Human Reproduction Update 1998, Vol. 4, No. 5 pp. 496–502

Myometrial zonal differentiation and uterine junctional zone hyperplasia in the non-pregnant uterus

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Human non-gravid myometrium differentiates in response to ovarian sex steroids into a subendometrial layer or junctional zone and an outer myometrial layer. Compared to the outer myometrial layer, the junctional zone myocytes are characterized by higher cellular density and lower cytoplasmic–nuclear ratio. These structural differences allow in-vivo visualization of the myometrial zonal anatomy by T2-weighted magnetic resonance (MR) imaging. The human myometrium is also functionally polarized. Video-vaginosonography studies have shown that propagated myometrial contractions in the non-pregnant uterus originate only from the junctional zone and that the frequency and orientation of these contraction waves are dependent on the phase of the menstrual cycle. The mechanisms underlying zonal myometrial differentiation are not known, but growing evidence suggests that ovarian hormone action may be mediated through cytokines and uterotonins locally released by the basal endometrial layer and endometrio–myometrial T-lymphocytes. Irregular thickening of the junctional zone due to inordinate proliferation of the inner myometrium, junctional zone hyperplasia, is a common MR finding in women suffering from menstrual dysfunction. Preliminary data suggest that junctional zone hyperplasia is further characterized by loss of normal inner myometrial function. Although irregular thickening of the junctional zone has been associated with diffuse uterine adenomyosis, the precise relationship between subendometrial smooth muscle proliferation and myometrial invasion by endometrial glands and stroma remains to be established.

Key words: MR imaging/myometrial zonal anatomy/ smooth muscle differentiation

Introduction

Magnetic resonance (MR), video-vaginosonography and immunohistochemical studies have revealed that the inner and outer layers of the human non-gravid myometrium are distinctly different during the reproductive years. The aim of this review is to address the potential mechanisms underlying myometrial zonal differentiation. In addition, we reassess if disruption and hyperplasia of the inner myometrial layer are predictive for the presence of diffuse adenomyosis.

Structural myometrial zonal differentiation in response to ovarian sex steroids

Myometrial zonal anatomy *in vivo* was first described by Hricak *et al.* in 1983 using MR imaging. In women of reproductive age, three layers can be distinguished in the myometrium on T2-weighted MR images: surrounding the high signal-intensity endometrial stripe there is a low signal-intensity junctional zone followed by an outer intermediate signal-intensity zone and a thin low signal-intensity subserosal zone (Figure 1A and B). The contrast in signal intensity

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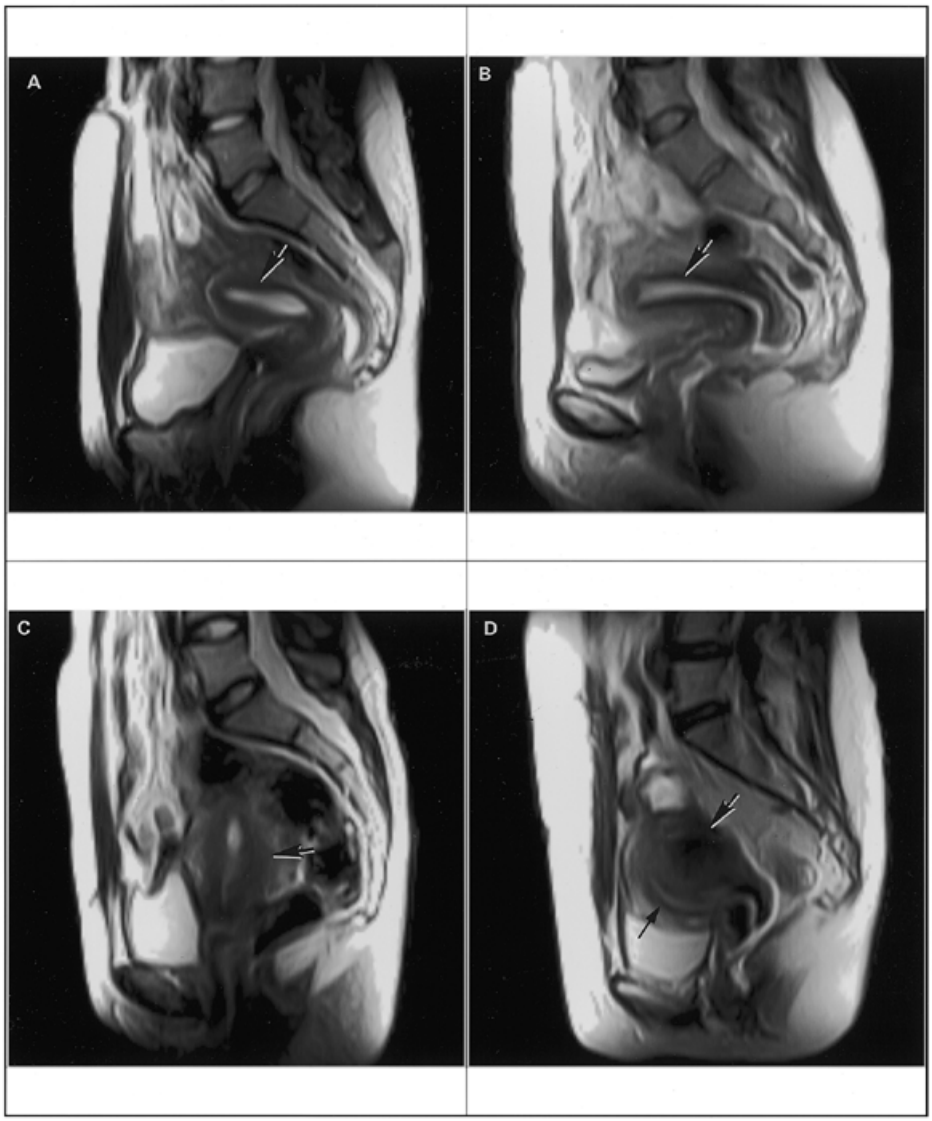

Figure 1. T2-weighted (2500/80 ms [TR/TE]) spin echo midline sagittal sections of uteri of women in reproductive years. (**A** and **B**) Normal myometrial zonal anatomy: although the low signal-intensity junctional zone (arrow) is regular in both cases, it appears distinctly thinner in **B**. The clinical significance, if any, of a very thin junctional zone (<3 mm) in the absence of exogenous steroid treatment is unknown. (**C**) Junctional zone hyperplasia: the inner myometrial zone is not only irregular but there is also diffuse thickening of this layer affecting predominately the posterior myometrial wall (arrow). (**D**) Large focal adenomyoma affecting the entire posterior uterine wall (fat arrow). Note that the junctional zone (thin arrow) is thin and regular, indicating that diffuse and focal adenomyosis may have distinctly different aetiologies.

between the outer myometrium and junctional zone is a striking feature on T2-weighted scans, and the reason for this difference in signal intensity has been the cause of considerable debate. Initially, it was postulated that the zonal polarity on MR images resulted from a higher vascular perfusion rate in the inner third of the myometrium (Lee *et al*., 1985) who demonstrated that the T1 and T2 values of unfixed myometrium were stable for at least 6 h after hysterectomy. Using image analysis of nuclear-stained myometrial sections, the same group demonstrated that, in comparison with the outer myometrium, the junctional zone is characterized by a threefold increase in nuclear area and decreased extracellular matrix per unit volume. The increased nuclear area reflects both a higher smooth muscle density and an increased nucleo-cytoplasmic ratio of the myocytes (Scoutt *et al.*, 1991). This latter observation is important, as it demonstrates that there are not only architectural differences but also cellular differences between myocytes in the junctional zone and outer myometrium.

Uterine zonal differentiation is dependent on gonadal hormones (Demas *et al*, 1985; McCarthy *et al.*, 1986; Andreyko *et al.*, 1988). In premenarchal girls and

postmenopausal women, the zonal anatomy is often indistinct, with a comparatively low signal intensity from the myometrium. Ovarian suppression with gonadotrophin-releasing hormone analogues leads to an MR appearance of the uterus mimicking that of postmenopausal women; while hormone replacement therapy in postmenopausal women results in re-appearance of myometrial zonal anatomy (Demas *et al*., 1985). Further evidence for hormonal responsiveness of the junctional zone is provided by the work of Wiczyk *et al.* (1988), who performed MR scans on five volunteers with normal ovulatory cycles on days 4, 8, 12, 16, 20 and 24 of the cycle. They demonstrated junctional zone thickness changes throughout the menstrual cycle in conjunction with endometrial thickness changes. The endometrium increased from 5.8 ± 1.1 mm on day 4 to a mean peak of 10.3 ± 1.7 mm on day 24, with the greatest growth occurring from days 8 to 16. Although the junctional zone showed substantially less growth, the pattern of growth was similar, mainly occurring between days 8 and 16 (from 5.1 ± 0.7 to 6.7 ± 0.7 mm).

The myometrial zonal anatomy changes profoundly during pregnancy. Focal disruption of the junctional zone occurs in early pregnancy (Turnbull *et al*., 1995). This has been observed as early as 7 days after ovulation in a patient on whom serial MR scans were performed fortuitously during a conception cycle. Interestingly, focal disruption of the junctional zone does not appear to occur with ectopic pregnancies, suggesting that local factors released at the implantation site mediate this effect (Barton *et al.*, 1993). During pregnancy, the junctional zone increases in signal intensity and the zonal differences become indistinct (Willms *et al.*, 1995). The normal zonal anatomy gradually reappears within 6 months of delivery (Willms *et al.*, 1995).

Functional polarity of the myometrium

Vaginal video-sonography is a new imaging technique which allows semiquantitative assessment of myometrial contractility waves (de Vries *et al*., 1990; Lyons *et al.*, 1991; Kunz *et al*., 1996). Several groups have reported that in the non-gravid uterus myometrial peristalsis emanates only from the inner myometrium and that the orientation, amplitude and frequency of the contraction waves correlate with the phase of the menstrual cycle. In the follicular and periovulatory phases, cervico-fundal subendometrial contractions can be seen, the amplitude and frequency of which increase notably toward ovulation. Short, asymmetrical myometrial waves are present during the luteal phase, but during menstruation propagated fundo-cervical subendometrial contractions waves are noted (de Vries *et al*., 1990; Lyons *et al.*, 1991; Chalubinski *et al*, 1993; Kunz *et al*., 1996).

In an elegant study, Kunz *et al*. (1996) used hysteroscintigraphy to demonstrate that rapid sperm transport through the female genital tract in the pre-ovulatory phase is provided by these subendometrial cervico-fundal contraction waves. Others have postulated that the asymmetrical myometrial peristalsis during the luteal phase serves to maintain the developing blastocyst within the uterine fundus. The role of fundo-cervical contractions during menstruation has not yet been elucidated, but one report noted hyperperistalsis in a woman suffering from excessive menstrual loss (Chalubinski *et al.*, 1993).

Junctional zone dysfunction has been implicated in the pathogenesis of pelvic endometriosis and subfertility (Salamanca and Beltrán, 1995; Leyendecker *et al*., 1996; Ijland *et al*., 1997). A recent study found marked hyperperistalsis during the early and mid-follicular phase and dysperistalsis during the late follicular phase in women with endometriosis. Hysterosalpingoscintigraphy studies in these women indicated that the hyperperistalsis was associated with a marked increase in the transport of inert particles from the vaginal depot to the peritoneal cavity, while the peri-ovulatory dysperistalsis resulted in a significant decrease of uterine transport capacity in comparison with healthy controls (Leyendecker *et al.*, 1996).

In-vitro studies also have confirmed the functional polarity of the myometrium (Daels, 1974). In contrast to the in-vivo observation, myometrial strips taken from the outer myometrium show spontaneous strong regular contractions which can be amplified by adrenaline or, to a lesser extent, by oxytocin. Muscle strips from the inner myometrium, however, display very few spontaneous contractions and are largely unresponsive to adrenaline and oxytocin stimulation (Daels, 1974).

Mechanism(s) controlling myometrial structural and functional polarity

In non-pregnant myocytes, oestrogen receptor (ER) expression is maximum in the late proliferative phase and declines sharply in the early secretory phase. An increase in ER immunoreactivity has been reported in the late secretory phase. Following an initial rise in progesterone receptor (PR) immunoreactivity in the proliferative phase, there is, by contrast, no significant change in PR expression throughout the menstrual cycle (Snijder *et al*., 1992). Until recently it was widely accepted that there are no zonal differences in steroid receptor expression in the myometrium, but a recent study has challenged this. Richards and Tiltman (1995) examined 20 hysterectomy specimens

obtained in the proliferative phase of the cycle and demonstrated that the subendometrial myometrium expresses significantly higher concentrations of ER, as measured by radioimmunoassay and by immunocytochemistry, than the outer myometrium.

The mechanism underpinning elevated ER expression in the junctional zone is not well understood. There is, however, increasing evidence for a complex interaction between gonadal steroids and locally expressed cytokines in the target tissue. For instance, interferon-gamma (IFN-γ) has been shown to induce a 30–50% increase in the ER content of ZR75–1 cells, an oestrogen-responsive breast cancer cell line (Solary *et al.*, 1991). In the uterus, IFN-γ is predominately produced by activated CD3-positive T cells which are found in characteristic lymphoid aggregates in the endometrio–myometrial junction (Stewart *et al*., 1992; Tabibzadeh *et al*., 1988, 1993). Recently, it has become clear that these T cells play an important role in polarizing the endometrial epithelium. The phenotypic responsiveness to sex steroids and the proliferative activity of the endometrial epithelium are maximal in the functional layer and gradually diminish towards the basalis. The low proliferative activity in the basal layer is associated with a markedly increased human leukocyte antigen (HLA-DR) expression by the glandular epithelium in this layer (Tabibzadeh *et al.*, 1986a,b; 1988). Tabibzadeh *et al.* (1993) elegantly demonstrated *in vitro* that IFN-γ released by activated T cells can induce high HLA-DR expression in endometrial epithelial cells and dramatically inhibit their proliferative activity. IFN-γ also attenuates the action of certain cytokines, such as transforming growth factor (TGF)-β (Arici *et al.*, 1995), which have been implicated in myocyte proliferation (Ishikawa *et al*., 1990) and endometrial stromal cell function (Arici *et al.*, 1996; Casey *et al.*, 1996). Although the role of IFN-γ and other cytokines released by the basal endometrium in the induction of a specific inner myometrial microenvironment remains speculative, the thickness of the junctional zone and its gradual blending with the outer myometrium is in keeping with an effect of locally produced factor(s) which diminishes with increasing distance from the site of production.

The mechanisms that govern the cycle-dependent contractions of the inner myometrium in the non-gravid uterus are not understood. It appears likely that the symmetrical, high amplitude propagated contraction waves in the late follicular phase require electrical and mechanical coupling of myocytes in the junctional zone. Gap junctions are intercellular communication channels and their expression is required for co-ordinated synchronous myometrial contractions. Structurally, each channel or connexon is composed of a hexamer of connexin proteins (Lowenstein, 1987). In the myometrium, the major gap junction protein is connexin-43 (Cx-43) and its expression is thought to be mediated through binding of the activation protein-1 (AP-1), a composite transcription factor consisting of c-Jun and c-Fos, to AP-1 binding sites in 5'-flanking promoter region of the Cx-43 gene (Piersanti *et al.*, 1995; Geimonen *et al*., 1996). In uterine tissues, ER activation not only results in elevated c-Fos concentrations but also results in stabilization of the AP-1 complex and hence increased promoter activity (Webb *et al*., 1995). The higher level of ER expression in the junctional zone in the proliferative phase may thus represent a preferential target for oestrogen regulation of Cx-43. In contrast, progesterone (Zhao *et al*., 1996), human chorionic gonadotrophin (Ambrus and Rao, 1994) and activators of the protein kinase A pathway (Cole and Garfield, 1986; Sakai *et al*., 1992) are thought to inhibit Cx-43 expression in myometrium. Down-regulation of Cx-43 expression could explain why the luteal phase is characterized by short, asymmetrical peristalsis. Differential zonal regulation of Cx-43 has been demonstrated in the bovine myometrium (Douall-Bell *et al*., 1995) but, to the best of our knowledge, no studies have as yet addressed the regulation of the connexin proteins in the inner 5 mm of the human non-gravid myometrium.

Although gap junction expression is likely to be essential, mere expression is not sufficient to trigger co-ordinated contractions. Several cytokines have been identified which might modulate myometrial peristalsis. For instance, epidermal growth factor is not only a potent mitogen for myometrial smooth muscle cells but it can also induce uterine contractions *in vitro*, both in intact tissue and in isolated myometrial cells (Gardner and Stancel, 1989). Specific binding sites for endothelins have also been found in the myometrium, and in tissue bath experiments, endothelin-1 markedly increased contractility of myometrial strips, an effect mediated through the endothelin-A receptor (Bacon *et al*., 1995). Interestingly, the greatest density of endothelin binding sites is found on glandular epithelium in the endometrio–myometrial junction, and recent evidence suggests that endothelins in the endometrium can induce release of prostaglandin F2α and further release of endothelins, in a paracrine and autocrine fashion (Bacon *et al*., 1995). These factors are potent uterotonins in non-gravid uteri and may therefore mediate the contractions of the underlying myometrium in a juxtacrine fashion.

The 'ontogeneic hypothesis' of human myometrial zonal differentiation

Early in fetal development the fused paramesonephric ducts are surrounded by a single layer of multi-potent mesenchymal cells (Konishi *et al.*, 1984). Light microscopy of transverse sections of the body of the uterus reveals that by 14 weeks gestation two layers of mesenchymal cells can be recognized. The outer, subserosal layer consists of elongated cells and is more cellular than the inner layer. Ultrastructurally, the mesenchymal cells of the uterus do not display characteristics of smooth muscle cells until 16–18 weeks gestation. The cells in the outer layer then resemble immature smooth muscle cells. At 26 weeks the thickness of the outer layer increases markedly, and by 31 weeks bundles of almost mature smooth muscle cells are present. These microscopic observations resulted in the hypothesis that mesenchymal cells of the outer layer differentiate into immature myofibroblasts and subsequently mature myocytes while those of the inner layer are the precursors of endometrial stromal cells in the adult uterus (Konishi *et al.*, 1984). An alternative explanation, first proposed by Werth and Grusdew in 1898, is that subserosal mesenchymal cells give rise to the outer myometrium while cells from the inner mesenchymal layer are the progenitors of both endometrial stromal cells and inner myometrial smooth muscle cells (Werth and Grusdew, 1898; Daels, 1974). A recent study showed that ER protein expression in the human fetal uterus is confined to a distinct zone at the junction of the inner and outer mesenchymal layers (Glatstein and Yeh, 1995). In contrast to the adult uterus, no oestrogen receptors were found in the outer layer or in glandular epithelial cells. It may be that differentiation of inner mesenchymal cells into smooth muscle cells is mediated by oestrogens and occurs independently from the cytodifferentiation of the outer mesenchymal cells. The observation that in-utero and neonatal exposure of mice to diethylstilbestrol results in severe disorganization of mainly the inner myometrial smooth muscle layer lends support to this conjecture (Ostrander *et al*., 1985). Teleologically, it is intriguing to surmise that human extravillous trophoblast invasion is limited to the decidua and inner myometrium because of the embryonic kinship of these compartments.

Immature myofibroblasts are not only found in the fetal uterus but also at the endometrio–myometrial junction in the adult uterus (Fuji *et al.*, 1989). These cells have more characteristics of smooth muscle cells in the luteal phase and in early pregnancy than in the follicular phase, which suggests that active metaplasia of stromal cells into myocytes (and vice versa) may occur at the endometrio–myometrial interface throughout the menstrual cycle.

Junctional zone hyperplasia

Irregular thickening of the junctional zone (Figure 1C) is a common finding in women suffering from menstrual dysfunction (Mark *et al*., 1987; Togashi *et al*, 1989; deSouza *et al.*, 1995) Pathologically, this has been shown to represent smooth muscle hyperplasia characterized by closely packed smooth muscle fibres that are poorly orientated and less vascular than the smooth muscle of the normal inner myometrium (Togashi *et al*., 1989). There is broad agreement in the literature that the 'normal' junctional zone is regular and measures ≤5 mm (Mark *et al*., 1987; Chernoff and Hricak, 1997) in thickness, but there is no consensus on what constitutes an 'abnormal' junctional zone on MR imaging. Widths between 6 and 12 mm have been proposed (Mark *et al*., 1987; Kang *et al*., 1996; Reinhold *et al*, 1997) The current confusion has arisen because most authors have attempted to correlate the junctional zone thickness *in vivo* with the presence or absence of adenomyotic islets on histology. These studies are likely to be inconclusive, as there is no agreement on the precise definition of superficial adenomyosis (Figure 1D). The Maryland Women's Health Study clearly illustrates this. Analysis of 1114 hysterectomy reports from 15 hospitals and 705 reports signed by 25 pathologists showed that the frequency of diagnosing adenomyosis ranged from 12 to 58% among hospitals and from 10 to 88% among pathologists (Seidman and Kjerulff, 1996). The wide variation could not be explained by differences in patient age, parity or other factors known to correlate with the incidence of adenomyosis (Seidman and Kjerulff, 1996). Furthermore, morphometric and demographic studies have questioned the concept that junctional zone hyperplasia is secondary to the presence of adenomyotic lesions. We reported that extensive thickening of the junctional zone can be present with minimal or no penetration of the mucosa into the myometrium (deSouza *et al*., 1995). Similarly, Reinhold *et al.* (1996) found that only if the junctional zone width is ≥12 mm does it becomes predictive of the presence of endometrial islets at 2.5 mm or deeper below the endometrio–myometrial junction. In addition, endometrial infiltration of the myometrium is commonly patchy and focal, while the myoproliferative changes often affect the entire inner myometrium, as demonstrated by diffuse thickening on MR imaging. Demographically, diffuse adenomyosis is regarded as a disease typical of multiparous women in the perimenopause (Azziz, 1989; Vercellini *et al.*, 1993). In contrast, a recent MR study reported a 54% incidence of junctional zone hyperplasia in subfertile patients suffering from menorrhagia or dysmenorrhoea. The mean age of these patients was only 34 years and 71% were nulliparous (deSouza *et al*., 1995).

Combined, these observations seem to indicate that disruption of the normal inner myometrial architecture due to excessive myocyte proliferation is a cause of diffuse adenomyosis rather than a consequence of it.

Our unpublished observation (Bickerstaff *et al.*) indicates that disruption of the inner myometrial architecture compromises subendometrial peristalsis throughout the menstrual cycle. Symptomatic, subfertile women with junctional zone hyperplasia were found to have a dramatic loss of both propagated fundo-cervical contraction waves during early menstruation and cervico-fundal waves in the late proliferative phase when compared to controls with normal junctional zone appearances on MR imaging. In some patients with junctional zone hyperplasia focal, sometimes convulsive, dysperistalsis was noted during the menstrual phase of the cycle. It appears likely that the hypoperistalsis or convulsive dysperistalsis during menstruation contributes to excessive menstrual loss, while pre-ovulatory hypoperistalsis may affect sperm transport through the uterus and tubes and thus compound the fertility problems observed in these patients.

Conclusion

The junctional zone is structurally and functionally different from the outer myometrium. We postulate that myometrial zonal differentiation is regulated by complex interactions between ovarian sex steroid hormones and cytokines and uterotonins produced locally at the endometrio–myometrial junction. The intimate interaction between the basal endometrial layer and the junctional zone during reproductive years may have its basis in the differentiation of the mesenchymal layers surrounding the fused Müllerian ducts in the fetal uterus. There may be merit in reassessing this hypothesis, first published almost a century ago, using contemporary molecular techniques.

We observed that irregular thickening of the junctional zone is a common finding in women suffering from dysfunctional uterine bleeding. Although the pathogenesis of junctional zone hyperplasia is not known, inordinate inner myocyte proliferation reflects a breakdown in the mechanisms that govern normal myometrial zonal differentiation. It therefore represents a pathological condition regardless of the presence or absence of adenomyotic foci on histology.

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