The role of the peritoneum in the pathogenesis of endometriosis

Vicky J. Young, Jeremy K. Brown, Philippa T.K. Saunders, and Andrew W. Horne*

MRC Centre for Reproductive Health, Queens Medical Research Institute, The University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, UK

Correspondence address. E-mail: andrew.horne@ed.ac.uk

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BACKGROUND: Endometriosis affects 6–10% of women of reproductive age and is associated with chronic pelvic pain, dysmenorrhea, dyspareunia and infertility. Endometriosis is defined by the presence of endometrial tissue outside the uterus, most commonly attached to the pelvic peritoneum. The endometrium in women with endometriosis is reported to be altered and there is increasing evidence that the phenotype of the pelvic peritoneum may also play a role in the establishment and maintenance of the disease. The aim of this review is to discuss the putative role of the pelvic peritoneum in the pathophysiology of peritoneal endometriosis.

METHODS: A review was undertaken of the published literature on (i) the anatomy and physiology of the peritoneum and (ii) the potential roles played by peritoneal cells in the establishment and maintenance of peritoneal endometriosis. The current understanding of the biology of peritoneal endometriosis is summarized and the potential interaction of the peritoneum with ectopic endometrial cells in endometriosis is highlighted.

RESULTS: Several studies indicate that differential expression of peritoneal mesothelial adhesion factors occurs in women with endometriosis, providing potential ectopic endometrial cell attachment sites for the establishment of endometriosis lesions. Changes in the peritoneal mesothelial cell phenotype, including loss of tight junctions, may allow ectopic cells to bind to, or early lesions to invade into, the extracellular matrix. Epithelial-to-mesenchymal transition of peritoneal mesothelial cells may also lead to an increase in lesion invasion and formation of fibrotic tissue in and around the lesion. There is evidence that the peritoneal mesothelium may also play a role in the invasion potential of ectopic cells by production of MMPs increasing local tissue remodelling. Peritoneal immune scavenging function may be lowered in women with endometriosis; for example there is a notable increase in macrophage-derived secretion products in women with endometriosis associated with increases in cell proliferation, cell adhesion and neovascularization.

CONCLUSIONS: The pelvic peritoneum appears to play a key role in the development and maintenance of endometriosis.

Key words: endometriosis / peritoneum / mesothelium / epithelial–mesenchymal transition / adhesion
**Introduction**

Endometriosis is a chronic disease characterized by the presence of endometrial tissue outside the uterus. Its prevalence is estimated to be 6–10% in the general female population and it is associated with chronic pelvic pain, dysmenorrhea, dyspareunia and/or infertility (Giudice, 2010). Surgery provides relief to women with endometriosis-associated pain but symptoms recur in up to 75% of cases within 2 years (Jacobson et al., 2009). The mainstay of medical management is ovarian suppression and includes treating patients with the combined oral contraceptive pill, progestogens or gonadotrophin-releasing hormone agonists (Giudice, 2010). However, progestogens and gonadotrophin-releasing hormone agonists are often only useful for limited periods due to their undesirable side effects, necessitating change or use of additional medication. It is now recognized there are three different types of endometriosis: peritoneal endometriosis, deep infiltrating endometriosis and ovarian endometriomas (cysts). The focus of this review is peritoneal endometriosis.

Our understanding of the aetiology of peritoneal endometriosis is limited. The most widely accepted (and supported) explanation is ‘Sampson’s hypothesis’, which suggests that it occurs due to retrograde menstruation, where endometrial tissue passes through the Fallopian tube into the peritoneal cavity where it implants (Sampson, 1927). However, this mechanism fails to explain why endometriosis only occurs in some women when retrograde menstruation is estimated to occur in 90% of women (Halme et al., 1984). Coelomic metaplasia, another common hypothesis, suggests that epithelium can be transformed into endometrium by metaplasia. However, this theory fails to explain why endometriosis is not common in males, why endometriosis is generally localized to the abdominal cavity and why the disease does not increase with age, as with other metaplastic diseases (Meyer, 1919). The implantation theory suggests the establishment of endometriosis in the peritoneal cavity requires endometrial tissue or cells to complete a process of adhesion, invasion and proliferation. The vast majority of studies to date have focused on differences within the endometrium of endometriosis patients or differences between the eutopic and ectopic endometrium (reviewed by Carvalho et al., 2011). Few studies have investigated whether the pelvic peritoneum plays a role in the establishment and maintenance of endometriosis or whether it is altered in women with endometriosis. The aim of this review is to discuss the putative role of the pelvic peritoneum, and in particular the peritoneal mesothelium, in the pathophysiology of peritoneal endometriosis. We focus on studies that relate to the implantation theory. In this context, a number of potential roles of the peritoneum in the pathophysiology of endometriosis are considered and discussed. These include provision of ectopic endometrial cell attachment sites, facilitation of endometrial cell invasion, potential epithelial–mesenchymal transition, changes to immune cell activation/recruitment and different expression of inflammatory cytokines (Table I).

**Methods**

**Search strategy and selection criteria**

We searched ‘Pubmed’ using the terms ‘peritoneum’ and ‘endometriosis’ for studies published between 1990 and 2012. The initial search identified 741 manuscripts and they were used as the basic material for this report. We initially focused on manuscripts published in the past 5 years, but also extended our remit to commonly referenced and important older publications. We also searched the reference lists of articles identified by this search strategy and selected those we judged as relevant. For a study to be included, it needed to be primarily focused on peritoneal physiology and the role of the peritoneum in the aetiology of endometriosis. Morphological changes and genes identified on this basis were then discussed in the context of endometriosis. Studies that were solely epidemiological in nature were not included.

**Results**

**Normal physiological role of the pelvic peritoneum**

The pelvic peritoneum is the largest serous membrane in the body (\(> 2 \text{ m}^2\)). It is composed of three layers: the mesothelium, its basement membrane and the underlying connective tissue (see Fig. 1).

The peritoneal mesothelium is an epithelial-like monolayer that occupies the surface of the peritoneal membrane (Fig. 1). The mesothelial cells form a simple monolayer that lines the peritoneum and are believed to play a multifunctional role in the physiological homeostasis of the peritoneal cavity. They reduce friction between the tissues, transport solutes and fluids to blood vessels and participate in host defense (Nagy and Jackman, 1998). The mesothelial surface is continuous with cells joined by tight junctions as well as a complex organization of membrane-bound proteins (e.g. integrins and cadherins) anchored to the actin cytoskeleton (Fukata, 1963; Bardi and Hope, 1964). The tight junctions regulate the passage of ions, water and other solutes via paracellular transport. Within the mesothelial cells there are a large numbers of cytoplasmic vesicles which can be round or elongated (Odor, 1954; Bardi and Hope, 1964) together with abundant intracellular and extracellular lamellae (Dobbie and Anderson, 1996). These are thought to be responsible for the intracellular transport of molecules across the mesothelium. The peritoneal mesothelium can participate in host defense by secreting several cytokines involved in the activation of peritoneal inflammation and attraction of neutrophils and macrophages (Jonic, 1992; Lanfrancone et al., 1992).

The basement membrane and connective tissue make up the extracellular matrix (ECM) of the peritoneal membrane. The basement membrane found between the mesothelial cells and the connective tissue is composed of type I and IV collagen, laminin and fibronectin (Witz et al., 2001a), all of which are produced by the mesothelial cells (Renard et al., 1984) and is essential for the adhesion of the mesothelial cells to the peritoneum. The connective tissue is a barrier for the diffusion of large molecules and comprises a network of aqueous channels through a ‘gel’ made from glycosaminoglycans together with collagen, elastin and fibronectin (Wayland and Silberberg, 1978; White et al., 2009) permeated by a variety of cell types including fibroblasts, macrophages and mast cells (Fig. 1).

Macrophages can be found on the surface of peritoneal mesothelial cells or in tissue close to the peritoneal basement membrane and vascular regions (Suassuna et al., 1994). Macrophages are the major source of cytokines that are released upon injury to the peritoneum and are involved in regulating inflammation (Topley et al., 1996). Fibroblasts within the membrane are also capable of secreting inflammatory cytokines and may also participate in host defense (Witowski et al.,...
Table 1 List of putative roles of the peritoneum in the establishment and progression of endometriosis.

<table>
<thead>
<tr>
<th>Process</th>
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<th>Activity</th>
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<td>Ectopic cell attachment</td>
<td>Peritoneal mesothelial cells</td>
<td>Expression of adhesion molecules</td>
<td>Expression of integrins, cadherins, VCAM-1 and ICAM-1 provide potential attachment sites for ectopic cells</td>
<td>Carter et al. (1990); Gulberg et al. (2004); Witz et al. (1998); Witz et al. (2000); van der Linden et al. (1994); Chen et al. (2002); Yanez-Mo et al. (2003); Groothuis et al. (1999); Kyama et al. (2008a, b)</td>
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<td>Changes to mesothelial cell morphology</td>
<td>Loss of tight junctions exposes ECM and provides potential attachment sites for ectopic cells</td>
<td>Ishimaru et al. (2004); Dunselman et al. (2001); Demir et al. (2000)</td>
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<td>Ectopic cell invasion</td>
<td>Peritoneal mesothelial cells</td>
<td>Changes to mesothelial cell morphology EMT</td>
<td>Loss of tight junctions exposes ECM and may facilitate invasion of attached ectopic endometrial cells</td>
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<td>Inflammation</td>
<td>Peritoneal macrophage</td>
<td>Secretion of cytokines</td>
<td>Presence of pro-inflammatory cytokines increases cell proliferation and neoangiogenesis</td>
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<td>Immune evasion</td>
<td>NK cells</td>
<td>Impaired scavenger function</td>
<td>Impaired scavenger function due to shedding of ICAM-1 and lower NK cell numbers allows for lesion establishment</td>
<td>Somigliana et al. (1996); Viganò et al. (1998); Wu et al. (2004)</td>
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EMT, epithelial mesenchymal transition; ICAM, intercellular adhesion molecule; NK cells, natural killer cells; VCAM, vascular cell adhesion molecule.

Figure 1 Diagrammatic structure of the peritoneal membrane showing the mesothelial layer, basement membrane and the submesothelial connective tissue with cell populations, structural proteins and vasculature.

2009). Fibroblasts are mainly found within the connective tissue where they produce the majority of the macromolecules (collagens and elastin), which make up the ECM (Davila and Crouch, 1993). The fibroblasts are involved in production of collagen during peritoneal membrane repair and are subsequently involved in peritoneal fibrosis (Jimenez-Heffernan et al., 2004). The connective tissue contains a
network of microvessels that function in the dialysing capacity of the peritoneal membrane and lymphatic vessels, which maintains the peritoneal fluid (Mactier et al., 1987; Michels et al., 2004).

Secreted molecules, for instance fibroblast growth factors, can bind to macromolecules such as heparin-like glycosaminoglycans within the ECM forming a reservoir of signaling molecules (Folkman et al., 1988). These molecules can act on local target cells either released following ECM digestion or by binding to specific carrier molecules (Powers et al., 2000). These reservoirs allow for large amounts of molecules to be activated quickly, and activation from the ECM may itself be regulated by several factors (Powers et al., 2000).

The peritoneal fluid
Peritoneal cavity fluid is formed from plasma transudate and ovarian exudate (Koninckx et al., 1980; Oral et al., 1996) and it changes in volume throughout the female menstrual cycle. The large surface area of the peritoneal cavity allows for passive dialysis of large quantities of substances between peritoneal fluid and blood plasma. It serves to reduce friction between peritoneal surfaces and contains a variety of immune cell types including natural killer cells (NKC) and macrophages (Oosterlynck et al., 1992; Weil et al., 1997; Giudice, 2010). The peritoneal environment including the mesothelium and immune cells are heavily influenced by factors within the fluid due to their close proximity to the fluid and dialysis of solutes within the peritoneal cavity. Therefore, the properties of the peritoneal fluid are an important contributor to the physiology and pathologies of the peritoneum and its regulation is the focus of current research efforts.

Peritoneal endometriosis
The pelvic peritoneum is the most common site for endometrial implants with endometriosis occurring in the Pouch of Douglas in over 80% of patients (Mahmood and Templeton, 1991). However, endometriosis can occur at sites throughout on the pelvic peritoneum (Jenkins et al., 1986) and is also very rarely found in extra abdominal mesothelial sites such as the pleura and pericardium (Cecconari et al., 2010). Endometriosis is traditionally defined by the presence of ectopic endometrial glands and stroma within the lesion; however, the occurrence of smooth muscle cells is common (Anaf et al., 2000). A study by Jones et al. (2009) examined the ultrastructure of endometrial—peritoneal lesions in women and reported that there was ‘significant heterogeneity and abnormalities in the tissue architecture of ectopic lesions’. For example, lesions often had immature endometrial cells with differing gland morphologies and only some lesions had infiltrated into the stroma (Jones et al., 2009). Lesions can appear as red glandular lesions (early), tight black lesions (advanced) or white fibrotic lesions (healed) when viewed macroscopically by laparoscopy but notably their appearance does not predict symptoms or severity of the disease (Stripling et al., 1998). Despite being classified as a non-malignant disorder, cells within endometriosis lesions share characteristics with tumours including increased cell proliferation (Zhang et al., 2010), invasion (Gaetje et al., 1997), neoangiogenesis (Gilabert-Estellés et al., 2007) and reduced apoptosis (Gebel et al., 1998).

Evidence from studies using animal models suggests a close intercellular interplay between peritoneum and the ectopic endometrial tissue. Hull et al. in 2008 used a mouse model in which human tissue xenografts were introduced into nude (immunosuppressed) mice to interrogate the endometrial—peritoneal interactions during lesion development. Staining of lesions 7 days after introduction of human tissue showed mouse peritoneal cells to be present in the outer regions of the lesion surrounding human endometrial tissue. By Day 14 mouse peritoneal cells were found mixed with human endometrial stromal cells throughout the lesion. This study therefore suggests a close association may exist between endometrial and peritoneal cells in endometriosis and contributes to the notion that peritoneal cells can contribute to the development, growth and progression of the lesions themselves (Hull et al., 2008).

The potential role of the peritoneum in the establishment and maintenance of endometriosis
Cell adhesion to pelvic peritoneal mesothelium
In order for endometriosis to become established, cells from retrograde menstruation must adhere to the peritoneal surface. It is not clear whether the ectopic endometrium adheres to the peritoneal ECM, and therefore requires the mesothelium to be damaged or missing, or if it can adhere directly to an intact mesothelial cell layer. Dunselman et al. (2001) reported in 2001 that an intact mesothelial cell layer lining prevents adhesion of shed endometrial tissue and that this tissue is much more likely to adhere to the underlying peritoneal ECM. The authors further concluded that menstrual effluent creates its own adhesion sites by damaging the mesothelial layer, thus exposing the ECM. Notably, primary endometrial cells from endometriosis patients were shown to be able to bind ECM components, fibronectin and collagen IV in an adhesion assay but this was not associated with presence or absence of mesothelial cells (Sillems et al., 1999). A more common observation is that ectopic endometrial stromal and epithelial cells adhere directly to the peritoneal mesothelium (Nisolle et al., 2000; Witz et al., 2003; Lucidi et al., 2005). However, the reason why attached cells proliferate and invade the underlying peritoneal tissue in some women and not in others is unclear. This may be due to differences in the ectopic cells or differences in the peritoneal mesothelium, or more likely both, in women with endometriosis but mechanistic regulators await further study (Sampson, 1927; Witz et al., 2000, 2001b, 2003; Lucidi et al., 2005; Griffith et al., 2010).

Exposure to surgical trauma may be considered as a risk factor in the development of endometriosis. A large study on the development of spontaneous endometriosis in the rhesus monkey showed that hysterotomies, but not laparoscopies, are a significant risk factor in the development of endometriosis (Hadfield et al., 1997). In contrast, a study of baboons concluded that exposure to repetitive laparoscopies was a risk factor for the development of endometriosis (D’Hooghe et al., 1992). The mechanisms relating to this increased risk are not clear but it is possible that surgical trauma leading to damage and/or inflammation in the peritoneal mesothelial cells can facilitate ectopic endometrial cell attachment sites on the ECM. Furthermore, the inflammatory response from such trauma may have downstream effects on cell proliferation, immune cell recruitment and expression of cell surface factors all of which may add to the initiation or progression of the disease.

Differences in the expression of mesothelial cell adhesion molecules and junctional complexes may be a key determinant in why some women develop endometriosis and others do not. Notably, it has been reported that mesothelial cells express cell surface proteins including cadherins and integrins that are implicated in the regulation of...
Integrins are cell surface adhesion receptors that can facilitate cell–cell, cell–matrix and cell–pathogen interactions. They are composed of an α and β subunits of which there are 18 different subunits and a possible 24 unique combinations reported in mammals (Luo et al., 2007; Carvalho et al., 2011). As adhesion molecules, integrins play key roles in attachment of cells to ECM and some, in particular α2 and α3, are involved in cell–cell adhesion (Carter et al., 1990; Nagy and Jackman, 1998; Gullberg et al., 2004). Integrin subunits α2, α3, α5 and αv were shown to be expressed in human peritoneal tissue and subunits β1, β2, α2, α3 and αv were expressed in cultured human mesothelial cells (Odor, 1954; Witz et al., 1998; Tietze et al., 1999; Bird, 2004). Additional studies have reported integrins αβ, αβ, and αβ to be present at the base (next to the basement membrane) and on the surface (exposed to peritoneal cavity) of the peritoneal mesothelial cells indicating a potential locus for ectopic cell adhesion (Fukata, 1963; Bardi and Hope, 1964; Witz et al., 1998, 2000). Supporting the argument that integrins may facilitate ectopic endometrial cell attachment to the peritoneum, integrins β and β were present in endometriosis lesions in a nude mouse model and shown to be of peritoneal origin (Hull et al., 2008). As αβ, αβ, and αβ were shown to be expressed in both endometriosis lesions as well as in peritoneum of patients without endometriosis, it is likely the integrins were of peritoneal mesothelial cell origin and not from ectopic endometrial cells (Odor, 1954; Bardi and Hope, 1964; van der Linden et al., 1994).

Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are members of the integrin adhesion protein family that bind leukocytes during inflammation. Several studies have shown peritoneal mesothelial cells to constitutively express ICAM-1 and VCAM-1 in vitro and in vivo (Suassuna et al., 1994; Dobbie and Anderson, 1996; Zeillemaker et al., 1996). They are most abundantly expressed on the microvilli surface of the peritoneal mesothelial cells. Their expression is up-regulated by several pro-inflammatory cytokines and may provide a potential site for ectopic cell adhesion (Jonjic, 1992; Lanfrancone et al., 1992; Suassuna et al., 1994; Zeillemaker et al., 1996). Women with endometriosis were found to have increased the expression of VCAM-1 in the peritoneum during the menstrual phase, in comparison with the luteal phase, whereas there was no difference in the expression in women without endometriosis (Suassuna et al., 1994; Klein et al., 1995; Kyama et al., 2008a). ICAM-1 polymorphisms have been studied in Italian, Japanese and Iranian women, in relation to endometriosis occurrence and severity but results of these studies remained inconclusive (Wayland and Silberberg, 1978; Aghajianpour et al., 2011) (Suassuna et al., 1994; Topley et al., 1996; Zeillemaker et al., 1996; Viganò et al., 2003; Kitakaki et al., 2006; White et al., 2009; Witowski et al., 2009).

The cadherins are the biggest class of cell adhesions factors in vertebrate tissues, originally named after the tissue in which they were discovered: E-cadherin in epithelial cells, N-cadherin on nerve cells, P-cadherin on placental cells, although all cadherins have been found in a variety of tissues. E-cadherin has been shown to be expressed in healthy peritoneal mesothelium and eutopic endometrial epithelium (Davila and Crouch, 1993; Groothuis et al., 1999; Yanez-Mo et al., 2003; Jimenez-Heffernan et al., 2004). The possibility of polymorphisms in the E-cadherin gene, CDH1, giving rise to a predisposition to endometriosis has been investigated in Chinese, Japanese and Indian women but the results remain somewhat inconclusive (Mactier et al., 1987; Michels et al., 2004; Shan et al., 2007; Govatati et al., 2012; Yoshida et al., 2012). A more recent study has shown peritoneal mesothelial cells expressed N-cadherin but not E-cadherin in contrast with those above (Matsuzaki and Darcha, 2012). P-cadherin was also revealed to be the predominant cadherin subtype present in the human peritoneum in one study and concentrations of mRNA were found to be significantly increased in peritoneal endometriotic lesions compared with matched eutopic endometrium, suggesting that P-cadherin may play a active role in mediating endometrial–peritoneal cell interactions in the development of endometriosis (Chen et al., 2002).

**Ectopic endometrial cell invasion into the peritoneum**

Once attached, ectopic endometrial cells must invade the peritoneal environment in order to establish themselves as lesions. Investigations using endometrial and peritoneal tissue from patients with and without endometriosis showed there was an increase in the invasion potential of the endometrial stromal cells from patients with endometriosis (Shi et al., 2011). Furthermore, the invasion of endometrial stromal cells through invasion chambers coated in matrigel was increased 10-fold when a peritoneal mesothelial monolayer was present (Nair et al., 2008). Supporting this, a recent study demonstrated that human peritoneal mesothelial cells from women without endometriosis could resist invasion by human stromal cells in an in vitro co-culture experiment. However, human peritoneal mesothelial cells from women with endometriosis could not resist invasion and this was associated with loss of adhesion between the mesothelial cell monolayer. This suggests that the peritoneal mesothelium may play an essential role in determining the invasion potential of ectopic endometrial cells and may have an altered response in endometriosis. Interestingly, the authors noted that the human peritoneal mesothelial cells from endometriosis patients disappeared after stromal cell invasion and this was shown to be due to apoptosis of the mesothelial cells (Chen et al., 2012). A time lapse analysis of the invasion process revealed that the endometrial epithelial cells and stromal cells spread over the surface of mesothelium and then through the mesothelial cell junctions followed by extension under the mesothelium, with vascularization by 12 h and lifting of mesothelium (Witz et al., 2003). The spread of endometrial stromal cells through mesothelial cell junctions during invasion is consistent with the loss of mesothelial cell adhesion described previously and follow-up studies seem merited.

Invasion of the mesothelium may be facilitated by the matrix metalloproteases (MMPs) a group of collagenase proteins that are capable of digesting and remodelling the ECM. MMPs are expressed in the endometrium, and have been shown to be present in the peritoneal fluid and endometriotic lesions (Gilbert-Estellés et al., 2007; Sotnikova et al., 2010; Itoh et al., 2012). Within the endometrium, MMPs play a critical role in the growth, development and regeneration of endometrial tissue throughout the menstrual cycle (Itoh et al., 2012). Steroid hormones and cytokines regulate MMPs in the endometrium and in particular progesterone acts as a blanket inhibitor of MMP activity (Itoh et al., 2012). Peritoneal mesothelial cells are also capable of secreting MMPs through regulation by TGF-β (Fig. 2) and this expression has been linked to ECM remodelling in peritoneal adhesion formation (Ma et al., 1999). Therefore, peritoneal expression of MMPs may facilitate the remodelling of the ECM during the development of endometriosis,
allowing the ectopic endometrial cells to invade and establish as a lesion. Notably, endometriosis lesions have been shown to express MMP 1, 2, 3, 7 and 13 in rat and chicken models of disease (Cox et al., 2001; Nap et al., 2004; Ramon et al., 2005). MMP-3 was expressed in the human peritoneum and its expression was significantly increased in women with endometriosis compared with healthy controls, furthermore its expression was significantly higher during the menstrual phase of the cycle suggesting possible hormonal regulation of MMPs in the peritoneum (Kyama et al., 2006). Array data showed MMP-7 and MMP-11 to be expressed in the peritoneal cells of lesions but not that of ectopic endometrial cells, when lesions were induced in a mouse model of endometriosis, thus linking the peritoneal cells with ECM remodelling during endometriosis (Hull et al., 2008). As progesterone is known to repress MMP activity (Nisolle et al., 2000; Witz et al., 2003; Lucidi et al., 2005; Itoh et al., 2012) and endometriosis is generally accepted to be associated with insensitivity to progesterone (Bulun et al., 2010), it may be assumed that increased MMP activity is a result of increased progesterone insensitivity in endometriosis patients. Indeed, an insensitivity to progesterone has been shown to be associated with a failure to suppress MMP expression in vitro, supporting this theory (Bruner-Tran et al., 2002). Additionally, in vivo studies inhibiting MMP activity have been successful in preventing the development of endometriosis in a chicken model of endometriosis, suggesting that MMP activity is essential for the establishment of lesions (Nap et al., 2004).

Changes in peritoneal mesothelial cell morphology in women with endometriosis

Changes to mesothelial cell morphology have been described in endometriosis. In 2001, Dunsleman et al. (2001) reported that damage to the mesothelial cells lining the pelvis after exposure to menstrual effluent resulted in an elongated columnar morphology leading to gaps between the cells and loss of tight junctions. Another group described changes in mesothelial cell morphology from cuboidal to columnar cell types in peritoneal biopsies adjacent to endometriosis lesions and this was observed most frequently in red endometriosis lesions (Ishimaru et al., 2004). There was also an increase in macrophages adjacent to red endometriosis lesions that the authors linked to increased hepatocyte growth factor concentrations which are known to stimulate cell motility and ECM breakdown (Ishimaru et al., 2004). The authors attributed this change in morphology to the coelomic metaplasia theory; however, these changes in morphology can also be seen in response to injury or inflammatory stimulus and often coincides with loss of tight junctions.
Given that these cells were adjacent to an inflamed endometriotic lesion and associated with an increase in macrophages, these changes may be simply due to the inflammatory microenvironment and not the primary cause of lesion formation. However, a change in cell morphology could be important in the progression of the disease contributing to the invasion of lesions by removal of the tight junctions in the mesothelial cell layer allowing adherent endometrial cells to infiltrate the underlying tissue. Another study reported that menstrual effluent induced morphological changes in peritoneal mesothelial cells associated with the changes with reorganization of the cytoskeleton rather than apoptosis or necrosis as initially suspected. The authors concluded that this change could lead to exposure of the ECM to the peritoneal environment and facilitate the binding of ectopic endometrial cells (Demir-Weusten et al., 2000). The distribution of peritoneal endometriosis lesions supports this theory with the most common sites of lesions found close to the Fallopian tube.

**Epithelial–mesenchymal transition of peritoneal mesothelial cell in women with endometriosis**

Peritoneal mesothelial cells, originate from the mesoderm via mesenchymal–epithelial transition (MET) and epithelial cells can return to the mesenchymal cell state through epithelial–mesenchymal transition (EMT) (Kalluri, 2009). EMT is a process defined by complete loss of epithelial cell traits and gain of mesenchymal cell characteristics and is an important process during embryonic development, fibrosis and tumorigenesis (Davidson et al., 2012; Kerosuo and Bronner-Fraser, 2012). Changes to epithelial cell morphology include a decrease in E-cadherin expression with increases in N-cadherin, loss of tight junctions, cytoskeleton reorganization, expression of vimentin and smooth muscle actin and gain in migration and invasion (Zeisberg and Neilson, 2009). Human peritoneal mesothelial cells have been found to undergo EMT during peritoneal dialysis and this EMT has been shown to be important in the development of fibrosis by peritoneal membrane injury and disruption of the mesothelium (Yanez-Mo et al., 2003; Jimenez-Heffernan et al., 2004).

A recent study has looked at EMT and MET in pelvic endometriosis and found that red peritoneal lesions expressed higher levels of the mesenchymal marker, vimentin, than menstrual endometrium (Matsuzaki and Darcha, 2012). However, black endometriosis lesions expressed higher levels of E-cadherin than menstrual endometrium. Although not all markers of EMT/MET were expressed, the authors proposed that endometrial cells might undergo an EMT-like process in red lesions and that this may be followed by a MET-like process in black lesions and that EMT and MET processes might be involved in the development of endometriosis (Matsuzaki and Darcha, 2012). Nevertheless, based on the results presented it was not possible to say if the mesenchymal cells detected resulted from the ‘transition’ of the epithelial epithelial cells or rather from peritoneal mesenchymal cells invading the lesion. Hull et al. (2008) have shown that the mesenchymal cells (identified by smooth muscle actin staining) are of peritoneal origin. Human peritoneal cells were shown to undergo an EMT-like process in vitro in response to menstrual effluent (Demir et al., 2004). The authors suggested this could be a cause of endometriosis with larger amounts of retrograde menstrual effluent causing a greater insult on the peritoneal tissue. N-cadherin, a marker of EMT, was found to be expressed in endometriosis lesions, suggesting an invasive phenotype. However, where these N-cadherin positive cells originated from, the peritoneal mesothelial cells or ectopic endometrial epithelial cells, is unclear (Koninckx et al., 1980; Zeitvogel et al., 2001). Comparisons with the mesothelium adjacent to lesions may shed more insight into the potential role of EMT in the pathogenesis of endometriosis by highlighting if these changes precede lesion development and determine whether EMT of peritoneal mesothelial cells is causative to endometriosis development by allowing ectopic cell attachment to the underlying ECM. Alternatively, EMT of the peritoneal mesothelium may result from the activity of inflammatory factors initiated by endometriosis.

**Changes in immune cell populations within the peritoneum**

There is accumulating data that suggest that a suppressed scavenger function in the peritoneum may lead to inadequate removal of retrograde menstrual tissue allowing ectopic endometrial cells to survive and implant on the peritoneal surface (Oosterlynck et al., 1992; Ho et al., 1995; Quaranta et al., 2006). Whether this suppressed scavenger function is a cause, or a result, of endometriosis is still to be determined. There are several reviews on the role of immune cells in endometriosis (Gazvani, 2002; Kyama et al., 2003; Khan et al., 2008). Here we have summarized findings related to immune cells from the peritoneal tissue only.

NKC are cytotoxic lymphocytes with the ability to lyse target cells. Peritoneal NK cells are thought to be involved in the clearance of retrograde menstruation and it is notable that patients with endometriosis showed a reduced NKC cytotoxicity compared with healthy controls suggesting a mechanistic reason as to why the ectopic endometrial cells may avoid detection by immune surveillance in some patients (Oosterlynck et al., 1991; Quaranta et al., 2006). This is further supported by the observation that a decrease in peritoneal NKC cytotoxicity correlated with endometriosis disease severity (Oosterlynck et al., 1992; Ho et al., 1995). There is still debate over the numbers of NKC in endometriosis patients; some reports suggesting that the numbers of NKC are decreased while others do not. However, a study by Aoki et al. (2006) using human endometrial tissue planted subcutaneously in a mouse model of endometriosis illustrated the importance of NKC in the establishment of endometriosis lesions. Lesions were established in only 40% of untreated nude mice in the study, whereas they were present in 100% of nude mice treated with an NK inhibitor.

ICAM-1 can mediate the adhesion of leukocytes to target tissue and increased ICAM-1 expression can augment immune responses and leukocyte accumulation in inflamed tissue. Adhesion molecules have been shown to be abundantly produced on the microvilli surface of the peritoneal mesothelial cells and leukocytes have been shown to bind and migrate across the mesothelial monolayer (Zeillemaker et al., 1996). It may be possible that changes in ICAM-1 expression/shedding, associated with endometriosis, may result in a decrease in mesothelial-associated leukocytes, leading to impaired immune surveillance of ectopic endometrial cells on the peritoneum and allowing for attachment and invasion into the peritoneum. ICAM-1 expression was shown to be down-regulated in women with endometriosis, supporting this theory (Kyama et al., 2006). Studies have identified the presence of soluble ICAM-1 (sICAM-1) in the peritoneal fluid and this is thought to originate from ectopic endometrial stromal cells and peritoneal mesothelial cells (Somigliana et al., 1996; Viganò et al., 1998). sICAM-1 is able to bind leukocytes inhibiting leukocyte epithelial cell adhesion and scavenger function (Becker et al., 1993) and has been shown to be associated with tumour progression though evasion of the immune system, suggesting that this may also allow endometriotic lesion progression though the same mechanism (Becker et al., 1991). One study looked at the ability of
The peritoneal mesothelium as a source of cytokines

Endometriosis is classified as a chronic pelvic inflammatory disease and a variety of pro-inflammatory cytokines and chemokines are elevated in the peritoneal fluid of endometriosis patients, including tumour necrosis factor α (TNFα), interleukin-1 (IL-1), IL-6, IL-8, monocyte chemotactic protein (MCP-1), MCSF-1 and transforming growth factor β1 (TGF-β1), as detailed in Table II. The majority of these molecules are secreted into the peritoneal fluid by macrophages. However, the peritoneal mesothelium, fibroblasts, NKC and ectopic endometrial cells can also contribute (reviewed in Gazvani, 2002).

The expression of cytokines and growth factors in the peritoneum of women has been shown to change during the menstrual cycle. For example, the expression of TGF-β and IL-6 by the peritoneum was shown to be significantly up-regulated during the menstrual phase of the cycle and there was a significant difference in TNFα expression between women with endometriosis compared with healthy controls (Kyama et al., 2006). TNFα is known to promote both adhesion and proliferation of endometrial cells, whereas IL-6 can up-regulate the expression of ICAM-1. The authors concluded that women with endometriosis have a biologically ‘different’ peritoneum when compared with healthy controls and that the increase in peritoneal cytokine release during menstruation may help to facilitate a pro-inflammatory environment which may in turn promote the development of endometriosis (Kyama et al., 2006). A follow-on study showed that even peritoneal tissue distal to endometriotic lesions expressed higher levels of IL-1β during the menstrual phase compared with peritoneal tissue from healthy controls (Kyama et al., 2008b). Higher IL-1β expression can increase shedding of ICAM-1 from the peritoneal mesothelial cells and may be the initiator in promoting neovascularization by inducing angiogenic factors such as VEGF and IL-6 (Kyama et al., 2008b). Peritoneal mesothelial cultures express IL-6, IL-8 and MCP-1 and this is increased in co-cultures with endometrial epithelial cells, indicating that endometrial epithelial cells might be seen as ‘foreign’ cells thereby initiating an pro-inflammatory response in the mesothelium that is chemotactic to macrophages (Song et al., 2003). As the mesothelial cells secreted MCP-1 and IL-6 at higher levels than the endometrial stromal and epithelial cells, it is thought that the mesothelium may play a significant part in the recruitment of macrophages (Song et al., 2003). In a second study, the expression of IL-6 was significantly increased in the peritoneal tissue from women with endometriosis in comparison with controls and the authors showed IL-6 to significantly increase haptoglobin production that may contribute to the altered peritoneal immune status in women with endometriosis as well as participate in neovascularization (Piva et al., 2001). Notably, when mouse endometrial epithelial and stromal cells were injected into the peritoneal cavity of mice there was an immediate inflammatory response and recruitment of macrophages. In this study, the mesothelium was shown to be at least in part responsible for the chemotactic recruitment of the macrophages and the inflammatory response (Cao et al., 2004). IL-6 has been shown to stimulate haptoglobin production in primary peritoneal mesothelial cells but not ectopic endometrial stromal cells from women with and without endometriosis. Haptoglobin promotes angiogenesis and can alter immune cell response, potentially contributing to the neovascularization associated with endometriotic lesions and to the altered peritoneal immune status in women with endometriosis and allowing a mechanism to escape peritoneal immune surveillance (Piva et al., 2001). Together these studies suggest that endometrial cells can trigger an inflammatory reaction in the peritoneal cavity and that mesothelial cells play an important role in this inflammatory response and in chemotaxis of macrophages during the development of endometriosis.
Table II  List of factors involved in the adhesion and invasion of ectopic endometrial cells during the establishment of peritoneal endometriosis together with their source and activity.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Abbreviation</th>
<th>Source</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocyte growth factor</td>
<td>HGF</td>
<td>Mesothelium</td>
<td>Changes to cell morphology, macrophage recruitment</td>
<td>Ishimaru et al. (2004)</td>
</tr>
<tr>
<td>Tumour necrosis factor α</td>
<td>TNFα</td>
<td></td>
<td>Ectopic cell adhesion and proliferation</td>
<td>Kyama et al. (2006)</td>
</tr>
<tr>
<td>Transforming growth factor β</td>
<td>TGF-β</td>
<td></td>
<td>Increases MMP expression</td>
<td>Ma et al. (1999); Kyama et al. (2006)</td>
</tr>
<tr>
<td>Interleukin-8</td>
<td>IL-8</td>
<td></td>
<td>Macrophage recruitment, angiogenesis, ectopic cell proliferation</td>
<td>Song et al. (2003)</td>
</tr>
<tr>
<td>Interleukin 1β</td>
<td>IL-1β</td>
<td></td>
<td>Increases shedding of ICAM-1, increases VEGF and IL-6 expression</td>
<td>Somigliana et al. (1996); Viganò et al. (1998); Kyama et al. (2008a, b)</td>
</tr>
<tr>
<td>Monocyte chemotactic protein</td>
<td>MCP-1</td>
<td></td>
<td>Macrophage recruitment</td>
<td>Song et al. (2003)</td>
</tr>
<tr>
<td>Vascular cell adhesion molecule I</td>
<td>VCAM-1</td>
<td>Mesothelium, endometrial epithelium</td>
<td>Ectopic cell adhesion</td>
<td>Zeilmaker et al. (1996); Suassuna et al. (1994); Kyama et al. (2008a, b)</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>–</td>
<td></td>
<td>Ectopic cell adhesion, ectopic cell invasion, EMT</td>
<td>Yanez-Mo et al. (2003); Groothuis et al. (1999); Matsuzaki and Darcha (2012)</td>
</tr>
<tr>
<td>Integrins</td>
<td>–</td>
<td>Mesothelium, endometriosis lesion</td>
<td>Ectopic cell adhesion</td>
<td>Carter et al. (1990); Gullberg et al. (2004); Witz et al. (1998); Witz et al. (2000); van der Linden et al. (1994)</td>
</tr>
<tr>
<td>P-cadherin</td>
<td>–</td>
<td></td>
<td>Ectopic cell adhesion</td>
<td>Chen et al. (2002)</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>–</td>
<td></td>
<td>Ectopic cell invasion, EMT</td>
<td>Zeitvogel et al. (2001); Matsuzaki and Darcha (2012)</td>
</tr>
<tr>
<td>Intercellular adhesion molecule I</td>
<td>ICAM-1</td>
<td>Mesothelium, endometrial epithelium, endometriosis lesion</td>
<td>Ectopic cell adhesion</td>
<td>Zeilmaker et al. (1996); Suassuna et al. (1994); Wu et al. (2004); Gonzalez-Ramos et al. (2007)</td>
</tr>
<tr>
<td>Matrix metalloproteases</td>
<td>MMP</td>
<td>Mesothelium, ectopic endometrial cells, endometriosis lesion</td>
<td>Ectopic cell invasion</td>
<td>Itoh et al. (2012); Gilbert-Estellés et al. (2007); Sotnikova et al. (2010); Ma et al. (1999)</td>
</tr>
<tr>
<td>Macrophage colony stimulating factor-1</td>
<td>M-CSF-1</td>
<td>Mesothelium and endometrial stromal cells</td>
<td>Macrophage recruitment</td>
<td>Nair et al. (2008)</td>
</tr>
<tr>
<td>Hapatoglobin</td>
<td>HP</td>
<td></td>
<td>Immune cell recruitment, angiogenesis, EMT</td>
<td>Piva et al. (2001)</td>
</tr>
<tr>
<td>Vimentin</td>
<td>–</td>
<td>Endometriosis lesion</td>
<td>Macrophage recruitment, angiogenesis, ectopic cell proliferation, increase ICAM-1 expression, increase HP expression</td>
<td>Matsuzaki and Darcha (2012)</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>IL-6</td>
<td>Mesothelium, macrophages</td>
<td>Macrophage recruitment, angiogenesis, ectopic cell proliferation, increase ICAM-1 expression, increase HP expression</td>
<td>Kyama et al. (2006); Song et al. (2003)</td>
</tr>
<tr>
<td>Soluble intercellular adhesion molecule I</td>
<td>sICAM-1</td>
<td>Endometrial stromal cells</td>
<td>Inhibition of NK cell activity</td>
<td>Somigliana et al. (1996); Viganò et al. (1998); Wu et al. (2004)</td>
</tr>
</tbody>
</table>

Discussion

The peritoneum is a multifunctional organ that plays a critical role in the dialysis of solutes and host defense against micro-organisms. The peritoneal membrane and fluid are rich in immune cells and cytokines and provide a unique environment within the body. According to the implantation theory of endometriosis, ectopic endometrial cells from retrograde menstruation can attach to, and invade, the pelvic peritoneal membrane to establish lesions. To date the majority of the literature reporting mechanisms responsible for establishment of endometriosis has focused on changes within the endometrial tissue itself and little has been reported on changes with the peritoneal microenvironment. New research into the role of the peritoneal cells has given us a better understanding of the aetiology of endometriosis and there is now increasing evidence that the pelvic peritoneal mesothelium may provide important contributions to the development and progression of peritoneal endometriosis.

The expression of cell surface adhesion factors on the peritoneal mesothelial cells gives the ectopic endometrial cells a focus for attachment and differential expression of these cell surface factors may explain why some women develop disease and others do not. Furthermore, changes in mesothelial cell morphology leading to gaps within the mesothelial layer have been observed and provide a locus for ectopic cell attachment directly to the ECM. Although attachment is an essential step in the implantation theory of endometriosis, it does not fully explain the cause of endometriosis as further steps in lesion development are needed. Alterations in the phenotype of peritoneal mesothelial cells resulting in the formation of myofibroblasts, through an EMT-like process, may result in disruption of tight junctions giving the ectopic endometrial cells...
an opportunity to attach to the underlying connective tissue and establish as a lesion. However, it remains unclear whether EMT processes happen as a result of the presence of endometrial tissue or that this change predates their adhesion. Regardless, it can be assumed that EMT processes will contribute to the development of endometriosis by increasing cell mobility and facilitating ectopic cell invasion of underlying ECM. This is further assisted by peritoneal production of several MMPs, allowing for local ECM remodelling.

The ability of ectopic endometrial cells to escape immune surveillance within the peritoneum is thought to be associated with an impaired scavenger response in the peritoneal membrane. Reduced NK cell numbers in the peritoneum may contribute to a defective clearance of retrograde menstrual tissue, augmented by shedding of sICAM-1 in response to inflammation, further reducing NK cell activity.

Local inflammatory responses are initiated by recruitment and activation of peritoneal macrophages and, although the number of macrophages in women with endometriosis remains a topic of debate, there is good evidence of increases in macrophage-secreted pro-inflammatory cytokines and growth factors. These products, such as IL-6, TNFα, IL-1β and HP, are responsible for initiating a wide range of outcomes including, but not limited to, increased cell proliferation, increased cell adhesion of ectopic tissues, increased shedding of ICAM-1 and hence lowered scavenger function, increased MMP production and increased neovascularization. This complicated inflammatory cascade is believed to play a central role throughout the initiation, establishment and progression of endometriosis.

Future research focusing on molecular changes within the peritoneal cells may help explain why only some women develop endometriosis. A recent study by Hull et al. clearly demonstrated that TGFβ deficiency in the peritoneum of mice reduced lesion development 11-fold demonstrating that TGFβ may be a key therapeutic target for peritoneal endometriosis (Hull et al., 2012). Furthermore, research into peritoneal–endometrial cell interactions with regard to cell morphology, invasion and inflammation are needed and might lead to the discovery of new therapeutic targets that are essential in the treatment of peritoneal endometriosis.

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