Treatment with anti-TNF monoclonal antibody (c5N) reduces the extent of induced endometriosis in the baboon


1 Department of Obstetrics and Gynaecology, Karolinska Hospital, Stockholm, Sweden, 2 Institute of Primate Research, Nairobi, Kenya, 3 Centocor Inc; Malvern, PA, USA, 4 Department of Obstetrics and Gynaecology, Leuven University Fertility Center, Leuven, Belgium and 5 Department of Obstetrics and Gynaecology, Danderyds Hospital, Stockholm, Sweden.

To whom correspondence should be addressed at: Department of Obstetrics and Gynaecology, Leuven University Fertility Center, UZ Gasthuisberg, Herestraat 49, B-3000, Leuven, Belgium. E-mail: thomas.dhooghe@uz.kuleuven.ac.be

BACKGROUND: Inflammatory cytokines, including interleukin (IL)-1, IL-6, IL-8 and tumour necrosis factor-alpha (TNF-α), are important in the pathogenesis of endometriosis. We assessed the efficacy of anti-TNF monoclonal antibody (mAb, c5N), known to prevent induced endometriosis in baboons, in reducing established endometriosis in baboons. METHODS: This prospective, randomized, blinded, controlled study was conducted in baboons at the Institute of Primate Research (IPR), Nairobi, Kenya. Endometriosis was induced in 18 adult female baboons (Papio anubis) with regular menstrual cycles and a normal pelvis; the extent of endometriosis was documented by videolaparoscopy 25 days later. The baboons were then randomly assigned to receive a single infusion of either placebo (n = 7, 5 ml/kg) or c5N (n = 11, 5 mg/kg). Follow-up laparoscopy was performed 25 days later to document any differences in the number, surface area and estimated volume of lesions between the two groups and between the first and the second laparoscopies in each group. Representative biopsies of at least one endometriotic lesion per baboon were obtained at the final laparoscopy. RESULTS: Significant reductions in total surface area, estimated total volume of endometriotic lesions and both number and surface area of red lesions were observed after treatment with c5N, but not after placebo treatment, when compared to the initial laparoscopy. Conversely, a significant increase in the number of typical and red lesions was observed after placebo treatment when compared to the initial laparoscopy. Neither c5N nor placebo treatment affected the menstrual cycle. CONCLUSION: In baboons with induced endometriosis, anti-TNF-mAb (c5N) treatment significantly reduced the extent of endometriosis, mainly due to reducing both the number and surface area of red lesions. These findings suggest that anti-TNF-mAb therapy may have therapeutic potential for active peritoneal endometriosis.

Key words: anti-TNF/baboon/cytokines/endometriosis/randomized controlled study

Introduction

Endometriosis is a major benign gynaecological disease defined by the presence of functional endometrial tissue fragments outside the uterine cavity. Endometriosis contributes to more than 100,000 hysterectomies worldwide each year (Carlson et al., 1994), and the estimated total hospitalization costs for women in the USA with endometriosis as the primary diagnosis was $540 million in 1991 (Zhao et al., 1998).

The cornerstone in the pathogenesis of endometriosis is the Sampson theory of retrograde menstruation (Sampson, 1927). However, retrograde menstruation occurs in most women and yet not all develop endometriosis (Blumenkrantz et al., 1981; Halme et al., 1984). Immunological factors and the inflammatory response have been reported to play important roles in the pathogenesis. Several cytokines, such as interleukin (IL)-1, IL-6, IL-8 and tumour necrosis factor-alpha (TNF-α), have been found to be elevated in women with endometriosis (Wieser et al., 2002). TNF-α from endometriotic lesions and mesothelial cells stimulate production of IL-8 and regulated on activation normally T cell expressed and presumably secreted (RANTES), which in turn promote neangiogenesis and recruitment of macrophages, T cells and eosinophils. The significance of TNF-α in the pathogenesis of endometriosis is supported by the proliferative effect of TNF-α observed on endometrial cells from women with endometriosis but not on cells from healthy controls (Braun et al., 2002). This proliferative effect was blocked by the anti-TNF-α agent etanercept (Braun et al., 2002), and recent studies suggest that apoptosis of monocytes and lymphocytes could be the mechanistic basis of TNF-α inhibitors (Di Sabatino et al., 2004; Shen et al., 2005). Furthermore, recent studies indicate that anti-TNF-α reduces pathological neovascularization (Gardiner et al., 2005).
and inhibits the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) by mucosal T cells (Agnholt et al., 2004). Thus, anti-TNF-α may act on several possible pathophysiological mechanisms of endometriosis. In both rats and baboons, anti-TNF-α therapy has been demonstrated to prevent the development of induced endometriosis (D’Antonio et al., 2000; D’Hooghe et al., 2006). Furthermore, a significant reduction of spontaneous endometriosis has been observed in baboons treated with anti–TNF-α agents (Barrier et al., 2003).

Current medical therapy (i.e. GnRH analogues and gestagens) for endometriosis induces a hypoestrogenic condition associated with several moderate to severe side effects, including the loss of the normal menstrual cycle. Since subfertility is a common symptom associated with endometriosis, new drugs would ideally maintain the reproductive potential during treatment.

The baboon is an established model for endometriosis research (D’Hooghe, 1997). One of the major advantages of the baboon model is the possibility of inducing endometriosis by injecting menstrual endometrial tissue, obtained through transcervical curettage, into the pelvic area under laparoscopic vision (D’Hooghe et al., 1995b). The induced lesions mimic the peritoneal lesions seen in women with endometriosis in terms of localization and shape (D’Hooghe et al., 1995b).

In this study, we hypothesized that treatment with a monoclonal antibody (mAb), to TNFα, can reduce the extent of induced peritoneal endometriosis in baboons without interfering with the normal, spontaneous menstrual cycle.

Materials and methods

Study design and population

This study was a prospective, randomized, blinded, placebo-controlled study. Eighteen adult female baboons (Papio anubis) with proven fertility in the wild were selected to participate. Their exact age was not known, but the animals had been in captivity at Institute of Primate Research (IPR, Kenya) between 10 and 42 months (median 10 months). The animals were all trapped in the wild and kept in quarantine for three months. During quarantine, the animals were screened for tuberculosis (TBC), simian T-lymphotrophic virus 1 (STLV-1) and Simian Immunodeficiency Virus (SIV). All animals had a normal menstrual cycle (mean ± SD of 39 ± 7 days) prior to the study. The Institutional Scientific and Ethical Committee (ISERC, Nairobi, Kenya) of IPR reviewed and approved the study protocol.

During the study, animals were kept in single squeeze-back cages for easy handling, monitoring of health and possible side effects of the study agent. The stage of the menstrual cycle was monitored daily by animal technicians through visual observation of inflation/deflation of the perineal skin. No hormonal assays were performed during this study.

Study agent

As infliximab does not bind to monkey TNF-α, a mouse–human version of a murine anti-TNF mAb (c5N, IgG2a subtype), was employed in this study (Song et al., 2002). c5N was chimerized in order to reduce immunogenicity and the antibody binds and neutralizes TNF of both monkeys and humans. c5N was produced and purified by Centocor (Malvern, PA, USA). Drug pharmacokinetics was not evaluated during this trial. The dosage used in the study was equivalent to the dose of infliximab (Centocor, Malvern, PA, USA) administered to humans for Crohn’s disease (5 mg/kg).

After surgery, the animals were randomized to receive either placebo (saline 5 ml/kg, n = 7) or c5N (5 mg/kg, n = 11). The administered volume/kg bodyweight was equal in both groups. The investigator was blinded to treatment assignment. Both c5N and placebo were given as a single intravenous infusion over 2 h. During infusion of test agent, a veterinarian documented respiration frequency, pulse and temperature every 15 min.

Laparoscopies

Anaesthesia and laparoscopies were performed as described previously (D’Hooghe et al., 1991). Peritoneal fluid (PF) was aspirated before the baboon was placed in the Trendelenburg position and stored for future analysis. A single investigator (HF) performed all laparoscopies.

The induction laparoscopy was performed on the first or second day of menstruation. During this laparoscopy, and prior to the induction process, all animals were screened for the presence of pelvic abnormalities. Transcervical curettage with a Novak curette was performed as previously described (D’Hooghe et al., 1995b). The endometrial tissue was weighed and minced through an 18-gauge needle. The average weight of the endometrial tissue was 1.65 ± 0.70 g (1.80 ± 0.55 and 1.55 ± 0.79 g for the placebo and c5N group, respectively). The tissue was then seeded under laparoscopic vision into the pelvic area. Induction sites were standardized to the uterosacral fold, urinary bladder, pelvic walls, anterior side of broad ligament, uterus and the pouch of Douglas. The ovaries were excluded due to the risk of extensive formation of tubal/ovarian adhesions, which could impair the inspection at follow-up laparoscopies.

The second videolaparoscopy was performed to stage the extent of induced endometriosis on day 25 after induction and was labelled the ‘pretreatment laparoscopy’. A detailed pelvic map was constructed, with systematic photographic and video documentation of the pelvis during each laparoscopy. Adhesions involving ovary, Fallopian tube and cul-de-sac were graded according to the revised classification system of the American Society for Reproductive Medicine (American Society for Reproductive Medicine, 1997). Other adhesions that were not related to the ovary, Fallopian tube and cul-de-sac and that were observed between individual peritoneal endometriotic lesions and pelvic organs were recorded separately. The surface area of an endometriotic lesion (and an endometriotic lesion-related adhesion) was determined by multiplying length (mm) × width (mm) in cases of a circular lesion, by using the formula Π r². The volume of a lesion was estimated by multiplying surface area (mm²) × depth (mm).

Lesion area and volume were calculated from measurements made with a lateral trocar with a 1 mm hole at the tip. Only a diagnostic laparoscopy was performed, no endometriosis biopsies were taken.

The third videolaparoscopy was performed on day 25 after c5N/ placebo administration and is defined as the posttreatment laparoscopy. Lesions were documented according to size, type and localization on video, photo prints and individual pelvic maps. At least one and at most two representative biopsies of an endometriotic lesion (preferentially red or blue-black) were taken by scissors and electrocoagulation from each baboon for pathologic confirmation of the disease. Only easily accessible lesions were excised, frozen in liquid nitrogen and stored at −80°C.

Documentation and classification of lesions and adhesions

Endometriotic lesions were characterized according to type (typical blue-black, red and white lesions), surface area and localization. The types were subgrouped into 10 different groups. Typical blue-black were labelled either as typical, puckered blue-black cysts or white plaque with pigmented spots. Red lesions were grouped as red vesicular, red haemorrhagic, red polypoid or red-white plaques. White lesions were divided into white plaques, white nodules or white clear
vesicles. The extent of endometriosis was classified according to rAFS scoring system (American Fertility Society, 1985) after modification for baboon size.

Adhesions caused by lesions were characterized either as dense or filmy. Localization of attachment and type of lesion causing the adhesion was documented.

**Histology**

Nineteen biopsy samples (two white lesions, seven red lesions and 10 blue-black lesions) were collected during posttreatment laparoscopic surgery and snap-frozen in liquid nitrogen. Prior to cryosectioning, samples were cut in half with a pre-cooled surgical blade. One half was kept frozen for future analysis, and the other half was thawed and embedded in TissueTek, OCT (Sakura Finetek, Zouterwoude, The Netherlands) to facilitate the cryosectioning. Cryosections were made with a Cryostat HM 560 (Microm International, Merelbeke, Belgium) at a constant temperature of -30°C. In the first series, 100 sections of 7 μm were prepared from each biopsy. Four consecutive sections were placed on each slide. Every second slide was fixed immediately in formalin, and a haematoxylin–eosin staining was performed. The unstained slides were prepared from each biopsy. Four consecutive sections were placed on each slide. Every second slide was fixed immediately in formalin, and a haematoxylin–eosin staining was performed. The unstained slides were kept frozen at -20°C. Using this approach, biopsies were stained at intervals of 28 μm. This interval was chosen to identify all possible detectable lesions since the smallest endometriotic lesion expected to be detectable at laparoscopy is estimated to be approximately 50 μm (Redwine, 2003). The stained sections were checked individually for the presence of endometriotic glands and stroma by three independent investigators. An additional 100 sections were prepared from the samples that were not found positive in the first series.

**Data analysis and statistics**

Initially, comparisons were made between the c5N and placebo groups following the pretreatment and posttreatment laparoscopies to determine any inter-group variances for total surface area, volume, total number of lesions, number and surface area of red lesions, adhesions and rAFS score.

Subsequently, to determine possible differences between the c5N and placebo groups, the surface area and volume and number of endometriotic lesions were compared between the pretreatment and posttreatment laparoscopies for both groups. Subanalyses were performed for changes in adhesions, red lesions and changes in rAFS score. Furthermore, the formation of new adhesions, new lesions and remodelling of endometriotic lesions (D’Hooghe et al., 1992) was also compared between the pretreatment and posttreatment laparoscopies in both groups. Remodelling was defined as lesions changing from white to either subtle red or typical lesions and vice versa.

Statistical analysis was performed with the Wilcoxon test for paired samples, the Mann–Whitney U-test and the paired Student’s t-test where appropriate, with P values less than 0.05 being considered statistically significant.

**Results**

Results of analyses for possible inter-group variances are shown in Table I. Total surface area, total number of lesions, number of red lesions, surface area of red lesions, rAFS score and number of adhesions were equally distributed between the two treatment groups prior to infusion. However, total lesion volume was significantly higher in the c5N group than in the placebo group after the induction of endometriosis, but prior to treatment with c5N.

Both surface area and volume of endometriotic lesions were significantly reduced after c5N treatment when compared to the pretreatment laparoscopy, whereas no significant changes were observed in the placebo group (Figure 1). The total number of lesions was significantly increased after both c5N and placebo administration when compared to the pretreatment laparoscopy (Figure 2). On average, two new lesions were formed in the baboons between the pretreatment and posttreatment laparoscopies.

A significant increase was observed in the total number of subtle red and typical lesions in the placebo group. In the c5N group, the number and surface area of subtle red lesions, and surface area of all subtle red and typical lesions, was significantly decreased when compared to the pretreatment laparoscopy.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Posttreatment</th>
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<tbody>
<tr>
<td>c5N</td>
<td>Placebo</td>
</tr>
<tr>
<td>c5N</td>
<td>Placebo</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
</tr>
<tr>
<td>Total surface area (mm²)</td>
<td>105.0 ± 18.9</td>
</tr>
<tr>
<td>Total volume (mm³)</td>
<td>111.5 ± 19.6</td>
</tr>
<tr>
<td>Number of total lesions</td>
<td>16.6 ± 1.4</td>
</tr>
<tr>
<td>Number of red lesions</td>
<td>7.9 ± 1.4</td>
</tr>
<tr>
<td>Area of red lesions (mm²)</td>
<td>67.8 ± 17.9</td>
</tr>
<tr>
<td>rAFS score</td>
<td>11.6 ± 1.4</td>
</tr>
<tr>
<td>Number of adhesions</td>
<td>2.2 ± 0.7</td>
</tr>
</tbody>
</table>

All data presented are mean ± SD.

*P < 0.05 c5N versus placebo.

**Figure 1.** Changes in total surface area and lesion volume. NS, not statistically significant.
Anti-TNF-mAb (c5N) reduces induced endometriosis in the baboon

Figure 2. Changes in total number of lesions.

Figure 3. Changes in number and surface area of red lesions. NS, not statistically significant.

(Figure 3, Table II). Typical lesions were a rare finding at pretreatment laparoscopy in both the c5N group (2 animals with 1 lesion each) and in the placebo group (no lesions; Table II). Following c5N administration, both number and surface area of typical lesions were increased when compared to the pretreatment laparoscopy (Table II). The number of adhesions and the rAFS score were comparable between the pretreatment and posttreatment laparoscopies in both groups (Table III).

Significantly, fewer subtle white lesions present at the pretreatment laparoscopy remodelled into subtle red or typical lesions at the posttreatment laparoscopy in the c5N group when compared to the placebo group. More lesions that were detected at pretreatment laparoscopy had disappeared at the posttreatment laparoscopy in the c5N group when compared to the placebo group (Table IV).

The total histological confirmation rate was 68% and comparable between the treatment groups. Two biopsies contained endometrial stroma only (n = 1, c5N group) or the endometrial glands only (n = 1, placebo). There was a non-significant trend towards a higher confirmation rate in blue-black lesions when compared to white and red lesions combined (Table V).

The menstrual cycle was monitored four months after the last laparoscopy. No changes in mean ± SD cycle length were observed after the study (39.8 ± 8.7 and 35.8 ± 6.6 days in the placebo and c5N groups, respectively) when compared with data before the onset of the study (39.0 ± 5.4 and 35.4 ± 5.3 days).

Unexpected events and observations during c5N/placebo infusions

Two animals in the c5N group died during the study. The first baboon died unexpectedly during the posttreatment laparoscopy, with postmortem/histopathology findings revealing signs of asphyxia with congestion of the liver, myocarditis and chronic pneumonia. The second baboon died 46 days after the final laparoscopy, possibly related to a self-inflicted finger trauma with subsequent sepsis. The postmortem/histopathology findings showed signs of endocarditis and acute pneumonia. Furthermore, it was noted that both animals had non-specific brain pathology with a marked astrocytosis.

The observations of pulse, temperature and respiration during the infusions of c5N/placebo demonstrated a significant bradycardia in the c5N group compared to the placebo group (mean ± SD pulse of 90 ± 22 and 73 ± 17 bpm, respectively, P < 0.05). The other vital sign parameters assessed were comparable between the groups.

Discussion

This study presents experimental evidence that treatment with anti-TNF-mAb (c5N) can reduce the surface area and volume of induced peritoneal endometriosis in baboons. The main effect of c5N treatment appears to be a significant decrease in number, surface area and volume of preferentially red lesions. In placebo-treated animals, the total number of typical and red lesions increased, thus reflecting the natural course of induced endometriosis in baboons. Furthermore, a significantly larger amount of white lesions remodelled into typical or red lesions in the placebo group as compared to the c5N group. Although new lesions developed in both groups, ‘older lesions’, i.e. lesions present before c5N/placebo administration, disappeared to a greater extent in the c5N group than in the placebo group. The apparent disruption of the natural progression of endometriosis could support the anti-proliferative effects of TNF-α inhibitors observed in recent in vitro experiments (Braun et al., 2002).
Subanalyses of changes in typical and subtle red lesions prior to (pre) and following (post) c5N/placebo administration

Histological confirmation rates for each type of lesion in both groups

Changes in number of adhesions and rAFS score prior to (pre) and following (post) c5N/placebo administration

Table II. Subanalyses of changes in typical and subtle red lesions prior to (pre) and following (post) c5N/placebo administration

<table>
<thead>
<tr>
<th></th>
<th>c5N</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Area</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Typical lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red lesions</td>
<td>0 (0–1)</td>
<td>1 (0–9)</td>
</tr>
<tr>
<td>Red lesions</td>
<td>8 (2–19)</td>
<td>5 (0–10)</td>
</tr>
<tr>
<td>Typical and red lesions</td>
<td>8 (2–19)</td>
<td>9 (0–14)</td>
</tr>
</tbody>
</table>

Table III. Changes in number of adhesions and rAFS score prior to (pre) and following (post) c5N/placebo administration

<table>
<thead>
<tr>
<th></th>
<th>c5N</th>
<th>Placebo</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Area</td>
</tr>
<tr>
<td>Number of adhesions</td>
<td>2 (0–6)</td>
<td>3 (0–8)</td>
</tr>
<tr>
<td>rAFS score</td>
<td>10 (4–23)</td>
<td>10 (4–18)</td>
</tr>
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</table>

Table IV. Number of lesions remodelling from one phenotype into another between the two follow-up laparoscopies

<table>
<thead>
<tr>
<th></th>
<th>c5N</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remodelling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White to red</td>
<td>1 (0–2)</td>
<td>4 (0–7)</td>
</tr>
<tr>
<td>Red to white</td>
<td>2 (0–11)</td>
<td>2 (1–2)</td>
</tr>
<tr>
<td>Lesion disappeared</td>
<td>0 (0–5)</td>
<td>0 (0–5)</td>
</tr>
</tbody>
</table>

Table V. Histological confirmation rates for each type of lesion in both groups

<table>
<thead>
<tr>
<th></th>
<th>Typical blue-black lesions</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White</td>
<td>Red</td>
</tr>
<tr>
<td>c5N</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Placebo</td>
<td>0%</td>
<td>67%</td>
</tr>
</tbody>
</table>

The diagnosis of endometriosis in humans has traditionally been made by the presence of typically puckered blue-black lesions during laparoscopy. However, endometriosis has been diagnosed in up to 90% of cases with subtle lesions (red or white), and red lesions are considered to be the most active type (Nisolle and Donnez, 1997), with the highest likelihood of histological confirmation. Neoangiogenesis is believed to be one of the most important pathogenetic factors for endometriosis, and studies of the stromal vascularization have shown that red lesions have a significantly higher capillary mean surface area when compared to typical and white lesions (Nisolle et al., 1993). Red lesions may later turn in to black lesions, which are considered typical for advanced endometriosis (Donnez et al., 1993; Nisolle et al., 1993; Donnez et al., 1998). White lesions are assumed to be healed or latent lesions but may also remodel into more active lesions (D’Hooghe et al., 1992). This remodelling process was clearly observed at the two subsequent laparoscopies in both groups of animals. In support of the favourable effect of c5N treatment, the remodelling from white lesions to red subtle lesions or typical lesions was less pronounced in the c5N group than in the placebo group.

Endometriosis was confirmed in 68% of the 19 analysed biopsies. There was a non-significant trend towards a higher confirmation rate in the placebo animals (75%) compared to the c5N animals (64%), indicating a higher ‘cure-rate’ in the c5N group. Similar results were observed previously with the use of TNFα-blocking protein (r-HTBP-1) in induced endometriosis in baboons; these findings could provide further support of the healing effect of TNFα blockade on endometriotic lesions (D’Hooghe et al., 2006). The overall confirmation rate in our study was slightly lower than previously reported (86%, Barrier et al., 2003). This could in part be explained by a selection bias during laparoscopy. Lesions located in easily accessible areas were preferentially biopsied, regardless of their appearance, while lesions that were situated close to large vessels or vital organs were avoided. Indeed, no white lesions were biopsied in the previously mentioned study, whereas two white lesions were biopsied and analysed by histology in the present study. It should also be noted that Barrier et al. analysed only eight biopsies in their study.

Changes in adhesions and rAFS scores were not statistically significant in this study. However, such changes would not typically be expected at only 25 days after study agent administration. It may be possible that a prolonged period of treatment is necessary to achieve significant differences. The rAFS scores were generally lower than previously reported (D’Hooghe et al., 1995a, 2006), probably because the ovarian/tubal adnexa were not seeded with menstrual endometrium at induction. In earlier studies (D’Hooghe et al., 1995b, 2006), menstrual endometrium was also seeded on ovarian and tubal...
surfaces during intrapelvic injection. This led to a higher rAFS score 25 days after induction when compared to the present study and is likely related to a higher degree of tubal and ovarian adhesions that considerably increase the score. In the present study, this ovarian/tubal seeding of menstrual endometrium was not performed to prevent the development of dense adnexal adhesions that may impair the accuracy and reproducibility of serial inspections of peritoneal endometriosis during follow-up laparoscopies. As in previous published data, superficial or deep cystic ovarian endometriosis was absent in this study (D’Hooghe et al., 1995a, 2006). It is therefore not possible to draw any conclusions regarding effects of c5N on ovarian endometriosis.

Currently used medical therapies in endometriosis have several major side effects. Some of the most important are the negative effects on the menstrual cycle, ovulation and fertility. The menstrual cycles of the baboons were carefully monitored during and after the study. No significant alterations in cycle length, when compared to the average prestudy cycles, were observed in either treatment group. This suggests that the reproductive potential (i.e. ovulation) is preserved during treatment with c5N.

The results from this study are consistent with previously published data by Barrier et al. on spontaneous endometriosis in baboons (Barrier et al., 2003). One could argue that induced endometriosis is experimental in contrast to spontaneous disease and thus limits the ability to extrapolate the results to humans. It is, however, important to keep in mind that screening for spontaneous endometriosis in baboons requires more animals to find the appropriate number for the experiment. With the induction of endometriosis, fewer animals are excluded during this process (no animals were excluded in this study, whereas 40% were excluded in the study by Barrier et al.). Furthermore, induction of endometriosis offers a more controlled and standardized method of achieving an appropriate experimental setting. Standardization is vital to the conduct of placebo-controlled trials, and the induction of endometriosis in our study resulted in two highly comparable study groups (Table I).

Two animals in the c5N group died during the study, which is uncommon during surgical experiments at IPR. The post-mortem and histopathology findings revealed signs of asphyxia in the first case and of endocarditis/acute pneumonia in the second case. The asphyxia was probably related to a presurgical lung condition (chronic pneumonia) in combination with general anaesthesia during surgery. The generalized infection observed in the second case may have been related to a finger wound. While minor cvciotomy-related traumas such as finger wounds are unfortunately rather common, they rarely lead to permanent injuries or infections if treated properly. Reports on adverse events and toxicity related to the use of TNF-α inhibitors include severe infections, induction of malignancies, systemic lupus erythematosus (SLE) and death (Colombel et al., 2004). The histopathology findings on the deceased animals raise the question whether c5N caused an increased susceptibility to minor infections with a higher risk of developing severe infections. Activation of latent tuberculosis (Keane et al., 2001) and the occurrence of other opportunistic infections (True et al., 2002; Slifman et al., 2003) have been reported during treatment of rheumatoid arthritis with TNF-α blocking agents. Since c5N has not been tested before, the possibility of a relation between c5N treatment and adverse events related to an increased risk of systemic immunosuppression will need to be explored further in dose–response studies. However, in the study by Barrier et al. no side effects were observed when a commercially available TNF-α inhibitor was employed.

Taken together, these data show that the anti-TNF-mAb c5N reduces the volume and area of active endometriotic lesions and inhibits the natural development of induced peritoneal endometriosis in baboons. The specific inhibitory effect on subtle red lesions suggests that not only the quantity but also the degree of activity of endometriotic lesions is reduced in the treated group. In conclusion, the anti-TNF-mAb c5N reduces the extent of endometriosis in baboons with established disease without affecting the menstrual cycle. Anti-inflammatory compounds such as c5N could be a breakthrough in the medical therapy of endometriosis. Studies on endometriosis-associated infertility are warranted and the baboon model is well suited for fertility trials (D’Hooghe, 1997).

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References


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