle in 4 primates. Four baboons underwent a second series of paired laparoscopies 6 months later. Again, the first laparoscopy was performed in the early luteal phase, followed by a second laparoscopy in the early luteal phase of the next cycle in all 4 animals.

In the second part of this study (1993), 53 laparoscopies were performed, as described in Table 1. The mean time interval was 4.5 ± 2.8 days, 3.6 ± 0.9 days, 3.2 ± 0.8 days, and 3 days between the first and second, second and third, third and fourth, and fourth and fifth laparoscopies, respectively. During these laparoscopies, only careful inspection of the ovulation stigma and corpus luteum (6) was performed, without any other concomitant operative procedure.

All of the baboons studied recovered well after each laparoscopy; none of them developed signs of pelvic or peritoneal infection.

Peritoneal Fluid Analysis

In the first part of this study (1991–1992), PF was measurable in 43 of 48 laparoscopies with use of a finely graduated syringe. The concentration of white blood cells (WBCs) was determined with a hemocytometer in 18 (9 paired) laparoscopies in eight baboons (four with a normal pelvis, one with spontaneous adapted revised AFS stage I endometriosis, and three with spontaneous adapted revised AFS stage II endometriosis).

In the second part of this study (1993), PF was measurable in 44 laparoscopies with use of a finely graduated syringe. It was not possible to analyze all PF study variables because of technical difficulties (remote location of Coulter counter and fluorescence-activated cell sorter; limited amounts of PF for all determinations). The concentration of WBCs and the distribution of lymphocyte subsets (CD3 [T-cells], CD4 [T-helper/inducer cells], CD8 [T-suppressor/cytotoxic cells], and CD20 [B cells]) could be determined after 19 laparoscopies in eight baboons (one with a normal pelvis, two with spontaneous adapted revised AFS stage II endometriosis, two with induced adapted revised AFS stage II endometriosis, two with spontaneous adapted revised AFS stage III endometriosis, and one with induced adapted revised AFS stage IV endometriosis).

A Coulter counter was used to measure the total WBC concentration. Flow cytometry with light scattering was used to determine the proportions of lymphocytes, monocytes/macrophages, and granulocytes. Phycoerythrin- and fluorescein isothiocyanate (FITC)-conjugated mouse antihuman monoclonal antibodies (Becton-Dickinson, Mountain View, CA) were used for the assessment of WBC subsets. PF cells were analyzed without Ficoll-Paque separation. Twenty microliters of FITC- or phycoerythrin-conjugated antibody was added to 200 μL of whole PF, in a 1:2 dilution with phosphate-buffered saline, and staining was performed at 4°C for 20 minutes. Subsequently, red blood cells were lysed with 2 mL of NH_4Cl (9.3 g/L) for 5 minutes. PF cells were then centrifuged, and the pelleted cells were fixed with 1 mL of 0.5%–1% paraformaldehyde and analyzed by flow cytometry using a FACStar Plus cell sorter (Becton-Dickinson).

PF samples from 16 laparoscopies in six baboons (1–4 laparoscopies per baboon) were analyzed by ELISA (R&D Systems, Minneapolis, MN) for the cytokines interleukin-6 (IL-6; lowest limit of sensitivity 0.7 pg/mL), transforming growth factor-β1 (TGF-β1; lowest limit of sensitivity 5 pg/mL), and IL-10 (lowest limit of sensitivity 2 pg/mL). These six baboons included two animals without endometriosis and four primates with spontaneous endometriosis (two with revised AFS stage II and two with revised AFS stage III disease). The IL-6 cytokine (R&D Systems) had been validated previously for use in PF (11) and in nonhuman primates (12).

Statistical analysis was performed with Kruskal-Wallis test, Wilcoxon test for paired samples, Mann-Whitney *U* test, factorial analysis of variance (ANOVA), and (un)paired Student's *t*-test where appropriate. $P<.05$ was considered statistically significant.

RESULTS

In the first part of this study, both mean (±SD) PF volumes and mean (±SD) PF WBC concentrations were distributed nonparametrically but were comparable ($P=.4$, Kruskal-Wallis and paired Wilcoxon tests) at the baseline laparoscopy (2.8 ± 2.3 mL [median, 2.4; range, 0–9 mL]; 3.0 ± 1.6 × 10^3 WBC/μL [median, 3.1; range, 0.8–5.2 × 10^3 WBC/μL]) and at the follow-up laparoscopies performed after 1 month (2.3 ± 2.1 mL [median, 2.0; range, 0–9 mL]; 3 ± 2.9 × 10^3 WBC/μL [median, 1.7; range, 0.4–7.5 × 10^3 WBC/μL]) and after 2 months (3.1 ± 2.8 mL [median, 3.1; range, 0–6 mL]).

TABLE 2

The effect of serial laparoscopies on PF volume and WBC concentration.

Laparoscopy	No. of subjects	Volume of PF (mL)*	No. of subjects	WBC concentration ($\times 10^3/\mu L$)†	Percentage of macrophages‡	Percentage of granulocytes‡	Percentage of lymphocytes	Percentage of CD2-positive cells§	Percentage of CD4-positive cells	Percentage of CD8-positive cells	Percentage of CD20-positive cells
First											
Preovulatory	8	2.6 ± 2.6	7	1.5 ± 0.6	66 ± 16	6 ± 10	27 ± 11	84 ± 4	27 ± 9	69 ± 8	4 ± 2
		2 (0.5–7.5)		1.6 (0.8–2.3)	—	3 (2–29)	23 (17–47)	—	28 (12–38)	70 (56–79)	5 (2–6)
Postovulatory	7	2.1 ± 2.0	5	2.1 ± 1.5	—	—	—	—	—	—	—
		1.5 (0.3–7)		2.3 (0.3–4.2)	—	—	—	—	—	—	—
All	15	2.3 ± 2.2	12	1.7 ± 1.1	66 ± 16	6 ± 10	27 ± 11	84 ± 4	27 ± 9	69 ± 8	4 ± 2
		1.5 (0.3–7.5)		1.6 (0.3–4.2)	—	3 (2–29)	23 (17–47)	—	28 (12–38)	70 (56–79)	5 (2–6)
Second											
Postovulatory	10	12.6 ± 12.9	6	4.4 ± 1.6	42 ± 14	17 ± 7	36 ± 25	85 ± 9	33 ± 20	62 ± 7	5 ± 2
		11.2 (1.5–45)		4.5 (2.3–6.1)	—	17 (7–25)	37 (19–54)	—	44 (10–51)	61 (54–71)	6 (2–9)
Third											
Postovulatory	10	7.6 ± 6.2	2	2.0 ± 2.3	32 ± 5	30 ± 20	36 ± 25	85 ± 11	33 (n = 1)	48 ± 33	7 ± 6
		8.0 (5–22)		2 (0.4–3.6)	—	30 (16–45)	36 (19–54)	—	—	48 (24–72)	7 (3–11)
Fourth											
Postovulatory	7	—	4	1.9 ± 1.1	37 ± 17	30 ± 17	39 ± 9	89 ± 2	36 ± 4	64 ± 6	5 ± 2
		8 (3–35)		2.5 (0.8–8.7)	—	30 (12–48)	35 (30–48)	—	34 (33–42)	64 (58–71)	4 (3–8)
Fifth											
Postovulatory	2	—	0	—	—	—	—	—	—	—	—
		6.7 (5.5–8)	—	—	—	—	—	—	—	—	—

Note: Values are mean ± SD, followed by median (range).

* $P=.002$ by Kruskal-Wallis test. $P=.02$ for first (n = 6) vs. second (n = 6) laparoscopy by Wilcoxon paired test. $P=.8$ for laparoscopy before vs. laparoscopy after ovulation by Mann-Whitney *U* test.

† $P=.04$ by Kruskal-Wallis test. $P=.02$ for first (n = 6) vs. second (n = 6) laparoscopy by Wilcoxon paired test. $P=.4$ for laparoscopy before vs. laparoscopy after ovulation by Mann-Whitney *U* test.

‡ $P<.05$ by Kruskal-Wallis test.

§ $P=.02$ for first vs. second laparoscopy by paired *t*-test.

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In the second part of this study, PF volumes (Table 2) were distributed nonparametrically but were comparable ($P=.8$, Mann-Whitney U test) in the seven baboons initially screened before ovulation (median, 2 mL; range, 0.3–7.5 mL) and in the eight animals initially screened after ovulation (median, 1.5 mL; range, 0.5–7 mL). The volume of the PF (Table 2) collected at the initial laparoscopy was significantly lower than that collected during follow-up laparoscopies ($P=.002$, Kruskal-Wallis test) and was approximately 10-fold lower (median, 1.5 mL; range, 0.3–7.5 mL) at the first laparoscopy than the volume collected at the second laparoscopy (median, 11.2 mL; range, 1.5–45 mL; $P=.02$, Wilcoxon test for paired samples). The PF volumes after the second laparoscopy were comparable among the baboons that had additional serial laparoscopies (Table 2).

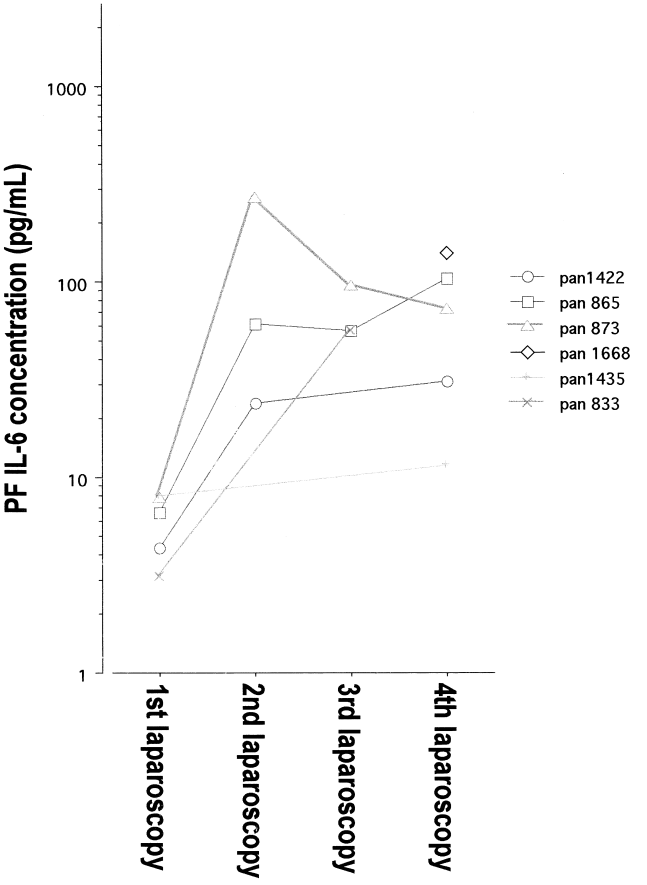
In the second part of this study, the PF WBC concentrations (Table 2) were also distributed nonparametrically and were comparable ($P=.4$, Mann-Whitney U test) in the baboons initially screened before ovulation (median, 1.6; range, 0.8–2.3 $\times$ 10^3 WBC/μL) and in the animals initially screened after ovulation (median, 2.4; range, 0.3–4.2 $\times$ 10^3 WBC/μL). The concentration of WBCs in the PF (Table 2) was significantly lower ($P=.04$, Kruskal-Wallis test) at the initial laparoscopy than at the follow-up laparoscopies and was approximately 3-fold lower (median, 1.6; range, 0.9–2.3 $\times$ 10^3 WBC/μL) at the first laparoscopy than the WBC concentration in PF collected at the second laparoscopy (median, 4.5; range, 2.3–6.1 $\times$ 10^3 WBC/μL; $P=.03$, Wilcoxon test for paired samples). The PF WBC concentrations after the second laparoscopy were comparable among the baboons that had additional serial laparoscopies (Table 2).

The subset of macrophages was higher at the initial laparoscopy ($P=.04$, Kruskal-Wallis test; $P=.02$, factorial ANOVA test) than at follow-up laparoscopies (Table 2). In contrast, the subset of granulocytes was lower at the initial laparoscopy ($P=.03$, Kruskal-Wallis test) than concentrations observed during follow-up laparoscopies, and no changes were observed in the subset of lymphocytes (Table 2). Within the population of lymphocytes, the CD2 lymphocyte subset was slightly lower at the baseline laparoscopy than at the second laparoscopy (paired t-test, $P=.01$), but no differences were observed in the CD4, CD8, and CD20 subsets between baseline laparoscopy and follow-up laparoscopies (Table 2).

In the second part of this study, the PF concentration of IL-6 (Fig. 1) increased 10-fold from the first laparoscopy (median, 6.6 pg/mL; range, 3.1–8 pg/mL) to the second laparoscopy (median, 61.1 pg/mL; range, 24–263 pg/mL) and remained elevated at the third laparoscopy (median, 56.6 pg/mL; range, 56.2–93 pg/mL) and the fourth laparoscopy (median, 71.6 pg/mL; range, 11.4–140.4 pg/mL). The concentration of TGF-β in the PF (Fig. 2) also increased 2-fold from the first laparoscopy (median, 1,800 pg/mL; range, 1,200–2,500 pg/mL) to the second laparoscopy (median, 6,150 pg/mL; range, 4,700–6,300 pg/mL) and remained elevated at the third procedure (median, 9,200 pg/mL; range, 6,900–10,900 pg/mL) and the fourth procedure (median, 8,300 pg/mL; range, 4,400–9,900 pg/mL). The PF concentration of IL-10 was beneath the detection limit of 2 pg/mL in 12 of the 16 PF samples. In the other 4 samples, values were 2.3, 3.2, 6.6, and 7.4 pg/mL and were unaffected by subsequent laparoscopy.

FIGURE 1

The effect of serial laparoscopies on the peritoneal fluid concentration of IL-6 (pg/mL).



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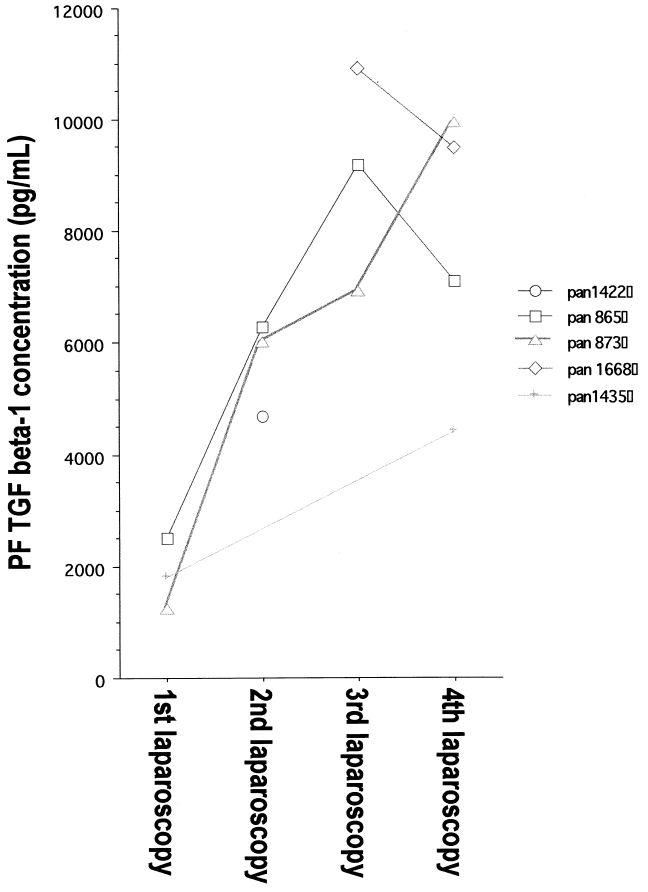
DISCUSSION

The results of this study indicate that diagnostic laparoscopies in baboons can cause local pelviperitoneal inflammation, as evidenced by the increased volume of PF, increased PF WBC concentration, and increasing concentrations of PF granulocytes, CD3 lymphocytes, IL-6, and TGF-β observed within 1 week of the baseline laparoscopy. Although surgical excision of endometriotic lesions and adhesions was not

FIGURE 2

The effect of serial laparoscopies on the peritoneal fluid concentration of TGF-β1 (ng/mL).



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performed during any of the laparoscopies performed in this study, it is possible that repeated manipulation of the pelvic peritoneum and organs facilitates at least microtrauma to the tissues, leading to inflammation. Unfortunately, systemic markers of acute-phase inflammation, such as C-reactive protein, were not assayed. Therefore, we cannot exclude that the observed peritoneal inflammation may also be secondary to a systemic inflammatory response to the anesthesia, the laparoscopy, and the associated stress of surgery.

Our observations may have important clinical implications regarding laparoscopy in women because subclinical pelvic inflammation may facilitate adhesion formation, which could affect female fertility. However, it seems more likely that the inflammation caused by laparoscopy is self-limited, resolving potentially within a maximum of 1 month after the initial laparoscopy. This speculation is supported by several observations. First, paired laparoscopies performed during the early luteal phase with an interval of 1 month did not significantly affect the amount of PF or the PF WBC concentration. Second, the PF volume and WBC concentration after the second laparoscopy were comparable among the baboons that had additional laparoscopies. This suggests that a maximal acute-phase inflammation is produced by the initial laparoscopy. Therefore, it seems unlikely that a diagnostic laparoscopy, while causing self-limited pelvic inflammation, will significantly affect fertility. In contrast, pelvic inflammatory disease caused by infection with *Chlamydia* and other microorganisms may lead to pelvic adhesions and tubal infertility.

In the second part of our study, no differences in either volume or WBC concentrations were observed in the PF obtained at laparoscopies performed before or after ovulation. However, most of the initial measurements in the preovulatory group were taken in baboons with endometriosis (n = 7) and compared with initial measurements in the postovulatory group, in which six of eight baboons had a normal pelvis. It can be argued that the lack of significant differences in the initial laparoscopy groups (for PF volume as well as WBC concentrations) may have occurred because the endometriotic baboons harbored a greater PF volume and WBC concentration at the late follicular phase versus the (possible) physiologic increase during the early luteal phase in the normal baboons.

This potential bias must be put in perspective and compared with published data about the association among PF volume and WBC concentration, cycle phase, and the presence or absence of endometriosis in baboons. In women, the PF volume has been reported to increase progressively during the follicular phase, to attain a maximum value during the early luteal phase, and to decline afterward (13, 14). In baboons, the effect of cycle stage and of the presence of endometriosis on PF volume and PF WBC concentrations seems to be more complex. First, in a cross-sectional study (15), an increased PF WBC concentration was observed only in baboons with spontaneous endometriosis and not in those with induced endometriosis, whereas in the current study, three of seven baboons in the preovulatory group had induced endometriosis. Second, the same study (15) demonstrated that the WBC concentration, PF macrophage concentration, and the percentages of lymphocyte subsets were comparable during menses, the follicular phase, and the luteal phase in baboons with and without endometriosis. Third, in another cross-sectional study (16), no statistically significant differences in PF volume were observed between baboons with and without either endometriosis or luteinized unruptured follicle syndrome. Fourth, an initial study evaluating the prevalence of spontaneous endometriosis did not find statistically significant differences in PF volume according to cycle stage (5).

On the basis of the previous two paragraphs, one cannot exclude the possibility that ovulation independently contributed to the 10-fold increase in PF volume and the 3-fold

increase in PF WBC concentration observed during the second laparoscopy. However, this bias seems to be relatively unimportant when considering published data (15, 16) and a recent study in baboons (17) that had 80% power to exclude a 60% (one-sided $\alpha = 0.05$) to 70% (two-sided $\alpha = 0.05$) increase in PF volume during the luteal phase when compared with the follicular phase. Therefore, it is unlikely that the 10-fold and 3-fold increases in PF volume and WBC concentration, respectively, at the second laparoscopy when compared with the first laparoscopy can be adequately explained by the effect of ovulation.

The acute peritoneal inflammatory effect at the second laparoscopy was reflected not only by the increased PF volume and WBC concentration, but also by the increased proportion of PF granulocytes and the increased PF concentrations of (pro)inflammatory cytokines, such as IL-6 and TGF-β. The increased proportion of granulocytes is not surprising because neutrophil granulocyte exudation from the blood into the peritoneal cavity would be expected to happen as one of the first events in the inflammation cascade (18). After the additional surgeries, their proportion increased from a mean ($\pm$SD) of 17% $\pm$ 7% at the second laparoscopy to a mean ($\pm$SD) of 30% $\pm$ 20% at the third laparoscopy. In contrast, the proportion of macrophages decreased from a mean ($\pm$SD) of 66% $\pm$ 16% at the first laparoscopy to a mean ($\pm$SD) of 42% $\pm$ 14% at the second laparoscopy and remained low at the third and fourth procedures. This finding occurred because, in conditions of acute inflammation, movement of monocytes from the blood into the peritoneal area with subsequent transformation to macrophages occurs later than the migration of granulocytes (18). It is possible that the expected increase in PF macrophages was not observed in this study because the repeated surgical interventions induced an acute inflammatory response at each laparoscopy.

The cytokines IL-6, TGF-β, and IL-10 were assessed in this study because they are potentially involved in inflammation, are produced by activated monocytes and T cells, and have potent pleiotropic and immunomodulatory activities. IL-6 is a proinflammatory cytokine and a principal growth factor for activated B cells late in the sequence of B-cell differentiation. IL-10 also stimulates the growth and differentiation of B cells and has a wide variety of immunosuppressive activities, including inhibition of proinflammatory cytokine production such as IL-6 and IL-1, inhibition of T-cell proliferation and IL-2 production, and inhibition of class II major histocompatibility complex antigen expression on monocytes (19). TGF-β1 inhibits the growth of many cell types while stimulating the proliferation of others and causes the synthesis of extracellular matrix proteins and the formation of new blood vessels (angiogenesis). Cytokines involved in inflammation such as TGF-β (20), tumor necrosis factor-α (21), and IL-6 (22) have also been implicated in the pathogenesis of endometriosis (23).

The findings of our study may have important implications relevant to the incidence and spontaneous evolution of endometriosis in baboons. In our previous studies (2), regular laparoscopic observations were performed over 30 months in 13 baboons with spontaneous endometriosis. Periods of development and regression of endometriosis were observed, resulting in overall disease progression as evidenced by a statistically significant increase in AFS score and in both the number and surface area of endometriotic lesions. Subsequently, in another of our studies (3), the incidence of spontaneous endometriosis in baboons with an initially normal pelvis was determined over 32 months; the cumulative incidence of minimal endometriosis (proved by histology) was found to be 64%. The 8 baboons that developed biopsy-proved endometriosis were followed during a longer period of time and had undergone more serial laparoscopies than the animals that did not get the disease (3).

It is possible, as hypothesized previously (1), that peritoneal trauma and irritation occurred during these repeated laparoscopies. Together with the potentially toxic effects of CO_2 insufflation and general anesthesia, these factors may have favored the eventual development of endometriosis through locally induced inflammation, as indicated in our current study. To test this hypothesis further, additional studies are needed to compare the natural history of endometriosis in baboons that undergo and do not undergo serial laparoscopies performed weekly.

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