Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process

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Inflammatory mediators in the cervix, placenta and fetal membranes play a crucial role in human parturition. The aim of this study was to determine whether the upper and lower segments of the myometrium are infiltrated by inflammatory cells during pregnancy and parturition. Myometrial biopsies were obtained from non-pregnant women, and pregnant women at term before and after the onset of spontaneous labour. Subpopulations of inflammatory cells were identified using immunocytochemistry. The intercellular adhesion molecules, 1 and 2, platelet endothelial cell adhesion molecule, vascular cell adhesion molecule and E-selectin were immunolocalized to investigate their involvement in leukocyte accumulation. Morphological analysis demonstrated that inflammatory cells, predominantly neutrophils and macrophages, infiltrate human myometrium during spontaneous labour at term. The infiltrate is predominant in the lower uterine segment but is also present in the upper segment. Increased expression of E-selectin was found on the vascular endothelium of biopsies obtained during labour, suggesting a role for this molecule in the accumulation of leukocytes. These results suggest that inflammatory cell infiltration is part of the physiological mechanisms that occur in the myometrium during parturition. Further understanding of this process may suggest new strategies aimed at preventing preterm delivery.

Key words: adhesion molecules/labour/macrophages/neutrophils/uterus

Introduction

Inflammatory mediators play a crucial role in human parturition (Kelly, 1996). Cervical ripening, an early event in normal labour, has been compared to an inflammatory reaction and is characterized by an accumulation of leukocytes in the cervical stroma (Junqueira et al., 1980; Liggins, 1981). The inflammatory infiltrate within the ripened cervix consists predominantly of neutrophils and macrophages and accumulates in the stroma before the onset of labour at term (Bokstrom et al., 1997). These leukocytes contribute to cervical remodelling by releasing proteolytic enzymes such as collagenase and elastase (Rajabi et al., 1988; Osmers et al., 1992). Elsewhere in the pregnant uterus, inflammatory cells are known to infiltrate the placenta, maternal decidua and the fetal membranes during parturition, and may play a role in spontaneous rupture of the membranes (Halgunset et al., 1994; Rosenberg et al., 1996).

There are no data on whether inflammatory cells infiltrate myometrium during labour. However, indirect evidence suggests that inflammatory cells may infiltrate the myometrium at the time of parturition. Rechberger and Woessner (1993) demonstrated that collagenase activity is increased in lower segment myometrium during labour and proposed that neutrophils may be responsible for much of the increase. Furthermore, Osmers et al. (1995) demonstrated a significant increase in interleukin-8 concentration in lower segment myometrium during labour, and proposed that this increase could result in an influx of neutrophils. The presence of these leukocytes in myometrium has been examined prior to the onset of labour only. Butterworth et al. (1991) found no neutrophils in the myometrium of both normal and pre-eclamptic women before the onset of labour.

The attachment and extravasation of circulating leukocytes is controlled by the expression of cell surface adhesion molecules on both the circulating cells and the vascular endothelium (Akyama et al., 1989; Bevilacqua, 1993). An increased expression of adhesion molecules on the endothelium occurs in inflammatory conditions and supports the recruitment and aggregation of leukocytes (Bevilacqua, 1993). The major adhesion molecules involved in leukocyte attachment and transendothelial migration include E-selectin, intercellular adhesion molecule-1 and 2 (ICAM-1 and 2), platelet endothelial cell adhesion molecule (PECAM) and vascular cell adhesion molecule-1 (VCAM-1).

The purpose of the present study was to investigate inflammatory cell populations in the human myometrium during pregnancy and labour. Specifically, we aimed to determine whether myometrium in each of the lower and upper uterine segments, like cervical stroma, maternal decidua and the fetal membranes, is infiltrated by leukocytes during parturition. Furthermore, the expression and distribution of the cell adhesion molecules, E-selectin, ICAM-1 and 2, PECAM and VCAM-1, were assessed in myometrium during labour to investigate possible mechanisms of leukocyte accumulation.

Materials and methods

Subjects and collection of tissue

Pregnant women delivered by lower uterine segment Caesarean section and non-pregnant women undergoing hysterectomy were
Inflammatory cells were identified in each of the biopsies obtained. Neutrophils, macrophages, T-lymphocytes and B-lymphocytes, immunocytochemistry, eosinophils and basophils were considered specific for cells of granulocytic lineage (neutrophils, stained for naphthol AS-D chloroacetate esterase activity, an enzyme which is not expressed in mammalian cell systems. Tonsillar tissue was used as positive controls for CD 3, CD 20 and CD 68.

Identification of inflammatory cells

Inflammatory cells were detected using immunocytochemistry, with a panel of antibodies as shown in Table I. Serial sections were stained for naphthol AS-D chloroacetate esterase activity, an enzyme considered specific for cells of granulocytic lineage (neutrophils, eosinophils and basophils).

Antigen, Cell type, Clone, Pretreatment, Dilution, Supplier

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cell type</th>
<th>Clone</th>
<th>Pretreatment</th>
<th>Dilution</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 15</td>
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<td>2.35.14</td>
<td>Microwave</td>
<td>1/60</td>
<td>SAPU</td>
</tr>
<tr>
<td>Neutrophil elastase</td>
<td>Neutrophils</td>
<td>NP 57</td>
<td>Nil</td>
<td>1/150</td>
<td>Dako Ltd</td>
</tr>
<tr>
<td>CD 68</td>
<td>Macrophages</td>
<td>PG-M1</td>
<td>Trypsin</td>
<td>1/300</td>
<td>Dako Ltd</td>
</tr>
<tr>
<td>Mast cell tryptase</td>
<td>Mast cells</td>
<td>AA1</td>
<td>Trypsin</td>
<td>1/300</td>
<td>Dako Ltd</td>
</tr>
<tr>
<td>CD 3</td>
<td>T lymphocytes</td>
<td>Polyclonal</td>
<td>Microwave</td>
<td>1/50</td>
<td>Dako Ltd</td>
</tr>
<tr>
<td>CD 20</td>
<td>B lymphocytes</td>
<td>L 26</td>
<td>Microwave</td>
<td>1/50</td>
<td>Dako Ltd</td>
</tr>
</tbody>
</table>

*SAPU, Carluke, UK; Dako Ltd, High Wycombe, UK.

Inflammatory cells were identified in each of the biopsies obtained before and after the onset of labour at term (>37 weeks gestation); (ii) Eighteen pregnant women who were delivered during active labour at term (cervical dilatation >4 cm and <9 cm). Women were excluded from the study if they had a multiple pregnancy, evidence of active infection, or following induction of labour; (iii) Thirty non-pregnant, pre-menopausal women with regular menstrual cycles undergoing hysterectomy for benign disease.

Identification of inflammatory cells

Inflammatory cells were collected from three groups of women: (i) Eighteen pregnant women who were delivered before the onset of labour at term (>37 weeks gestation); (ii) Eighteen pregnant women who were delivered during active labour at term (cervical dilatation >4 cm and <9 cm). Women were excluded from the study if they had a multiple pregnancy, evidence of active infection, or following induction of labour; (iii) Thirty non-pregnant, pre-menopausal women with regular menstrual cycles undergoing hysterectomy for benign disease.

Identification of inflammatory cells

Mast cells

Mast cells were localized in non-pregnant and pregnant myometrial biopsies. Mast cells were localized using immunocytochemistry, with a panel of antibodies as shown in Table II. Sections 5 μm thick were cut from the paraffin embedded tissues and mounted on silane-coated slides, heated to 60°C for 30 min, and deparaffinized in xylene and rehydrated in a graded alcohol series. The sections were then pre-incubated with 20% normal goat serum (SAPU, Carluke, UK) was first diluted in 1.5% horse serum and 1.5% normal human serum. After washing as before, sections were incubated for 10 min in 3% hydrogen peroxide in methanol at room temperature. The sections were then incubated for 90 min with a monoclonal antibody raised against IgG1 Aspergillus niger glucose oxidase (Dako Ltd, High Wycombe, UK), an enzyme which is not expressed in mammalian cell systems. Tonsillar tissue was used as positive controls for CD 3, CD 20 and CD 68.

Mast cells

Mast cells were localized in non-pregnant and pregnant myometrial biopsies. The paraffin embedded sections were prepared as before and digested in a trypsin solution to retrieve the antigen (Table I). The sections were then pre-incubated with 20% normal goat serum (SAPU, Carluke, UK) in PBS (10 mM sodium phosphate, pH 7.5, 120 mM sodium chloride) for 30 min at room temperature. They were then incubated for 90 min with a monoclonal antibody raised against mast cell tryptase (Dako Ltd) diluted 1/300 in 2% normal goat serum. The primary antibody was omitted from the negative control slides. Next, sections were washed in PBS before incubation with goat anti-mouse IgG alkaline phosphatase (Sigma) diluted 1/100 in Tris.Cl pH 7.6, and appeared as a brown end-product. Negative controls included sections incubated without the primary antibody and sections incubated with a mouse monoclonal antibody against IgG1 Aspergillus niger glucose oxidase (Dako Ltd, High Wycombe, UK), an enzyme which is not expressed in mammalian cell systems. Tonsillar tissue was used as positive controls for CD 3, CD 20 and CD 68.

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Table II. Primary antibodies used for cell adhesion molecule immunocytochemistry

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Dilution</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1</td>
<td>BBIG-I1</td>
<td>1/500</td>
<td>R&amp;D Systems</td>
</tr>
<tr>
<td>ICAM-2</td>
<td>CBR-IC2/2</td>
<td>1/200</td>
<td>Chemicon</td>
</tr>
<tr>
<td>PECAM</td>
<td>9G11</td>
<td>1/500</td>
<td>R&amp;D Systems</td>
</tr>
<tr>
<td>VCAM</td>
<td>BBIG-V1</td>
<td>1/500</td>
<td>R&amp;D Systems</td>
</tr>
<tr>
<td>E-selectin</td>
<td>BBIG-E4</td>
<td>1/250</td>
<td>R&amp;D Systems</td>
</tr>
</tbody>
</table>

*R&D Systems, Abingdon, UK; Chemicon, Harrow, Middlesex, UK.

Cell adhesion molecules

Sections 5 μm thick were cut from the frozen tissue and mounted on silane-coated slides. The sections were fixed in acetone for 10 min, washed in TBS, and placed in 0.5% hydrogen peroxide in methanol for 30 min at room temperature. The sections were then washed...
Leukocytes infiltrate the myometrium during parturition

Figure 1. Identification of granulocytes in myometrium. The granulocytes are localized by staining for naphthol AS-D chloroacetate esterase activity and appear as violet granulation. (A) Granulocytes are sparse in myometrium collected from women not in labour (arrow) and (B) are abundant in myometrium obtained during labour. Bar = 50 µm.

Figure 2. Identification of neutrophils in lower segment myometrium obtained (A) before and (B) during labour. The neutrophils are immunolocalized using an antibody directed against the enzyme human neutrophil elastase. Neutrophils are abundant in myometrium obtained during labour and sparse in myometrium obtained before the onset of labour (arrow). The negative controls (see text) exhibited no reactivity. Bar = 50 µm.

before and pre-incubated with 20% (w/v) normal goat serum (SAPU) in TBS for 30 min at room temperature. The sections were then incubated for 16 h at 4°C with the primary antibody diluted in 1.5% horse serum. Table II shows the characteristics of the primary antibodies. The primary antibody was omitted from the negative control slides. Next, sections were washed in TBS before incubation for one h with biotinylated goat anti-mouse immunoglobulin (Dako Ltd), diluted in 2% normal goat serum and 1.5% normal human serum. The sections were thoroughly washed again, then incubated for 30 min with streptavidin horseradish peroxidase (Dako Ltd) in TBS before final washing. The antigen was localized as previously described using DAB.

Figure 3. Identification of macrophages in lower segment myometrium obtained (A) before and (B) during labour. The macrophages are immunolocalized using an antibody directed against the antigen CD 68. Whilst a population of immunoreactive macrophages is identified before the onset of labour (arrows), significantly more macrophages are localized in myometrium following the onset of labour. The negative controls (see text) exhibited no reactivity. Bar = 50 µm.

Figure 4. Identification of mast cells in (A) non-pregnant myometrium and (B) term, pregnant myometrium. The mast cells are immunolocalized (arrows) using an antibody directed against the enzyme, mast cell tryptase. There are significantly more mast cells in non-pregnant myometrium than in pregnant myometrium. The negative controls (see text) exhibited no reactivity. Bar = 50 µm.

Identification of granulocytes

Granulocytic cells were identified by staining for naphthol AS-D chloroacetate esterase activity. This activity was determined using a commercial kit (Sigma Diagnostics, Poole, UK). Briefly, 1 ml of sodium nitrite solution (0.1 mol/l) was added to 1 ml of Fast Red Violet LB Base solution (15 mg/ml fast red violet LB base in 0.4 mol/l hydrochloric acid with stabilizer) and allowed to stand for 2 min. This solution was then added to 40 ml prewarmed (37°C) deionized water, 5 ml of Trizma 6.3 buffer concentrate (TRIZMA maleate, 1 mol/l with surfactant, pH 6.3) and 1 ml of naphthol AS-D chloroacetate solution (naphthol AS-D chloroacetate 8 mg/ml and stabilizer). The slides were incubated in this solution for 15 min at...
37°C and then rinsed thoroughly in deionized water. Sites of activity showed violet granulation.

**Quantification of inflammatory cells and statistical analysis**

The inflammatory infiltrate was quantified (i) to determine whether inflammatory cells infiltrate the myometrium during labour at term (ii) to compare the density of the inflammatory cell infiltrate in upper and lower uterine segment myometrium and (iii) to compare mast cell density in non-pregnant and pregnant myometrium. Inflammatory cells were identified by either brown staining (neutrophils, macrophages, T cells and B cells) or red staining (mast cells). In each section of myometrium, the number of positive cells was counted in a high powered field (×400 magnification within parts of a lined grid covering an area of 0.02 mm²). Six different fields were counted by two observers who were blinded to the specimen details. Areas containing blood vessels were avoided and leukocytes within vessels were not included. The average number (arithmetic mean) of positive cells recorded per field by each observer was calculated, and then a mean of these two values obtained. Statistical comparisons of the means were performed using three-factor analysis of variance (ANOVA) with Scheffé’s S as a post hoc test. Significant differences between groups were explored using the Mann–Whitney U-test. The sites of expression of each of the cell adhesion molecules were recorded by the observers who were blinded to the specimen details. Differences in the expression of these molecules before and after labour were analysed using the χ² test.

**Results**

Staining for naphthol AS-D chloroacetate esterase activity, which identifies inflammatory cells of granulocytic lineage, revealed that myometrial biopsies removed from labouring women [group (ii)] showed a marked inflammatory infiltrate in the muscle connective tissue in 17 of the 18 specimens (Figure 1). In contrast, the myometrium in the non-labouring women [group (i), n = 18] did not exhibit an inflammatory infiltrate. The infiltrate appeared most dense at both the luminal (decidual) edge of the myometrium and also in and around blood vessels. Further, the infiltrate was most striking in the myometrium obtained from the lower uterine segment, but was also present in the myometrial biopsies obtained from the uterine fundus during labour.

Analysis of individual cell types using immunocytochemistry showed significantly more inflammatory cells in labouring myometrium compared to non-labouring biopsies (Scheffé’s test, P = 0.0001), and significantly more inflammatory cells were present in the lower uterine segment compared to the upper uterine segment myometrium (Scheffé’s test, P < 0.02).

The leukocyte subpopulations were characterized as follows:

**Neutrophils**

Neutrophils were sparse in myometrium obtained before the onset of labour and abundant in biopsies obtained during labour (Figure 2). A significant increase in myometrial neutrophil density occurred following the onset of labour in both the lower (Table III), and the upper uterine segments (Table IV). During labour, the neutrophil density was significantly greater in the lower than in the upper uterine segment (P < 0.02). Within the labouring biopsies, the elastase antigen was localized both within the neutrophil cytoplasm and extracellularly in the vicinity of the leukocytes, suggesting that a proportion of these cells had degranulated.

**Macrophages**

A population of immunoreactive macrophages was identified in myometrium before the onset of labour. The number of macrophages was significantly increased in both lower and upper uterine segments of the myometrium following the onset of labour (Figure 3, Tables III and IV). Following the onset of labour, there was no significant difference in macrophage density between the upper and lower uterine segments.

**Lymphocytes**

There was a significant increase in the density of T-lymphocytes in lower segment myometrium following the onset of labour (Table III). B-Lymphocytes were sparse in lower segment myometrium and no significant change in their density occurred with the onset of labour. There was no significant change in either T or B-lymphocyte density following the onset of labour in upper segment myometrial biopsies (Table IV).

<table>
<thead>
<tr>
<th>Table III. Number of inflammatory cells in lower segment myometrium before and during labour at term, median (interquartile range) per high powered field</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell type</strong></td>
</tr>
<tr>
<td><strong>(n = 18)</strong></td>
</tr>
<tr>
<td>Neutrophils</td>
</tr>
<tr>
<td>Macrophages</td>
</tr>
<tr>
<td>T-lymphocytes</td>
</tr>
<tr>
<td>B-lymphocytes</td>
</tr>
<tr>
<td>Mast cells</td>
</tr>
</tbody>
</table>

*ns = not significant.*

<table>
<thead>
<tr>
<th>Table IV. Number of inflammatory cells in upper segment myometrium before and during labour at term, median (interquartile range) per high powered field</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell type</strong></td>
</tr>
<tr>
<td><strong>(n = 7)</strong></td>
</tr>
<tr>
<td>Neutrophils</td>
</tr>
<tr>
<td>Macrophages</td>
</tr>
<tr>
<td>T-lymphocytes</td>
</tr>
<tr>
<td>B-lymphocytes</td>
</tr>
<tr>
<td>Mast cells</td>
</tr>
</tbody>
</table>

*ns = not significant.*

<table>
<thead>
<tr>
<th>Table V. Presence of mast cells in human myometrium. Values are shown as median (interquartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of mast cells per high powered field</strong></td>
</tr>
<tr>
<td><strong>Non-pregnant (n = 13)</strong></td>
</tr>
<tr>
<td>Term, not in labour (n = 17)</td>
</tr>
<tr>
<td>Term, in labour (n = 18)</td>
</tr>
</tbody>
</table>

*P < 0.0001 compared with each pregnant group (term not in labour and term in labour).*
Leukocytes infiltrate the myometrium during parturition

Figure 5. Localization of cell adhesion molecules to the vascular endothelium in serial cryosections of myometrium. There are no changes in the expression of (A) ICAM-1 (B) ICAM-2, (C) PECAM or (D) VCAM in the vascular endothelium following the onset of labour. E-selectin is not expressed in any of the biopsies obtained before the onset of labour but is identified in three of the six biopsies obtained during labour. (E) Localization of E-selectin in the capillary vascular endothelium in myometrium obtained during labour. The negative controls (see text) exhibited no reactivity. Bar = 50 µm.

Mast cells
There was no significant difference in mast cell density in myometrium before and after the onset of labour at term (Table III). Furthermore, mast cells were sparse in both upper and lower uterine myometrial biopsies (Table IV), with no significant differences in mast cell density between the biopsy sites. We assessed mast cell density in non-pregnant myometrium [group (iii)], and found that this was significantly greater than in biopsies obtained from each of the pregnant groups, term not in labour [group (i)], and term in labour [group (ii)] (Figure 4). These results are summarized in Table V.

Cell adhesion molecules
The cell adhesion molecules were localized as brown staining in serial cryosections of lower segment myometrium before \( (n = 6) \) and after \( (n = 6) \) the onset of labour (Figure 5). There were no changes in the immunolocalization of ICAM-1, ICAM-2, PECAM or VCAM following the onset of labour. ICAM-1 immunostaining was of weak intensity and ICAM-2 staining was of moderate intensity in the myometrial vascular endothelium, although not all vessels expressed these molecules. PECAM expression was strong and consistent in all of the vascular endothelium. In contrast, VCAM expression was weak and absent in some vessels. E-selectin was not expressed in any of the tissues obtained before the onset of labour. However, three of the six biopsies obtained during labour showed immunostaining for E-selectin on the vascular endothelium \( (\chi^2 P < 0.05) \).

Discussion
We have demonstrated that inflammatory cells, predominantly neutrophils and macrophages, infiltrate human myometrium
during spontaneous labour at term. The inflammatory infiltrate
is predominant in the lower uterine segment, but is also present
in the myometrium of the uterine body. We also observed a
significant increase in T-lymphocyte density in lower segment
myometrium following the onset of labour. There was no
change in B-lymphocyte and mast cell densities in the myo-
metrium during parturition.

In this study, we have examined both lower and upper
uterine segment myometrium for the presence of inflammatory
cells. Whether lower segment tissue reflects the state of the
cervix or that of the uterine fundus, has aroused considerable
interest over the last half century (Calder, 1994). Some
workers contest that lower uterine segment biopsies provide
an alternative source of tissue that closely resembles cervix
(Rajabi et al., 1988). Because of the difficulties in obtaining
cervical tissue for study, lower segment biopsies have been
used in place of cervical tissue to investigate collagenase
activity during cervical dilatation (Rechberger and Woessner,
1993). Our results indicate that lower uterine segment myo-
metrium behaves quite differently from reported behaviour in
cervix, and has similarities to upper segment myometrium.
Bokstrom et al. (1997) demonstrated an abundance of neutro-
phils and macrophages in cervical biopsies in late pregnancy
before the onset of labour with no significant increase in their
densities during labour. In contrast, we have shown that
neutrophils and macrophages are sparse in lower and upper
segment myometrium before, and abundant during labour.
Although the upper and lower uterine segments behave in a
similar manner, they are not identical. During labour, the
inflammatory infiltrate is more dense in lower segment myo-
metrium than in upper segment myometrium. Consistent with
this histological finding, functional studies have demonstrated
that in normal labour the upper uterine segment contracts more
strongly than the lower, a situation which is reversed in
abnormal labour (Caldeyro-Barcia and Poseiro, 1960; Margono
et al., 1993).

Activated neutrophils and macrophages are a rich source of
inflammatory mediators. These include plasminogen activators,
eicosanoids, collagenase and elastase, and proinflammatory
cytokines, including interleukin-1 and tumour necrosis factor-
α (Nathan, 1987; Osmers et al., 1992; Casatella, 1995). Since
these mediators have many diverse functions, the inflammatory
infiltrate could have different roles in different regions of the
uterus. Within the lower segment it could be involved in tissue
remodelling and thereby facilitate cervical dilatation and
passage of the fetus. In the upper segment, leukocyte products,
including eicosanoids, interleukins and tumour necrosis factor-
α, may stimulate uterine contractions directly, or indirectly by
facilitating the production of uterotonics in prostanoids (Casey
et al., 1990). Furthermore, inflammatory mediators may also
initiate tissue remodelling in the uterine body. Granstrom et al.
(1989) demonstrated that the connective tissue of the uterine
isthmus (lower segment) and the uterine body undergoes a
biochemical ripening process similar to that found in the
cervix, with an increase in collagenolytic activity following
the onset of labour. A breakdown of the connective tissue
within the myometrium may facilitate the co-ordination of
uterine contractions by allowing the formation of gap junctions
(Garfield and Hayashi, 1981).

We found no change in myometrial mast cell density before
and after the onset of labour at term. The function of mast
cells in the pregnant uterus remains unclear, although it has
been proposed that myometrial mast cells regulate uterine
contractility during labour (Rudolph et al., 1993). Mast cells
produce mediators (histamine and serotonin) and prosta-
glandins which can induce strong contractions in human
myometrium in vitro (Cruz et al., 1989; Rudolph et al., 1990,
1993). Further, these cells are considered to play a pivotal role
in wound healing, fibrosis and tissue remodelling (Galli, 1993),
and might be involved in promoting collagen degradation and
uterine involution in the postnatal period (Jeffrey et al., 1991).
We were surprised at the low density of mast cells in the
pregnant myometrium since this is in contrast with the
known distribution of mast cells in non-pregnant myometrium
(Mori et al., 1997). Since mast cell mediators are capable of
stimulating uterine contractions, the low density of mast cells
in pregnant myometrium may be involved in the maintenance
of myometrial quiescence as the uterus expands during pregnancy.
Whilst we could demonstrate no change in myometrial mast
cell density following the onset of labour, mast cell mediators
might be capable of stimulating uterine contractions at term
since the sensitivity of human myometrium to histamine and
serotonin is upregulated at the end of pregnancy (Cruz et al.,
1989). This means that myometrial smooth muscle cells might
be stimulated by mast cell mediators even when the mast cell
density is reduced. In non-pregnant myometrium, the high
density of mast cells has been proposed to have a role in
implantation (Brandon and Evans, 1983; Hore and Mehrotra,
1988), or in remodelling uterine smooth muscle and extracellu-
lar matrix during the menstrual cycle (Mori et al., 1997).

The mechanisms involved in the accumulation, extravasation
and degradation of inflammatory cells in uterine tissues
during parturition are poorly understood. Chemotactic cyto-
kines, including interleukin-1, tumour necrosis factor-α and
interleukin-8, seem to play a role (Barclay et al., 1993;
Chwalisz et al., 1994; Osmers et al., 1995), as well as other
chemotactic agents, such as C5a (El Madany et al., 1995).
E-selectin is involved in the infiltration of leukocytes to
the maternal decidua and fetal membranes during labour
(Rosenberg et al., 1996).

Since the leukocytes in our myometrial biopsies were
concentrated in and around blood vessels, we postulated that
an up-regulation in the expression of vascular cell adhesion
molecules in lower segment myometrium was involved in the
accumulation of leukocytes in this tissue. ICAM-1, ICAM-2,
PECAM and VCAM are members of the immunoglobulin
superfamily (Frenette and Wagner, 1996). ICAM-1 is important
in the adhesion of monocytes, lymphocytes and neutrophils
to activated endothelium, whilst VCAM binds to leukocyte
integrins on many cells including eosinophils and activated T
lymphocytes. E-selectin, a member of the selectin family of
adhesion molecules, is expressed by cytokine activated
endothelial cells and has a major role in attracting neutrophils,
monocytes, eosinophils and some lymphocytes (Lasky 1992;
The expression of cell adhesion molecules in the endometrium of the non-pregnant uterus is well described (Tawia et al., 1993; Tabibzadeh et al., 1994). A recent report has identified ICAM-1, VCAM, PECAM and E-selectin in pregnant human myometrium (Winkler et al., 1998). We have shown that ICAM-1, ICAM-2, PECAM and VCAM are expressed on the vascular endothelium in myometrium obtained before the onset of labour at term and we propose that these molecules play a role in regulating leukocyte trafficking into this tissue. We found no change in the localization and intensity of staining of ICAM-1, ICAM-2, PECAM and VCAM in the biopsies obtained during labour compared with those obtained before the onset of labour. In contrast, E-selectin expression was absent in all of the biopsies collected before labour, but was expressed in three of the six biopsies obtained during labour, suggesting a role for this molecule in the recruitment of leukocytes in at least some of the tissues. These results are in broad agreement with Winkler et al. (1998), who also found that E-selectin expression was up-regulated during labour. Both our own study and that of Winkler et al. (1998) have employed immunocytochemistry, a qualitative technique. In order to confirm the changes in cell adhesion molecule expression during parturition, further studies are required using quantitative techniques.

Factors responsible for the initiation of parturition remain obscure. We have demonstrated that leukocytes infiltrate both the upper and lower uterine segments of the myometrium during spontaneous labour at term, and we propose that these cells play a fundamental role in normal parturition. A better understanding of the mechanisms involved in the initiation of labour both at term and preterm would allow the development of novel strategies to prevent premature delivery. Our results suggest that strategies aimed at preventing the influx of inflammatory cells into the myometrium could be crucial in averting preterm delivery, and thus in reducing the excess perinatal mortality and morbidity associated with this condition.

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References


Leukocytes infiltrate the myometrium during parturition


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