Significance of mast cell distribution in placental tissue and membranes in spontaneous preterm birth

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Introduction

Despite major advances in perinatal care, the rate of preterm birth increased by 22% between 1991 and 2011 in Australia, where more than 25,000 babies are now delivered preterm each year.¹ Preterm birth is well recognized as a major cause of adverse neonatal and childhood outcomes, affecting not only survival in the short term but also longer term quality of life for children.² The more remote from term a child is born, the greater the burden of morbidity and mortality,³ but even late preterm birth (from 34 to 36 weeks) has the potential for adverse consequences.⁴ In addition to the direct health effects on children, preterm birth imposes emotional and social strains on a family and the care of children born preterm is very expensive.

Established preterm labor is difficult to treat, and the use of tocolytic agents is associated with a delay of only 1 or 2 days before birth.³ Fortunately, even an additional 24 hours allows time for the use of steroids to reduce neonatal respiratory morbidity, safe transfer of mothers to hospitals that can provide suitable care for the preterm neonate, and the use of magnesium sulfate for neuroprotection in very preterm births.

Studies suggest that preterm labor in women who do not have underlying risk factors (such as multiple pregnancy or cervical shortening) is commonly associated with inflammation at the maternal–fetal interface. There is some indirect evidence that mast cells (MCs) might represent a link between hormonal influences and local reactions leading to the onset of labor.

Patients and methods: The placentas and membranes of 51 uncomplicated spontaneous term births were compared to those from 50 spontaneous preterm births. Immunohistochemical staining for MC tryptase was undertaken allowing MC concentration, location, and degranulation status to be determined. Regression modeling was used to compare results.

Results: There were no significant differences in the demographic characteristics of the two cohorts. There were significantly more MCs in the decidua for term births than preterm births (P=0.03). The presence of histological chorioamnionitis did not affect MC concentrations.

Conclusion: Despite evidence suggesting a possible role for MCs in spontaneous preterm birth, this study found that the concentration of decidual MCs was in fact significantly lower in preterm compared to term birth.

Keywords: preterm birth, mast cells, inflammation, cohort study, regression modeling
necessarily peripheral blood, suggesting that inflammation at
the maternal–fetal interface might play an important role.6,7
Unfortunately, preventive strategies for inflammatory causes
of preterm birth have not been particularly successful.

Some evidence has arisen suggesting that mast cells
(MCs) might represent a link between hormonal influences
and local reactions that lead to the onset of labor. MCs are
plentiful in the reproductive tract, and their concentration is
increased in inflammatory conditions of the cervix.8 A study
of decidual tissue in spontaneous human early pregnancy
loss reported “a dramatic increase in the number of MCs”
compared to normal pregnancy.9 In rat models, MC degranu-
lation was “prominent” after antigestagen treatment, and
MC-stabilizing agents inhibited the antigestagen-induced
cervical ripening.10 MCs express estrogen receptors and their
responsiveness to degranulating agents appears to increase
with higher environmental estrogen concentrations.11
Estriol itself has been shown to stimulate MC degranula-
tion and this is blocked by tamoxifen, suggesting that
estriol-induced MC degranulation could result from
receptor activation.11 Progesterone appears to inhibit MC
migration and to downregulate receptor expression on the
MC surface,12 and progesterone also appears to inhibit his-
tamine secretion.13

We have not been able to identify previous studies of
MCs in the decidua and membranes of women with preterm
labor, and aimed to determine whether there was an increased
number, or distribution pattern of MCs in these tissues in
women with a spontaneous preterm birth compared to those
who labored spontaneously at term.

Patients and methods
Prospective ethics approval for this study was obtained
from the ACT Health Human Research Ethics Committee
(EthLR11.085). This study did not require patient consent
following formal Ethics Committee consideration, as the
samples to be used were collected for routine diagnosis and
subsequently stored, and were then retrieved for diagnostic
research that was not envisaged at the time of collection
(Exemption 3, Category 3, of the ACT Health Human
Research Ethics Committee Guidelines). The study group
comprised 51 women who had an uncomplicated spontaneous
term birth (≥37 weeks gestation), and 50 women with a sponta-
nous pre term birth (≤34 weeks gestation). Samples from
the placenta disc with membranes were obtained using routine
laboratory methods. Sections from the paracentral placental
block that included the fetal membranes and decidua were
examined. The tissues were fixed in 10% neutral buffered for-
malin before processing using standard laboratory methods.

Immunohistochemistry assessment was performed on the
Leica Bond Automated System (Vision BioSystems, Leica,
Bannockburn, IL, USA) using a standard protocol. Briefly,
4 μm thick sections were cut and dried in an oven at 60°C
for 1 hour. Antigen sites were unmasked by heat treatment
for 20 minutes using the Bond Epitope Retrieval Solution
2 from Leica Microsystems (Sydney, NSW, Australia), pH
8.9–9.1. The primary antibody used was a mouse monoclonal
MC tryptase (ab2378 1/15,000 dilution; Abcam, Cambridge,
UK) with an incubation time of 15 minutes. The detection
kit used was the Leica Bond Polymer Refine Detection Kit
(a polymeric horseradish peroxidase [HRP]-linker antibody
conjugate system) from Leica Microsystems. All sections
were counterstained with hematoxylin to allow visualization
of the nuclei, coverslipped, and viewed. Known positive and
negative controls for each antibody were used.

The number of MCs was determined across ten
high-power fields (magnification 40×, Olympus microscope)
within both the decidua and membranes for each subject.
MCs were assessed for degranulation status and location
within the tissues (Figure 1). Two independent pathologists
(MF and JD) were responsible for the assessment, and were
blinded to the patient cohort at the time of assessment.

In addition to descriptive statistics, generalized linear
models were used to test the association between gesta-
 tion (preterm or term) and the concentration of MCs in the
decidua, and the number of MCs in the chorion. A Poisson
log-linear model was used in the regressions. A probability
of <0.05 was considered significant on two-sided χ2 tests.
SPSS version 22.0 was the statistical package used.

Results
All births were vaginal, and maternal characteristics of
the study groups are presented in Table 1. Based on the

Figure 1 Photomicrograph showing mast cells (stained brown) within the decidua
(mast cell tryptase immunohistochemistry, original magnification x400).
generalized linear models, there were significantly more MCs in the decidua for term births than preterm births (Table 2 and Figure 2, \(P=0.03\)). The distribution of MCs according to gestational age is presented in Table 3. There was a scattered distribution of the MCs in the decidua with no obvious concentration around blood vessels. In the membranes there was a higher concentration of MCs in the preterm samples than term samples; however, the association did not reach statistical significance (Table 2 and Figure 2, \(P=0.06\)). The number of MCs was not related to the presence or absence of chorioamnionitis in the placental membranes. While in a few placenta the MCs were noted to be degranulating, there was no difference between preterm and term births.

### Discussion

The aim of this study was to determine whether there were differences in the numbers and distribution of MCs in the membranes and decidua of women, who had a spontaneous preterm birth compared to women delivering at term. The results showed a statistically significant difference in the concentration of MCs in the decidua between spontaneous term and preterm birth. However, the association was the opposite of that we had hypothesized, with a decrease in the concentration of MCs in the decidua in preterm births. The difference was subtle, and it is unclear as to whether this represents a clinically significant difference. It is possible that the MC numbers in the decidua are related simply to gestational age and might not have a role in preterm labor in women with no known underlying risk factors. It is difficult to know whether the differences in the number of MCs between the two groups represents a clinically important difference, or whether such influences as the administration of steroids for fetal lung ripening in the preterm group might have an effect.

Studies in humans suggest that MC numbers increase in pregnancy.\(^{14-16}\) Studies in mice have shown that degranulation occurs during birth, and histamine concentrations within MCs reach a peak toward the end of pregnancy, then

### Table 1 Demographics of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Preterm (n=50)</th>
<th>Term (n=51)</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal age (years)</td>
<td>27.5</td>
<td>28.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean gestational age (weeks)</td>
<td>27.3</td>
<td>39.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Fetal factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Twins</td>
<td>1 (2%)</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth restriction</td>
<td>3 (6%)</td>
<td>2 (3.92%)</td>
<td>1.56</td>
<td>0.2, 14.1</td>
<td>0.68</td>
</tr>
<tr>
<td>Chromosomal abnormality</td>
<td>2 (4%)</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Maternal comorbidity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>6 (12%)</td>
<td>3 (5.88%)</td>
<td>2.2</td>
<td>0.45, 11.8</td>
<td>0.32</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>4 (7.84%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Prolonged ruptured membranes &gt;18 hours</td>
<td>7 (14%)</td>
<td>13 (25.49%)</td>
<td>0.48</td>
<td>0.15, 1.45</td>
<td>0.21</td>
</tr>
<tr>
<td>Placenta previa</td>
<td>2 (4%)</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Antepartum hemorrhage</td>
<td>4 (8%)</td>
<td>2 (3.92%)</td>
<td>2.1</td>
<td>0.31, 17.7</td>
<td>0.44</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1 (2%)</td>
<td>1 (1.96%)</td>
<td>1.02</td>
<td>0.03, 38.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Note: –, Analysis was not performed.

**Abbreviations:** OR, odds ratio; CI, confidence interval.

### Table 2 Estimated marginal mean number of mast cells per microscopic field in decidua (95% CI) and membranes (95% CI) of placenta for preterm vs term babies

<table>
<thead>
<tr>
<th>Gestational status</th>
<th>Decidua</th>
<th>P-value</th>
<th>Membranes</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm (n=50)</td>
<td>9.12 (8.32, 10.0)</td>
<td>0.03</td>
<td>8.15 (7.38, 8.99)</td>
<td>0.06</td>
</tr>
<tr>
<td>Term (n=51)</td>
<td>10.47 (9.62, 11.4)</td>
<td>0.06</td>
<td>7.12 (6.42, 7.90)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Abbreviation:** CI, confidence interval.

### Figure 2 Estimated marginal mean number of mast cell per microscopic field in term placenta versus preterm placenta.
The inflammatory cell influx to the myometrium and endometrium associated with labor coincides with upregulation of several mediators including interleukin 8 (IL-8), all of which are ligands for receptors (such as CXCR1 and others) found on MCs.\textsuperscript{24,25} Inflammatory mediators released by MCs are also known to play a role in several labor-related biological processes including stimulation of smooth muscle cell contraction and promotion of angiogenesis.\textsuperscript{26} There is evidence that MC degranulation products contribute to both cervical angiogenesis in pregnancy and to cervical ripening.\textsuperscript{27} In vitro studies have reported that compounds causing MC degranulation can induce myometrial contraction, and that pretreatment with MC-stabilizing agents diminishes this effect.\textsuperscript{28} Histamine induces myometrial cell contraction in vitro, both through a direct effect on the H\textsubscript{1} receptor\textsuperscript{16,29} and indirectly by inducing prostaglandin production, including prostaglandin F\textsubscript{2α} secretion from decidual cells.\textsuperscript{30} A number of prostaglandins including E\textsubscript{2} and F\textsubscript{2α} can be directly secreted by MCs.\textsuperscript{31,32} Of note, in this study the rate of chorioamnionitis between the two groups was similar, making it unlikely to be an explanation for the difference in concentration of MCs between the two groups.

Serotonin secreted by MCs has uterotonic properties\textsuperscript{33} and oxytocin has been shown to inhibit serotonin uptake by uterine MCs, an effect that might increase its local bioavailability during labor.\textsuperscript{34} Release of MC tryptase is also known to stimulate production and release of matrix metalloproteinases from endometrial stromal cells, and increases in endometrial matrix metalloproteinase expression have been observed in the perimenstrual phase.\textsuperscript{35,36}

**Conclusion**

Despite a body of indirect evidence pointing to a possible role for MCs in spontaneous preterm birth, this study found that the concentration of MCs was significantly lower in the decidua in preterm compared to term birth. Whether this might represent an effect of gestation, or may even be related to administration of steroids, remains indeterminate.

**Acknowledgment**

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**Disclosure**

The authors report no conflicts of interest in this work.
References


