The role of mast cells and their mediators in reproduction, pregnancy and labour

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BACKGROUND: Mast cells (MCs) are the classical mediators of allergy, however, their importance in the development of innate and adaptive immune responses is increasingly being recognized. Herein, the present MC literature is summarized, with particular focus on studies of MCs in the endometrium and myometrium, and their involvement in fertility, implantation, pregnancy and labour.

METHODS: Recent developments in MC biology were identified by systematic searches of PubMed, Medline and Google Scholar from 2000 to November 2009. To specifically examine the role of MCs in fertility and pregnancy, we then performed a systematic review of English literature cited in the PubMed, Medline and Google Scholar databases, but extended the search period, from 1980 to January 2010

RESULTS: MCs can respond to immunoglobulin E-independent innate immune stimuli and are present within the endometrium, with activation and release of mediators occurring prior to menstruation and in association with endometriosis. With respect to pregnancy, MCs are redundant during blastocyst implantation and although their mediators can induce myometrial contractility, there is no epidemiological link of preterm birth with allergy, suggesting a non-essential role or robust regulation. In males, MCs are present in the testes and are increased in oligo- and azoospermia, with MC mediators directly suppressing sperm motility in a potentially reversible manner.

CONCLUSIONS: MCs are prevalent in the female and male reproductive tract. However, whether MCs are absolutely required for a successful pregnancy or are fundamental to reproductive pathology, and thereby a therapeutic target, remains to be determined.

Key words: mast cells / pregnancy / parturition / preterm labour / inflammation

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Introduction

Since the first description of mast cells (MCs), or ‘Mastzellen’, by Paul Ehrlich in the 19th century, the multifunctional role of these cells in the immune system continues to be elucidated (Metz and Maurer, 2007; Beaven, 2009). Classically, MCs are viewed as mediators of allergic reactions, but it is now recognized that their immune function extends far beyond this, playing critical roles in a variety of non-allergic innate (Marshall and Jawdat, 2004; Stelekati et al., 2007) and adaptive (Galli et al., 2005; Nakae et al., 2005) immune responses. In addition, MCs contribute to several autoinflammatory diseases (Rottem and Mekori, 2005), regulate tissue homeostasis, play a role in cancer and aid the development of peripheral tolerance (Lu et al., 2005). These roles depend on the expression of a multitude of biologically active products, and receptors for a wide variety of immunological and pathological mediators. Moreover, MC responsiveness is highly sensitive to the tissue cytokine environment, and controlled by synergistic, or inhibitory, interactions among the various MC receptor systems (Beaven, 2009).

A successful pregnancy requires conception and implantation; the establishment of a vascular supply for optimal fetal growth and development; and the subsequent remodelling of the uterus. These different stages require complex molecular and cellular dialogues, and it is clear that MCs have the potential to contribute to all aspects of this co-ordinated process. The aim of this review is to summarize recent developments in MC biology and discuss the possible contribution of MCs to fertility, pregnancy and labour.

Methods

Recent developments in MC biology were identified by a systematic search of MC literature in PubMed, Medline and Google Scholar from 2000 to November 2009. The most relevant literature was selected and divided into themes. To specifically examine the role of MCs in fertility and pregnancy, we then performed a systematic review of English literature cited in the PubMed, Medline and Google Scholar databases, but extended the search period from 1980 to May 2010 and used the following terms: cervix, conception, chemokine, endometrium, fertility, infertility, MCs, immunoglobulin (Ig)E, migration, myometrium, parturition, human parturition, pregnancy, preterm labour and smooth muscle. Both original articles and reviews were selected based on their relevance to human fertility, implantation, pregnancy and labour, as well as gynaecological disorders. After consideration of the literature based on human studies, the scope of our selection was widened to also consider animal studies. Our selection method enabled us to gain a clear insight into the field of MC biology, and the current published literature concerned with immune cells, pregnancy and labour.

The development, phenotype and localization of MCs

MC progenitors are generated from CD34+ haematopoietic stem cells in the bone marrow (Kirshenbaum et al., 1991; Drew et al., 2002), which then enter mucosal or connective tissues where they complete their differentiation (Kitamura and Fujita, 1989; Okayama and Kawakami, 2006). The cytokine stem cell factor (SCF), which binds the receptor c-kit (CD117), is required for MC development and proliferation, and promotes MC survival through inhibition of apoptosis (Iemura et al., 1994). c-kit expression is common to all haematopoietic progenitors, but is progressively lost as cells become terminally differentiated. MC progenitors, however, retain c-kit expression throughout differentiation (Ashman, 1999), and mature resident tissue MCs undergo further proliferation in the presence of SCF (Kitamura and Fujita, 1989; Tsai et al., 1991a, b). The central position of SCF and c-kit in MC maturation is evident in c-kit null mice which have a profound deficiency in mature tissue MCs, despite the presence of rudimentary MCs at birth (Kitamura et al., 1978; Galli and Kitamura, 1987; Tono et al., 1992; Wolters et al., 2005).

A series of other cytokines also have critical, but targeted, roles in MC development. Consistent with such central but restricted roles, interleukin (IL)-3 (Dahl et al., 2004) in combination with SCF is sufficient to generate MC from human umbilical cord blood mononuclear cells (Dvorak et al., 1993; Mitsui et al., 1993), while their derivation from bone marrow in vitro can be driven independently of IL-3 (Shimizu et al., 2008). Similarly IL-3 contributes to, but is not essential for, in vivo development of murine MCs, since IL-3-deficient mice are not MC deficient (Helb et al., 2008), but IL-3 blockade prevents the increase in MC numbers that is commonly observed with helminth Nippostrongylus brasiliensis infection (Madden et al., 1991).

Recently, much attention has focused on the newly described epithelium-derived cytokine IL-33, which is a member of the IL-1 family and signals via the T1/ST2 receptor (Schmitz et al., 2005). IL-33 prolongs the survival and adhesion of human MCs in the absence of SCF (Ikura et al., 2007), and can accelerate the in vitro maturation of CD34+ MC precursors (Allakhverdi et al., 2007). In addition, mouse studies have revealed that MCs are the only cell lineage that expresses the T1/ST2 receptor at high levels from as early as Day 15.5 of gestation in fetal blood and throughout their maturation (Moritz et al., 1998), suggesting that IL-33 may play a key role in MC development. Pregnancy is associated with Th2-type immunity (Lin et al., 1993; Wegmann et al., 1993), and since IL-33 induces the secretion of Th2 cytokines and chemokines, and drives the recruitment of classical Th2 inflammatory cells (Trajkovic et al., 2004; Allakhverdi et al., 2007), it seems possible that IL-33 and MC T1/ST2 may play a role in pregnancy-related alterations in the uterine environment. However, this still remains to be addressed.

In humans, MCs preferentially reside in tissues that contact the external environment, such as the skin and airways, placing them in a prime position to encounter pathogens and other external inflammatory stimuli. MCs exhibit clear tissue-specific phenotypes (Kitamura, 1989), for example, MCs in skin, lung, heart and tonsil clearly differ with respect to their receptor expression and inflammatory mediator content (Bradding, 2009). Early studies on MCs isolated from human myometrium (Massey et al., 1991) revealed that they share more functional characteristics with lung MCs than skin MCs. For example, uterine MCs responded to IgE stimulation by release of histamine, leukotriene C4 and prostaglandin (PG) D2, with the quantities released being slightly greater than those produced by similarly treated lung MCs.

MCs are often categorized by their granule protease content (Irani et al., 1986; Kitamura, 1989; Irani and Schwartz, 1994; Welle, 1997). Tryptase and chymase are serine proteases (Harvima et al., 1999; Peijler et al., 2007) which, when secreted, contribute to host defence mechanisms through promoting inflammation and tissue...
remodelling (Saito, 2005; Caughhey, 2007). MCs in humans are described as either MC\textsubscript{TC}, which contain tryptase and chymase (and also carboxypeptidase and cathepsin G) or MC\textsubscript{T}, which contain only tryptase. MC\textsubscript{TC} are associated primarily with the skin, while MC\textsubscript{T} dominate in mucosal tissues (Craig and Schwartz, 1989; Irani et al., 1989; Hogan and Schwartz, 1997; Pejler et al., 2007). This distinction is thought to be linked to their maturation status, and studies support the concept of a linear maturation pathway from MC\textsubscript{T} to MC\textsubscript{TC} (Li and Krulis, 1999). By comparison, MCs in mice cannot be readily segregated according to expression of proteases but are nonetheless classified as either mucosal tissue type or connective tissue type. Moon et al. have recently reviewed in detail the variations in phenotype that exist between human and mouse MCs (Moon et al., 2009).

**MCs and the endometrium**

The number of MCs within the endometrium is low, and those that are present are predominantly localized to the basal layer (Sivridis et al., 2001). Although there are no significant differences in the number of MCs present within the endometrium throughout the secretory, proliferative or premenstrual stages of the menstrual cycle (Drudy et al., 1991; Mori et al., 1997a; Sivridis et al., 2001), MCs with low granule content, suggesting prior activation, have been found within the endometrium at the premenstrual stages (Sivridis et al., 2001). Matrix metalloproteinases (MMPs) degrade extracellular matrix components, and the endometrial expression of a number of these proteases increases dramatically prior to and during menstruation (Hampton and Salamonsen, 1994). Release of tryptase from MCs stimulates the production of MMPs by endometrial stromal cells, in a manner that does not require cell–cell contact (Hampton and Salamonsen, 1994; Rawdanowicz et al., 1994; Zhang et al., 1998). The trigger for endometrial MC activation is currently unknown, although corticotrophin-releasing hormone has been suggested to play a role (Zhang et al., 1998; Kempuraj et al., 2004).

A 7-fold increase in activated MCs has been found in endometrial polyps compared with normal endometrium (Al-Jefout et al., 1999). MCs are also increased in benign nasal and colonic polyps compared with normal tissue, suggesting that MC increases may be a common feature of polyps (Otsuka et al., 1993; Gounaris et al., 2007). The role of MCs in this context is unclear, but tryptase, acting through protease activated-receptors, is a growth factor for certain epithelial cells. Moreover, MCs can exert pro-tumorigenic effects in some animal models of cancer, possibly through the regulation of angiogenesis.

Increased numbers of activated MCs, with concomitant increased SCF production, have also been found in association with peritoneal and ovarian endometriotic deposits, where they may contribute to fibrosis through the release of inflammatory mediators (Osuga et al., 2000; Konno et al., 2003; Kempuraj et al., 2004; Sugamata et al., 2005). MCs have also been shown to be located around blood vessels and the interstitium of endometrial cysts, where again their anatomic location would support a role for the development of fibrosis and adhesions (Fujinara et al., 2004). Notably, MCs can also contribute to endometriosis-related chronic and neuropathic pain (Anaf et al., 2006). It is thought that this might result from cross-talk between MCs and neurons. Thus, MCs mediators, including histamine (Mizumura et al., 2000; Herbert et al., 2001), tumour necrosis factor (TNF)-\(\alpha\) (Sorkin et al., 1997) tryptase (Vergnolle et al., 2001), PGs (Syriatowicz et al., 1999), serotonin (Dines and Powell, 1997), IL-1 (Sommern et al., 1999) and nerve growth factor (NGF) (Shu and Mendell, 1999; Skaper et al., 2001; Zhang and Nicol, 2004) can sensitize and/or activate primary nociceptive neurons, while neurotransmitters, such as substance P or NGF, can trigger MC degranulation and promote MC chemotaxis (Mazurek et al., 1986; Ebertz et al., 1987; Matsuda et al., 1989). Activation of MCs may also contribute indirectly to the development of pain by the recruitment of neutrophils and macrophages, which also release algic mediators (Liu et al., 2000; Perkins and Tracey, 2000). In deeply infiltrating endometriosis, a particularly painful form of the disease, MC numbers are markedly increased in the endometriotic deposits and are in close proximity to nerves, with evidence of degranulating MCs even within the perineural structures (Anaf et al., 2006). As a consequence of these studies, MCs have been proposed as a novel therapeutic target for the suppression of pain associated with endometriosis. In animal models, inhibition of MC degranulation by sodium cromoglycate can suppress the development of hyperalgesia. Treatment with histamine receptor antagonists can also suppress the development of hyperalgesia after nerve injury, and alleviate it once established (Zuo et al., 2003). An alternative approach could involve the direct suppression of MC activation through inhibition of Janus kinase 3, which is required for the full expression of IgE receptor-mediated MC inflammatory sequelae (D’Cruz and Uckun, 2007). Most recently, a combination of palmitoylethanolamide and polydatin, which are known to regulate MC activation, have been used to improve pain control in a pilot study of women with endometriosis (Indraccolo and Barbieri, 2010).

**MC involvement in implantation and placental development**

Cells and cytokines of the immune system are known to play a key role in the early stages of implantation (Dekel et al., 2010). Despite studies suggesting that histamine (produced by MCs) is released in response to blastocyst implantation in the uterus (Cocchiara et al., 1988), experiments carried out in rats and mice indicate that MCs are redundant during blastocyst implantation (Humphrey, 1967; Salamonsen et al., 1996). For example, rats treated with a MC stabilizing compound (FPL 55618) before and during the first 7 days of pregnancy, had equivalent frequency of ovulation and implantation sites in comparison with untreated controls (Salamonsen et al., 1996). Moreover, mice deficient in MCs (W/W\(^\text{6}\)) or W/W\(^\text{v}\) (MC expression decreased compared with WKY positive) are infertile despite being infertile owing to a germ cell defect, are able to successfully implant transplanted 3.5-day-old wild-type embryos (Wordinger et al., 1986).

Some evidence indicates that MCs and their products, including histamine, may participate in the development of the placenta through regulation of trophoblast invasion and growth (Szkiewicz et al., 1999). In particular, histamine can increase trophoblast expression of \(\alpha \beta \text{3}\) integrin, a key marker of extravillous trophoblasts (\(\alpha 6\beta 4\)-integrin negative and \(\alpha 5\beta 1, \alpha 1\beta 1, \alpha V\beta 3\) integrin positive) (Szewczyk et al., 2006). An alteration in the expression of these adhesion molecules has been associated with impaired placental invasion and pre-eclampsia. In pre-eclampsia, placental histamine levels and MC numbers are elevated (Szewczyk et al., 2008): this supports increased histamine and MC recruitment in pre-eclampsia as compensatory responses to promote trophoblast differentiation towards the
extravillous phenotype. Recent studies suggest that histamine binding to the H1 receptor can promote cell turnover by initiating apoptotic pathways in trophoblastic cells (Pyzlik et al., 2009), however the significance of this finding needs further investigation.

MCs and the myometrium

SCF mRNA has been detected in the cytotrophoblasts, endometrium and myometrium, with evidence that SCF is directly produced by myometrial myocytes (Mori et al., 1997a, b). Consistent with this, MCs are found in close proximity to myometrial smooth muscle cells (Mori et al., 1997a, b). Within the non-pregnant uterus, MCs are found in greatest numbers within the inner half of the myometrium, with an equal ratio of MC<sub>TC</sub> and MC<sub>T</sub> in this region (Mori et al., 1997a). In contrast, in the non-pregnant outer myometrium there are fewer MCs, and MC<sub>TC</sub> predominate (Mori et al., 1997a; Garfield et al., 2006). During pregnancy, myometrial MC numbers are increased and the ratio of subtypes is reversed with MC<sub>T</sub> dominating (Garfield et al., 2006).

MC migration

Upon initiation of an inflammatory response, additional MC progenitors are recruited from the circulation to add to the MC pool already present within the tissue. In addition to being important for MC survival, SCF in both its soluble and membrane-bound forms has been shown to be chemotactic for MCs (Meininger et al., 1992; Nilsson et al., 1994, 1998). In vitro studies using both human umbilical cord-derived MCs and HMC-1 cells (a human MC line) showed that the chemotactic response to SCF was dose-dependent and also required binding of the cells to extracellular matrix proteins (Nilsson et al., 1994).

In addition to SCF, it is thought that chemokines and their receptors play key roles in regulating MC migration. Chemokines are small secreted proteins that attract immune cells into tissues and help to determine their microanatomical localization within the tissue. In humans, 44 chemokines have been identified and are classified into four groups based on variations of a conserved cysteine motif i.e. C, CC, CXC and CX<sub>3</sub>C chemokines. Chemokines stimulate migration by signalling through heptahelical G-protein-coupled chemokine receptors on leukocyte surfaces, and 18 such receptors have been identified. Despite the confusing literature, many studies, as summarized in Table 1, have attempted to identify the chemokine receptors present on MCs and their progenitors (Scott and Bradding, 2005). It appears that a broad spectrum of chemokine receptors is found on MC progenitors in bone marrow and blood (Fig. 1a and b), and that within different tissues of the same individual, mature MCs present varying chemokine receptor profiles. The precise role of each chemokine receptor in MC biology remains to be determined. To our knowledge, there is currently no evidence that these receptors aid the movement of MC progenitors from the bone marrow into the circulation. However, CXCR2, but not CCR2, CCR3 or CCR5, appears to be required for the constitutive movement of MC progenitors from the blood into the small intestine in mice, a process which also requires α4β7 interaction with MadCAM-1 (Abonia et al., 2005). It seems reasonable to expect that other chemokine receptors will regulate MC progenitor recruitment into other tissues, particularly during inflammation. Interestingly, chemokine receptor activation can also control MC degranulation and it has been shown that CCR1 provides a key co-activation signal for the release of mediators from MCs during allergic responses in the conjunctiva (Toda et al., 2004; Miyazaki et al., 2005).

In addition to expressing receptors for chemokines, activated MCs are also a rich source of pro-inflammatory chemokines which may be critical for initiating and maintaining leukocyte recruitment during the inflammatory cascade. For example, bone marrow-derived MCs can produce CCL22, CCL17 and CCL2 in response to stimulation with SCF and/or IgE (Oliveira and Lukacs, 2001). CCL22 and CCL17, acting through CCR4, contribute to the recruitment of Th2 cells during Th2-mediated inflammatory conditions, such as airway hypersensitivity (Yamashita and Kuroda, 2002). CCL2 on the other hand, which operates through CCR2, is chemotactic for inflammatory monocytes and many other leukocytes, and has also been shown to be a chemoattractant for both non-activated and IgE-activated MCs in vitro (Taub et al., 1995). Thus, CCL2 could drive a positive feedback loop where MC activation leads to recruitment of MCs from within the tissue, and possibly MC progenitors from the blood, which in turn leads to further production of MC-attracting chemokines.

The ability of MCs and their progenitors to respond to chemokines may be important in the biology of these cells during pregnancy and labour. Chemokines are abundantly produced by the decidua and the placenta throughout pregnancy (Red-Horse et al., 2004; Hannan et al., 2006) and are released in response to the myometrial smooth muscle cell stretch that occurs as the uterus grows to accommodate the growing fetus (Loudon et al., 2004; Shynlova et al., 2008). Moreover, a specific chemokine profile is associated with the onset of labour, as revealed by transcriptomic comparisons of myometrial and cervical biopsies from women in spontaneous labour at term versus those not in labour at term (Bollapragada et al., 2009) and supported by other studies (Wood et al., 1999; Young et al., 2002; Osman et al., 2003; Bollapragada et al., 2009; Shynlova et al., 2009). This is thought to drive the leukocyte influx associated with labour onset in humans and in mice, and it is notable that CXCL3, CXCL5, CXCL8 (IL-8) and CCL2, all robustly up-regulated during labour, are ligands for receptors (CXCR1, CXCR2 and CCR2) known to be present on various MC populations and their progenitors (Ochi et al., 1999; Oliveira and Lukacs, 2001; Brightling et al., 2005a). Significantly, since SCF is produced from the myometrium (Mori et al., 1997a, b), MCs and MC progenitors recruited in response to chemokine production would encounter an environment that would support their survival and differentiation. These ideas are summarized in Fig. 1, which illustrates a working hypothesis for the role of chemokines in MC and MC progenitor recruitment within the human uterus during pregnancy and labour.

MC activation and degranulation

MCs are the primary cells responsible for mediating the allergic response, primarily by responding to antigen-cross-linked IgE. However, MCs are also now recognized as key sentinels within the immune system, initiating and shaping inflammatory responses through their ability to rapidly respond to IgE-independent innate immune stimuli (Galli et al., 1999, 2005; Marshall, 2004; Stelekati et al., 2005).
Table I  Human mast cell chemokine receptors.

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Receptor associated ligands</th>
<th>Mast cell type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR1</td>
<td>CCL5 (RANTES); CCL3 (MIP-1α); CCL7 (MCP-3)</td>
<td>Bone marrow-derived</td>
<td>Oliveira and Lukacs (2001); Fifadara et al. (2009)</td>
</tr>
<tr>
<td>CCR2</td>
<td>CCL2 (MCP-1); CCL7 (MCP-3); CCL8 (MCP-2); CCL13 (MCP-4)</td>
<td>Bone marrow-derived</td>
<td>Oliveira and Lukacs (2001)</td>
</tr>
<tr>
<td>CCR3</td>
<td>CCL5 (RANTES); CCL13 (MCP-4); CCL11 (Eotaxin); CCL28 (MEC)</td>
<td>Bone marrow-derived</td>
<td>Oliveira and Lukacs (2001); Collington et al. (2010)</td>
</tr>
<tr>
<td>CCR4</td>
<td>CCL17 (TARC); CCL22 (MDC)</td>
<td>Lung; Bone marrow-derived</td>
<td>Amin et al. (2005); Brightling et al. (2005a)</td>
</tr>
<tr>
<td>CCR5</td>
<td>CCL3 (MIP-1α); CCL4 (MIP-1β); CCL5 (RANTES)</td>
<td>Bone marrow-derived</td>
<td>Amin et al. (2005); Brightling et al. (2005a)</td>
</tr>
<tr>
<td>CCR7</td>
<td>CCL19 (ELC/MIP-3β); CCL21 (SLC)</td>
<td>Bone-marrow-derived</td>
<td>Ochi et al. (1999)</td>
</tr>
<tr>
<td>CXCR1</td>
<td>CXCL6 (GCP-2); CXCL8 (IL-8)</td>
<td>HMC-1 cell line</td>
<td>Lippert et al. (1998, 2004)</td>
</tr>
<tr>
<td>CXCR2</td>
<td>CXCL1 (Gro-α); CXCL2 (Gro-β); CXCL3 (Gro-γ); CXCL5 (ENA78); CXCL7 (NAP-2); CXCL8 (IL-8)</td>
<td>In vitro cord blood derived</td>
<td>Inamura et al. (2002); Brightling et al. (2005a)</td>
</tr>
<tr>
<td>CXCR3</td>
<td>CXCL9 (MIG); CXCL10 (IP-10); CXCL11 (TAC)</td>
<td>Lung</td>
<td>Ochi et al. (1999)</td>
</tr>
<tr>
<td>CXCR4</td>
<td>CXCL12 (SDF-1)</td>
<td>In vitro cord blood derived; HMC-1 cell line; Bone marrow-derived</td>
<td>Brightling et al. (2005a, b)</td>
</tr>
<tr>
<td>CXCR6</td>
<td>CXCL16</td>
<td>Bone marrow-derived; Lung</td>
<td>Ochi et al. (1999); Juremalm et al. (2000, 2002); Brightling et al. (2005a); Brightling et al. (2005a)</td>
</tr>
</tbody>
</table>

IL, interleukin.
The most notable and widely studied consequence of MC activation is degranulation (Fig. 2), which leads to the release of a spectrum of mediators (Hogan and Schwartz, 1997) some of which are preformed and stored in cytoplasmic granules, while others are synthesized de novo. Preformed mediators within MCs include histamine, serotonin, heparin, tryptase and chymase, while PGs, leukotrienes, cytokines and chemokines [e.g. CXCL8 (IL-8)] are synthesized de novo as the MC is stimulated and activated. One important pro-inflammatory mediator is TNF-α, which is found within MC granules and released on MC activation (Gordon and Galli, 1990, 1991). MC derived TNF-α has been shown to further promote inflammation by enhancing T cell activation (Nakae et al., 2005, 2006) and inducing cytokine release from a variety of cell types, and is thought to play an important role in lymph node ‘shutdown’ which temporarily prevents lymphocyte departure from draining lymph nodes to enhance adaptive immune responses (McLachlan et al., 2003).

The mediators and cytokines released by MCs influence a variety of biological processes. They act to encourage inflammation by promoting the infiltration of other immune cells, including T cells, neutrophils and monocytes, as well as stimulating the contraction of smooth muscle cells and promoting angiogenesis (Norrby, 2002). The ability to modulate these processes is of particular relevance to pregnancy and labour. For example, MC mediators are thought to contribute to angiogenesis within the cervix during pregnancy, and so contribute to the process of cervical ripening prior to labour onset (Sennstrom et al., 1997).

MCs mediators and the myometrium

One way in which MCs may influence the biology of the myometrium is through the regulation of smooth muscle cell proliferation and function. Significant evidence for an effect of MCs on smooth muscle has come from studies on asthma (Fanta, 2009). Pathologically, asthma is
characterized by a mucosal inflammatory infiltrate and remodelling of the airway architecture, accompanied by airway smooth muscle (ASM) hyperplasia and hypertrophy (Bradding, 2007). MCs infiltrate the airways during asthma, being particularly abundant within the bronchial smooth muscle bundles and airway epithelium (Brightling et al., 2002; Bradding et al., 2006). The MC mediators tryptase (Brown et al., 1995), platelet-derived growth factor (Hirst et al., 1996) and TNF-α (Amrani et al., 1996) have been shown in vitro culture models to induce ASM proliferation, and MCs can influence ASM contractile properties, with ASM/MCs co-culture inducing a contractile phenotype in ASM cells (Margulis et al., 2009). In addition, contact with ASM promotes MC differentiation, and promotes the survival of lung-derived MCs and HMC-1 cells (Hollins et al., 2008). Clearly, there is significant cross-talk between MCs and ASM in asthma that likely contributes to the pathology of this disease.

By extension, the physical relationship between myometrial smooth muscle cells and MCs has led to the hypothesis that MC mediators modify the actions of the myometrial layer, as illustrated in Fig. 1c. Numerous studies have investigated the impact of MC mediators on myometrial contractility (summarized in Table 2), and interestingly, pelvic pain akin to uterine contraction is associated with catastrophic MC degranulation during anaphylaxis (Rudick et al., 2008). The impact of MC degranulation on contraction has been studied using compound 48/80 and cromolyn sodium (sodium cromoglycate), which activate or inhibit MC degranulation, respectively (Bytautiene et al., 2004a). Compound 48/80 induced contraction of myometrium in an organ bath, while pretreatment with cromolyn sodium completely abolished the effect of compound 48/80 (Bytautiene et al., 2004a). Moreover, in a mouse model of pharmacologically induced labour, MCs were shown to be necessary for the uterotonic effect of the drug etohodin, whose effects include induction of MC degranulation (Rudolph et al., 1997).

One key MC mediator is histamine, a biogenic amine with roles in gut physiology (Hirschowitz, 1985), neurotransmission (Marshall, 1981), airway contractility and acute allergic reactions (Maintz and Novak, 2007). Four histamine receptors (H1, H2, H3 and H4) have

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**Figure 2** MC activation. MC activation can be classified into mechanisms where the MC directly interacts with the antigen (Ag) and mechanisms where MCs become activated indirectly by antibodies (Ab) or molecules which have been released from other cells that have bound antigen. In addition, these mechanisms can be classified with respect to their dependence on the antibody IgE. Ultimately, as a result of activation, MCs degranulate and release a range of mediators which perpetuate the immune response in a number of ways.
been identified to date. The H1 receptor is found on smooth muscle cells (Mitsuchashi and Payan, 1989), endothelium (Carrier et al., 1984) and within the central nervous system (Simons et al., 1995), and upon binding to this receptor, histamine can induce vascular smooth muscle relaxation and ASM contraction. The H2 receptor is primarily involved in regulation of gastric acid secretion within the gut (Pattichis and Menzies, 1993), and upon binding to this receptor, histamine can induce vascular smooth muscle relaxation and ASM contraction. The H2 receptor is primarily involved in regulation of gastric acid secretion within the gut (Pattichis and Menzies, 1993), while binding to the H3 receptor results in changes to neurotransmitter release (Chazot and Hänn, 2001). The H4 receptor plays a role in the chemotaxis of immune cells, including MCs (O’Reilly et al., 2002; Hofstra et al., 2003), and is also found on neurons (Connelly et al., 2009).

Histamine is thought to modulate the progression of labour, through both direct and indirect mechanisms (Mainzt et al., 2008). Histamine acts directly as an oxytocic agent, and studies have consistently shown that histamine induces contraction of myometrial strips in organ bath experiments (Cruz et al., 1989; Rudolph et al., 1993; Bytautiené et al., 2003; Garfield et al., 2006; Willets et al., 2008) by signalling through the H1 receptor (Bytautiené et al., 2003; Willets et al., 2008). Histamine acts indirectly to modulate myometrial contractility through its ability to induce PG production, in particular PGF2a from decidual cells (Schrey et al., 1995), which can evoke myometrial contractions (Rudolph et al., 1993; Smith, 2007). PGs are key players in labour (Challis et al., 1997; Olson, 2003; Khan et al., 2008) and have been reported to contribute to many processes necessary for labour, including stimulation of extracellular matrix proteins to allow fetal membrane rupture (McLaren et al., 2000) and cervical ripening (Keirse et al., 1983; Keirse, 1993). PGD2, PGE2 and PGF2a are all produced and released by MCs themselves (Chock and Schmauder-chock, 1988; Kawata et al., 1995), although their contribution to myometrial contractility has not been determined and many cells within the uterus, particularly in the fetal membranes and decidua, are rich sources of PGs ( Olson et al., 1995).

The ability of tryptase and chymase to directly modulate myometrial contractility has not been studied directly. However, high levels of tryptase have been associated with the induction of spontaneous miscarriages through its ability to induce inflammation (Pladhappan et al., 2003). MC tryptases may also be involved in the remodelling of the uterus post-partum because they are known to promote fibrinogenesis (Schwartz et al., 1985) and tissue remodelling (Saito, 2005; Caughey, 2007). Little is known about the effect of chymase on the myometrium during pregnancy and labour, although overproduction has been linked with severe pre-eclampsia (Mitani et al., 2002).

Serotonin, another MC mediator, is also considered to be a uterotonic agent and has been shown to induce myometrial contraction in a concentration-dependent manner (Cruz et al., 1989; Rudolph et al., 1993). Moreover, oxytocin administered during the labour process to aid contractions can inhibit serotonin uptake by uterine MCs in an estrogen-dependent manner (Rudolph et al., 1998) thereby acting to increase the bioavailability of this substance and further influence myometrial smooth muscle contractility.
Influence of female sex hormones on MC mediator release

Natural killer cells, macrophages, dendritic cells, T and B cells have all been identified within the uterus and are known to be sensitive and reactive to the presence of the female sex hormones (Klein, 2000a, b; Verdel et al., 2001; Beagley and Gockel, 2003). MCs are also sensitive and reactive to the presence of the female sex hormones, the levels of which are critical throughout pregnancy and during the initiation of parturition. Progesterone is necessary for the maintenance of pregnancy and plays a key role in maintaining cervical integrity prior to labour induction. Progesterone can prevent the migration of MCs in response to chemokines and down-regulate surface chemokine receptor expression (Belot et al., 2007). In addition, MC function can be altered by the presence of high concentrations of progesterone. For example, progesterone inhibits the secretion of histamine from MCs (Vasiadi et al., 2006). Notably, these observations would suggest that MCs present within the uterus during pregnancy are quiescent and inhibited by high levels of progesterone, and also that recruitment of MC progenitors from the circulation may be limited.

By term, functional responsiveness to estrogens levels predominates over progesterone. Estrogens are important for parturition as they increase the number of oxytocin receptors on myometrial cells and promote the formation of gap junctions between cells to allow a co-ordinated contraction. MCs express the high-affinity estrogen receptor (Pang et al., 1995) and studies have shown that estrogens augment their activities: in the presence of high levels of estrogens, MC responses to compound 48/80 are increased, leading to more substantial degranulation and release of histamine and serotonin (Vlagicofis et al., 1992; Kim et al., 2001; Zaitsu et al., 2007). This would suggest that as the uterus prepares for labour, the hormone environment supports the release of mediators from MCs, which further influences the contractility of myometrial smooth muscle cells.

Allergies and pregnancy

Given the increasing evidence that MCs can alter trophoblast invasion and myometrial contractility, is there epidemiological evidence linking MCs to adverse pregnancy outcomes? At present the prevalence of allergies, including allergic rhinitis, hayfever, eczema, food allergies and urticaria, is rising (Gupta et al., 2007). For example, allergic rhinitis currently affects ∼20–30% of women of childbearing age (Keles, 2004) and eczema affects 16% of women in the UK (Weatherhead et al., 2007). Despite this, there is little evidence to suggest any adverse pregnancy outcomes in women with these milder allergic conditions, suggesting that myometrial MCs are sufficiently regulated to prevent adverse outcomes. In contrast, in severe allergy, as in the recent report of shellfish allergy, MC activation was sufficient to induce preterm labour: notably, anaphylaxis treatment, including use of antihistamines and steroids, ceased the uterine contractions (Romero et al., 2010). Further studies are required to determine the prevalence of allergy-induced preterm labour.

Interestingly, animal studies have also indicated that myometrial function may be altered as a result of allergen-induced MC activation. For example, although guinea pigs sensitized during pregnancy to ovalbumin had a pregnancy that was indistinguishable from non-sensitized controls, the length of pregnancy was significantly shortened (by 4–5 days) if sensitized animals were subsequently challenged intraperitoneally with ovalbumin, a protocol that induces anaphylaxis in guinea pigs (Bytautiene et al., 2004b). Moreover, treatment with the H1 receptor antagonist ketotifen prevented this allergy-induced preterm labour (Bytautiene et al., 2004b).

MCs and male fertility

Although our discussions have focused exclusively on the role of MCs in females, there is some evidence that MCs may also influence male fertility. MCs are present in normal human testes and seminal fluid (Allam et al., 2009) and, under normal circumstances, can be detected in the interstitium and the lamina propria of the testes, with MCg being the dominant subtype (Yamanaka et al., 2000). Interestingly, however, there is an increase in testicular MCs in infertile men (Maseki et al., 1981; Nagai et al., 1992; Yamanaka et al., 2000). In addition, in obstructive azoospermia, idiopathic azoospermia and varicocele, the testicular MC phenotype changes, and the MCg subtype dominates (Yamanaka et al., 2000). This selective expansion of the MCg population may influence ongoing dysfunctional spermatogenesis and, through MC activation of fibroblasts and promotion of collagen synthesis, could contribute to testicular fibrosis. Indeed, the number of tubules with sclerosis and fibrosis is increased in association with MC numbers (Yamanaka et al., 2000). In addition to these direct effects on testicular tissue, MC-derived tryptase can also impair sperm motility (Cincik and Sezen, 2003), while reversal of this inhibitory effect is feasible by sperm washing or anti-tryptase antibody (Weidinger et al., 2003). Although the direct effect of other MC mediators on sperm remains to be determined, histamine similarly impairs sperm motility, with reversal by the H1 receptor antagonist ketotifen (Oliva and Multignier, 2006).

Conclusions

MCs are found in a diverse range of tissues and have the ability to adapt their function to the microenvironment. This results in the development of tissue-specific MC phenotypes that are tailored to respond appropriately to the needs of the tissue. Studies into MC function have tended to concentrate on their ‘classical’ roles during allergic responses and on their function and phenotype within the skin and the lung. However, MCs are found in many other tissues, including the endometrium, the uterus and the testes, and it is clear that their role extends far beyond allergy. Uterine MCs are found in close proximity to the smooth muscle of the myometrium, and there is clear evidence that MCs and their mediators can influence smooth muscle cell behaviour. Moreover, a body of evidence supports the hypothesis that MCs and their mediators can promote inflammation and, as such, it is possible that they contribute to the onset of labour. However, the importance of MCs and their mediators during implantation, menstruation and pregnancy, and their contribution to the initiation and progress of labour, is not yet fully understood. More detailed studies are required to (i) determine how MC number and phenotype changes in the human uterus during pregnancy and labour, (ii) reveal how these MCs are activated and regulated, and characterize in detail the mediators they release, (iii) define the impact of these mediators on tissue function and (iv) determine their indispensable roles during reproduction, employing, among other tools, MC-deficient mice. It
may then be possible to determine whether modulation of MC function could be used to influence fertility, labour and the development of disorders of the female reproductive system.

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References


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