Characterization of iron deposition in endometriotic lesions induced in the nude mouse model

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BACKGROUND: Pelvic endometriotic lesions are often laden with hemosiderin. In order to investigate the potential source of such iron deposits, we examined whether the seric and erythrocyte fractions of menstrual effluent might influence the occurrence of iron deposition within lesions in a murine model of endometriosis.

METHODS: Endometriosis was induced in 57 nude mice by intraperitoneal injection of unfractionated human menstrual effluent, endometrial fragments plus serum, endometrial fragments plus erythrocytes or endometrial cell fraction alone. The number of implants, histologic aspect, proliferative activity and iron deposition in lesions was assessed.

RESULTS: On day 5, lesions were evidenced in all 10 mice receiving menstrual effluent, in 9/13 of those injected with the cellular fraction, in 10/13 with the cellular fraction plus serum and in 10/12 with the cellular fraction plus erythrocytes. Iron conglomerates were observed at the interface between the lesion and peritoneum when menstrual effluent (47 deposits/mm²) and the cellular fraction with erythrocytes (20 deposits/mm²) were injected, but were scarce when the cellular fraction was injected without erythrocytes, either alone (4 deposits/mm²) or with serum (2 deposits/mm²) (P < 0.05).

CONCLUSIONS: Iron conglomerates, typically found in the stroma of endometriotic lesions, were induced by erythrocytes present in menstrual effluent. This may be one of the factors triggering oxidative damage and chronic inflammation.

Key words: endometriosis/erythrocytes/iron/menstrual effluent/nude mice

Introduction
Endometriosis is a common gynaecologic disease characterized by the presence and growth of endometrial tissue outside the uterine cavity. This pathology affects about 10–15% of women in their reproductive years. However, despite a growing number of reports on endometriosis, the pathophysiology of this disease remains poorly understood.

In order to obtain further information on the initial events involved in the histogenesis of endometriosis, animal models have been established in the monkey, rabbit, rat and mouse (Donnez et al., 1987; Awwad et al., 1999; D’Hooghe and Debrock, 2002; Sharpe-Timms, 2002) and in vitro approaches have also been developed (Dunselman et al., 2001).

Using immunodeficient mouse strains has an advantage over other animal models in allowing the transplantation of human tissue instead of autologous endometrium. Previous studies have shown that human secretory or proliferative endometrium can be successfully implanted onto the peritoneum of severe combined immuno-deficient (SCID) mice and athymic nude mice (Zamah et al., 1984; Awwad et al., 1999; Nisolle et al., 2000; Béliard et al., 2002; Hull et al., 2003).

We recently developed a model of experimental endometriosis using nude mice as recipients of human menstrual endometrium, instead of endometrium collected during the secretory or proliferative phases of the cycle (Nisolle et al., 2000). Adhesion of endometrial stromal cells already occurred 1 day after injection and, 5 days after injection, the implants were reorganized into endometriosis-like lesions with glands and stroma.

Pelvic endometriosis appears to be associated with an increase in iron metabolism within the peritoneal cavity (Van Langendonckt et al., 2002a). Arumugam and Yip (1995) have shown that the concentration of iron in peritoneal fluid is correlated to the severity of the disease. The presence of siderophages, known as iron-storing macrophages and the occurrence of iron pigments are considered to be typical features of endometriotic lesions (Gaulier et al., 1983; Moen and Halvorsen 1992; Stowel et al., 1997; Van Langendonckt et al., 2002b). We have previously shown that hemosiderin conglomerates can be found in healthy peritoneal biopsies in 20% of patients with or without endometriosis (Van Langendonckt et al., 2002b). Their presence is likely to be related to the presence of erythrocytes within the peritoneal cavity, observed during the peri-menstrual period in 90% of women with patent tubes (Halme et al., 1984). However, the occurrence of iron deposits was found to be much higher in peritoneum close to red endometriotic lesions, leading to the formation of densely stained hemosiderin deposits in 50% of
cases (Van Langendonckt et al., 2002b). It was not clear whether these deposits arose from menstrual flow that appears to be more abundant in women with endometriosis (Vinatier et al., 2000; D’Hooghe and Debrock, 2002), or resulted from bleeding of ectopic endometriotic foci.

The most widely accepted theory on the pathogenesis of pelvic endometriosis is the implantation theory of Sampson (1927), postulating that reflux of menstrual effluent into the peritoneal cavity, via the Fallopian tubes, may carry viable endometrial cells which could subsequently implant and grow on peritoneal surfaces. Our hypothesis is that iron, together with apoptotic endometrial fragments and activated macrophages, may induce oxidative stress within the pelvic cavity of patients with endometriosis, as recently reviewed (Murphy et al., 1998; Van Langendonckt et al., 2000a). As a potential inducer of genes encoding cell adhesion molecules, immunoregulators and inflammatory cytokines, iron-induced oxidative stress may help to trigger the chain of events that leads to the development of endometriotic lesions.

The present study was designed to examine whether the presence of erythrocytes in menstrual effluent and within menstrual endometrial tissue might influence the occurrence and histologic aspect of lesions induced in nude mice and affect iron deposition. For this purpose, menstrual effluent was collected from women during their menstrual periods and fractionated into cellular, seric and erythrocyte fractions. Endometriosis was induced in nude mice by injecting endometrial fragments either alone or in combination with unfractionated menstrual effluent, serum or erythrocytes into the pelvic cavity of the animal. The presence of implants and their morphologic and histologic features were examined 5 days later and the occurrence of iron deposition, the final degradation product of erythrocytes, was assessed by Prussian blue staining.

Materials and Methods

Collection and fractionation of human menstrual effluent

The use of human tissue for this study was approved by the Institutional Review Board of the Universite Catholique de Louvain. Menstrual effluent was obtained from 12 reproductive-aged women (range 27–44, mean age 35) without laparoscopically proven endometriosis, undergoing surgery for benign conditions. Samples were collected during menstrual bleeding. The effluent was recovered by gentle aspiration via a syringe without any anticoagulants and transported immediately to the laboratory for further processing.

Endometrial fragments from menstrual effluent were cut into pieces of 1 mm³. A 0.1 ml sample of effluent and four pieces of 1 mm³ endometrium were used for unfractionated menstrual effluent injection. The remaining effluent was divided into three fractions as follows: seric fraction, erythrocytes, and cellular fraction. The effluent containing endometrial fragments was centrifuged for 10 min at 600 g and the seric supernatant fraction was recovered. The pellet was washed in HEPES-buffered phenol red-free DMEM/F12 medium (Life Technologies, Merelbeke, Belgium) supplemented with 2 mM glutamine, 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin and resuspended in the same medium.

Four millilitres of the cell suspension was layered on top of 3 ml of a Ficoll–Paque gradient (Pharmacia Biotech, Roosendaal, the Netherlands) and centrifuged for 10 min at 400 g, according to the manufacturer’s instructions. The pellet containing only erythrocytes, as checked by microscopy, was washed in serum-free DMEM/F12 medium. The washed erythrocyte pellet was resuspended in a final volume of medium corresponding to the initial effluent volume. The cellular fraction recovered from the interface between the Ficoll and aqueous phases of the gradient was further separated from the remaining erythrocytes by a second Ficoll–Paque gradient and washed in serum-free DMEM/F12 medium. Four pieces of endometrial tissue from the final pellet were resuspended in 0.1 ml of the seric fraction, four pieces in 0.1 ml of the erythrocyte fraction and four pieces in serum-free DMEM/F12 medium. One piece of each endometrial fragment specimen was fixed for histologic and immunohistochemical analyses. The viability of the cells prior to injection ranged between 89–100%, as checked by trypan blue (0.4% solution, Sigma-Aldrich, Bornem, Belgium) exclusion (Phillips and Terryberry, 1957), following tissue dissociation by a 1 h incubation of endometrial fragments in DMEM/F12 medium supplemented with 40 U/ml collagenase A (Sigma-Aldrich).

Injection into nude mice

The guidelines for animal care were approved by the Committee on Animal Research of the Universite Catholique de Louvain.

Fifty-seven 8-week-old nude female mice (Swiss nu/nu) with a deficient T-lymphocyte system were used in this study. This murine model has been described in detail elsewhere (Nisolle et al., 2000).

Menstrual effluent collected from one donor patient was fractionated, as described above. Immediately after fractionation, one mouse was injected with unfractionated menstrual effluent, one with the cell fraction from the same effluent resuspended in menstrual serum, one with this cell fraction combined with erythrocytes and one with this cell fraction alone, originating from the same effluent. The experiment was repeated 13 times using one different menstrual effluent for each series since the amount of menstrual tissue available from each donor patient was only sufficient for four to five mice (except in one case where the effluent from one donor was used for two series of experiments). In the first five series of experiments, one mouse injected with menstrual serum was also included.

The mice were anesthetized with an intraperitoneal injection of 0.07 ml Imalgene 500 (Merrial, Brussels, Belgium) and 0.16 ml Rompun 2% (Bayer, Brussels, Belgium), 1/100 vol/vol. A small incision of less than 3 mm was made and 0.1 ml of the various human menstrual effluent fractions was injected into the peritoneal cavity (day 0). The cuts were sutured with a 4/0 nylon thread. Four mice died following anesthesia. The remaining animals were killed 5 days after injection. Their abdominal cavities were examined under a dissecting microscope and lesions showing the macroscopic characteristics of endometriosis were removed.

Seventy-five lesion samples were necropsied and put into 4% buffered formaldehyde fixative, and embedded in paraffin. Six-micrometre-thick serial sections were stained either with Gomori’s trichrome for histologic evaluation, or with specific antibodies for Ki 67 immunohistochemical analysis and with Prussian blue for iron staining. Characterization of ectopic lesions was performed by standard histologic methods (Nisolle et al., 1990).

Proliferative activity, presence of iron deposits, and neovascularization were evaluated in endometrial samples fixed on day 0 and lesions removed on day 5.
Staining of iron deposits

Ferric iron deposits were evidenced using Prussian blue staining according to Perls’ reaction (Perls, 1867). Briefly, dewaxed 6 μm-thick sections were stained for 20 min in a solution of kalium hexacyanoferrate 1% and HCl 2%, vol/vol, and nuclei were counterstained with nuclear fast red (Gabe, 1968).

The presence or absence of iron deposits was recorded in the stroma and interface area between the endometrial implant and murine tissue. The number of deposits per mm² was evaluated by a 2-D image analysis program set on Vidas 21 (Kontron Bildanalyse GmbH, Eching, Germany). Results were expressed as the mean number of deposits per mm². Deposits were classified either as extra- and/or intracellular Prussian blue stained granules and pale blue cytoplasmic staining, or as densely stained conglomerates stored in siderosomes (Iancu, 1992), as illustrated in Fig 1.

Measurement of proliferative activity

Ki 67 immunostaining was carried out as previously described (Nisolle et al., 1997). Briefly, after inhibition of endogenous tissue peroxidase, unmasking of the antigen and inhibition of non-specific reactions in a blocking solution containing 10% normal goat serum and 1% bovine serum albumin, sections were kept overnight in a 1/100 dilution of the monoclonal MIB-1 antibody (Immunotech, Marseille, France). Staining was performed with peroxidase-labelled secondary antibody and liquid diaminobenzidine as the chromogen (Dako, Glostrup, Denmark).

Proliferative activity was measured by counting Ki 67-positive and -negative nuclei (stroma average number: 3210, range 360–20000; glandular epithelium average number: 460, range 40–1740) and evaluating their distribution in the glandular epithelium and stroma of each lesion sample, as previously described (Nisolle et al., 1997).

Statistical analysis

The chi square test was used to compare the number of mice with lesions and proliferation indices. Proliferation indices were expressed as median value and interquartile (25% and 75%) ranges and were normalized by logarithmic transformation before analysis in order to correct for the skewed distribution.

Data on iron deposits were normally distributed and expressed as mean number of deposits and standard deviations. The differences between treatment groups were assessed using two-way ANOVA (fraction injected, experiment replication). Differences between treatments and control (menstrual effluent) were tested by Dunnett’s Post-Hoc Test. The analyses were performed using SPSS statistical software (SPSS Inc, Chicago, IL, USA). Differences were considered significant when \( P < 0.05 \).

Results

Macroscopic analysis

On day 5, macroscopic lesions were observed on the peritoneal wall in the 10 control mice (100%) that were given unfractionated menstrual effluent. No endometrial explants were found inside the peritoneal cavity of the five mice treated with menstrual serum but explants were detected macroscopically in 11 of the 12 mice injected with the cellular fraction with erythrocytes, and in 10/13 of those injected either with the cellular fraction alone or the cellular fraction plus seric fraction \( (P < 0.05) \). Implantation occurred on incision sites as well as intact mesothelium.

Histologic analysis

The presence of endometriosis-like features, i.e. stroma and endometrial glands at the fatty tissue–muscular layer interface, was confirmed by histologic analysis of the excised explants (Nisolle et al., 1990). On day 5, the lesions all looked similar, regardless of the type of fraction inducing their development. All the implants showed a well restructured columnar and/or
Table I. Morphological, histologic and immunohistochemical results according to the fraction injected.

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>Cell fraction Day 0</th>
<th>Menstrual effluent</th>
<th>Seric fraction Day 5</th>
<th>Erythrocytes + cell fraction Day 5</th>
<th>Cell fraction alone Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.7</td>
<td>0.5</td>
<td>1.3</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>13 (100%)</td>
<td>13</td>
<td>10 (83)</td>
<td>9 (69)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10 (77)</td>
<td>10 (83)</td>
<td>9 (69)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 deposits/mm²</td>
<td>12 deposits/mm²</td>
<td>9 deposits/mm²</td>
<td>2 deposits/mm²</td>
<td>4 deposits/mm²</td>
<td>0 deposits/mm²</td>
</tr>
<tr>
<td>0.5 deposits/mm²</td>
<td>1.3 deposits/mm²</td>
<td>1.3 deposits/mm²</td>
<td>0.5 deposits/mm²</td>
<td>0.0 deposits/mm²</td>
<td>0.0 deposits/mm²</td>
</tr>
<tr>
<td>Proliferation index* in glandular epithelium</td>
<td>0.1 (0.6, 4.1)</td>
<td>16.4 (7.3, 22.0)</td>
<td>37.4 (20.9, 40.2)</td>
<td>29.4 (16.0, 37.4)</td>
<td>19.1 (11.5, 45.4)</td>
</tr>
<tr>
<td>Proliferation index* in the stroma</td>
<td>1.0 (0.5, 7.2)</td>
<td>0.04 (0.02, 0.15)</td>
<td>0.2 (0.1, 0.4)</td>
<td>0.2 (0.6)</td>
<td>0.4 (0.2, 0.6)</td>
</tr>
</tbody>
</table>

*Proliferation indexes are expressed as median values and (25th, 75th percentiles).
**Significantly different (P < 0.05) from the rates observed when menstrual effluent was injected (χ² test).
**Significantly different (P < 0.05) from values observed in menstrual effluent samples before injection into the peritoneal cavity of mice (χ² test).
Significantly different (P < 0.05) when compared to menstrual effluent injection

Cuboidal glandular epithelium with a cytogenic stroma. A nascent capillary network was present at the interface between the implant and the murine tissue. Endometriosis-like lesions were found in all 10 mice receiving unfractonated menstrual effluent, 10/12 of the mice injected with the cellular and erythrocyte fractions, 10/11 of those injected with the cell fraction alone and 10/13 of those receiving the cell fraction supplemented with effluent serum (Table I).

The number of lesions observed per mouse ranged between one and three in mice exhibiting lesions, the mean number of lesions being similar among the different groups: 1.4 in the group receiving unfractonated menstrual effluent and 1.3 in the other three groups.

**Proliﬁerative activity**

The proliferative activity was assessed immunohistochemically in endometriosis-like lesions using an antibody specific for human Ki 67 (Table I).

The proliferatin index was low in menstrual endometrium before injection (day 0) in both stromal cells (median = 1.0; lower-upper quartile = 0.5–7.2) and glandular epithelial cells (median = 0.1; lower-upper quartile = 0–4.1). On day 5, a signiﬁcant increase was observed in glandular proliferative activity in endometrial explants, compared to values observed on day 0, the indices ranging between 16.4 and 37.4, depending on the fraction injected; while proliferation indices in stromal cells from explants were in the same range as noted before injection, varying between 0.04 and 0.4.

No statistical difference in proliferative activity was noted between the series of experiments.

When the results were examined according to the fraction injected, the proportion of Ki 67-positive nuclei in glandular epithelium on day 5 did not differ signiﬁcantly after injection of unfractonated effluent (median = 16.4; lower-upper quartile = 7.3–22.0), the cell fraction alone (median = 19.1; lower-upper quartile = 11.5–45.4), the cell fraction resuspended in serum (median = 37.4; lower-upper quartile = 20.9–40.2) and the cell fraction combined with erythrocytes (median = 29.4; lower-upper quartile = 16.3–37.4). In stromal cells, proliferation indices on day 5 were signiﬁcantly higher after injection of the cell fraction (median = 0.4; lower-upper quartile = 0.2–0.6) than after injection of unfractonated menstrual effluent (median = 0.04; lower-upper quartile = 0.02–0.15). Higher stromal proliferative activity was also detected in implants induced by the cell fraction combined with serum (median = 0.2; lower-upper quartile = 0.1–0.4) and erythrocytes (median = 0.2; lower-upper quartile = 0–0.6), although the differences were not signiﬁcant.

**Iron deposits**

Before injection, most washed menstrual endometrial fragments were devoid of iron deposits, as evidenced by Prussian blue staining. A few iron-containing granules were found in 25% of tissue samples. However, it is noteworthy that, on day 0, most fragments contained erythrocytes in the stroma. After washing and gradient fractionation, erythrocytes remained entrapped within endometrial tissue and could not be removed without dissociating tissue.

Five days after injection, 86.4% of endometrial implants induced by injection of unfractonated effluent showed cytoplasmic, granular and conglomerate deposits in the stroma, whereas no deposits were found in glandular epithelium. Deposits were particularly numerous in the interface area between the implant and the host tissue (Fig. 1). Iron deposits were rarely observed at the level of the murine peritoneal wall.

No signiﬁcant difference in iron deposition in the stroma or at the interface was observed between the series of experiments.

The number of cytoplasmic and granular deposits in the stroma of lesions differed according to the fraction injected (P = 0.01, ANOVA); fewer deposits were found in the stroma of endometriosis-like lesions after injection of the cell fraction alone (10 ± 12 deposits/mm²) or the cell fraction supplemented with serum (3 ± 4 deposits/mm²) than in the group injected with unfractonated effluent (83 ± 86 deposits/mm²), as shown in Fig. 2. In the group receiving unfractonated menstrual effluent, an average of 26 ± 24 conglomerate deposits were found per mm² in the stroma of lesions, while stromal deposits were scarce in lesions developing after injection of the other fractions (2 ± 2 deposits/mm², 1 ± 1 deposit/mm² and 4 ± 9 deposits/mm² for the cell fraction alone and the cell fraction supplemented with serum and erythrocytes respectively) (P = 0.04, ANOVA).

At the interface between the stroma of the implant and the murine peritoneum, deposits were found after injection of the cell fraction (27 ± 24 deposits/mm²) and the cellular
fraction resuspended in serum (29 ± 34 deposits/mm²), although they were significantly less numerous than after injection of unfractionated menstrual effluent (177 ± 86 deposits/mm²) or the cellular fraction supplemented with erythrocytes (72 ± 59 deposits/mm²) (P = 0.002, ANOVA). The accumulation of deposits resulted in the formation of intensely stained conglomerates at the interface when menstrual effluent (47 ± 50 deposits/mm²) and the cellular fraction with erythrocytes (20 ± 25 deposits/mm²) were injected, whereas iron conglomerates were uncommon when the cellular fraction was injected without erythrocytes either alone (4 ± 5 deposits/mm²) or with serum (2 ± 5 deposits/mm²) (P = 0.02, ANOVA).

**Discussion**

The present study confirms that injection of human menstrual effluent into the pelvic cavity of nude mice, under conditions mimicking menstrual reflux, results in the development of endometriosis-like lesions in all cases (Nisolle et al., 2000). Lesions were also observed when menstrual endometrium was injected, either alone or in combination with menstrual serum or erythrocytes, although to a lesser extent than with unfractionated menstrual effluent. The histologic aspect of lesions induced in mice was similar regardless of the fraction injected, except for the presence of iron deposits, as discussed below. When only menstrual serum was introduced into the peritoneal cavity, no lesions were found, indicating that endometriosis-like foci result from the transplantation of endometrial cells and not from metaplasia induced by menstrual serum.

It is well known that menstrual endometrium possesses the remarkable property of being able to survive within the peritoneal environment and to reorganize itself after adhesion into well-structured lesions (Nisolle et al., 2000). Nevertheless, the normal fate of these desquamated endometrial cells, with a low proliferation index as confirmed in the present study, would be to undergo apoptosis (Vinatier et al., 2000). In our model, the adhesion, survival and growth of menstrual endometrium was not related to an intrinsic property of eutopic endometrium from patients with endometriosis, since the endometrium originated from patients unaffected by the disease.

The adhesion and implantation of endometrial fragments onto peritoneal mesothelium may be favoured by factors present in menstrual reflux. Indeed, menstrual effluent has been reported to contain factors that induce alterations in the morphology of peritoneal mesothelium (Koks et al., 2000). Retrograde menstruation carries erythrocytes, releasing highly pro-oxidant factors into the peritoneal cavity, such as haeme and iron. Several lines of evidence suggest that haeme and iron overload occurs in the peritoneal cavity of patients with endometriosis (Van Langendonckt et al., 2000a). Increased concentrations of erythrocytes have been reported in the peritoneal cavity of women with endometriosis during menstruation (D’Hooghe and Debrock, 2002) and higher levels of iron were found in the peritoneal fluid of patients with endometriosis (Arumugam and Yip, 1995; Van Langendonckt et al., 2002b). Moreover, iron pigment is more frequently encountered in endometriotic than in non-endometriotic pelvic lesions (Stowel et al., 1997; Van Langendonckt et al., 2002b). Levels of ferritin, the major iron-storing molecule, and
transferrin, ensuring iron transport, were found to be increased in the peritoneal fluid of women with endometriosis. Our hypothesis is that iron-induced oxidative stress may play a role in the regulation of the expression of genes encoding immunoregulators, cytokines, monocyte chemoattractants and adhesion molecules implicated in the pathogenesis of endometriosis (Van Langendonckt et al., 2000a). However, since erythrocytes are found in the peritoneal cavity of most women (Halme et al., 1984), it remains unclear why only some patients develop the disease. It could be that, in some women, peritoneal defence mechanisms are swamped by menstrual effluent because of the abundance of reflux, as recently suggested (Van Langendonckt et al., 2000a).

Our murine model has allowed us to gather further information on the origin and type of iron deposits within endometriotic lesions and peritoneal tissue. Erythrocytes appear to be quickly metabolized, as corroborated by the fact that already 1 day after injection, the reddish menstrual endometrial tissue turned white, as previously reported (Nisolle et al., 2000). In the present study, 5 days after injection, iron deposits were found in the stroma of endometrial implants. Deposits in lesions are likely to originate from erythrocyte lysis, since iron deposition was barely detected in endometrial tissue before injection.

There is a lack of information on the degradation of erythrocytes within the peritoneal environment. Activated macrophages recruited within the pelvic cavity of patients with endometriosis may play an important role in the metabolism of erythrocytes, as suggested by the presence of numerous iron-laden macrophages known as siderophages.

Metabolization of haemoglobin, a by-product of erythrocyte lysis, also occurs within the endometrial implant. One of our previous studies showed that haeme oxygenase, the enzyme catalysing the degradation of the haeme moiety of haemoglobin into iron, carbon monoxide and biliverdin, is strongly expressed in active red endometrial lesions (Van Langendonckt et al., 2000c). Iron is sequestered within tissue and bound to proteins such as ferritin in a soluble, non-toxic and bioavailable form (Crichton, 2001). After oxidation of ferrous iron, accelerated by apoferitin, iron undergoes hydrolysis and is deposited as ferrhydrite within the protein shell of ferritin, leading to the formation of iron deposits such as those observed in the present study.

Our results indicate that the localization and type of deposits induced depend on the fraction of menstrual effluent injected. Indeed, when the cellular fraction was injected without free erythrocytes, either alone or in combination with menstrual serum, some cytoplasmic and granular iron deposits were evidenced by Prussian blue staining in the interface area between the lesion and the murine mesothelium. These deposits probably correspond to a ferritin iron storage pool that may later be mobilized to meet the iron requirements of proliferating endometrial cells.

On the other hand, the injection of free erythrocytes into the peritoneal cavity appears to enhance iron deposition at the interface. The occurrence of granular and cytoplasmic deposits was much more extensive when the cellular fraction was injected together with erythrocytes or as unfraccionated menstrual effluent, compared to injection of the cellular fraction alone or with serum. This accumulation of iron in the external stromal cell layers of endometrial tissue resulted in the formation of densely stained iron conglomerates. Conglomerates were also observed deeper in the stroma of the lesion when unfraccionated menstrual effluent was injected. These conglomerates consist of haemosiderin, another iron storage form, which is found in conditions of iron overload, usually associated with toxic pathological states in humans (Crichton, 2001). Haemosiderin, which is insoluble, is thought to be derived from the intralysosomal degradation of ferritin (Ioncu, 1992; Crichton, 2001). An excess of iron, unless appropriately chelated, may induce oxidative damage and chronic inflammation.

Taken together, the results of the present study appear to suggest that the presence of an increased number of erythrocytes within the pelvic cavity may have implications on the development of endometriotic lesions. The number of lesions induced in nude mice tended to be higher when unfraccionated menstrual effluent was injected, and injection of erythrocytes into the pelvic cavity of the mice resulted in an accumulation of ferritin-iron within endometrial tissue and the formation of haemosiderin deposits. In patients with endometriosis, the amount of retrograde menstruation does appear to be greater. This may result in increased reflux of erythrocytes into the pelvic cavity, leading to excess storage of iron within lesions, as observed in our murine model. This could thus be one of the factors triggering the inflammatory response associated with endometriosis.

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