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REVIEW *Gynaecology*

Role of TGF-βs in normal human endometrium and endometriosis

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Endometriosis is characterized by presence of endometrial tissue outside the uterus. Prevalence is estimated at 6–10% in the general female population and many patients experience pain and/or infertility. Diagnosis is achieved by laparoscopic intervention followed by histological confirmation of viable endometriotic tissue. Mild cases are managed medically with contraceptive steroids and non-steroidal anti-inflammatory agents. Surgery provides relief to women in pain but symptoms recur in 75% of cases within 2 years. Starting with menstruation, we have categorized endometriosis into six stages, namely (1) shedding of cells, (2) cell survival, (3) escape from immune surveillance, (4) adhesion to peritoneum, (5) angiogenesis and (6) bleeding. In most of these biological processes, which resemble metastasis, transforming growth factor-beta (TGF-βs) and their high-affinity receptors are involved directly or indirectly. TGF-βs are abundantly and differentially expressed in the endometrium under hormonal control. Although they are preferentially synthesized in the stroma, glands and macrophages also secrete TGF-βs into the uterine fluid, where interaction with preimplantation embryos is suspected. Because mRNA and protein expression of all three TGF-βs is increased around menstruation, we suggest that TGF-βs might be involved in initiation of menstruation. Furthermore, because of high postmenstrual TGF-β3 levels, we suppose that it might participate in scarless postmenstrual regeneration of endometrium. Our suggestions pave the way to novel routes of investigation into the roles of TGF-βs during menstruation and endometriosis.

Key words: endometriosis / TGF-beta / menstruation / diagnosis

Introduction

Endometriosis is an estrogen-dependent chronic gynecological disorder usually associated with pelvic pain and infertility. It is characterized by the presence of uterine endometrial tissue outside the uterus most often in the pelvic peritoneum (Fig. 1) or ovaries, but may also occur in retro-vaginal septum and rarely in the pericardium, pleura or brain (Giudice and Kao, 2004). Prevalence is estimated to be 6–10% in the general female population and 35–50% of the patients experience pain and/or infertility (Snesky and Liu, 1980; Houston, 1984; Cramer, 1987). Severe cases may result in extensive pelvic adhesions and distortion of pelvic anatomy and could lead to infertility (Giudice and Kao, 2004). Diagnosis is achieved by laparoscopic intervention followed by histological confirmation of viable ectopic endometrial glands and stroma. Because of the variability of symptoms and confusion with other disorders, diagnostic periods are long (6–9 years, Husby *et al.*, 2003). Mild cases are managed medically with contraceptive steroids, progestagens, agonists of gonadotrophin-releasing hormone (GnRH) androgens and non-steroidal anti-inflammatory agents (Lessey, 2000; Valle and Sciarra, 2003; Practice Committee of the American Society for Reproductive Medicine, 2004). However, because of undesirable side-effects, anti-hormonal treatments are useful for limited periods making it necessary to change or use additional medication. Surgery provides relief to women in pain but symptoms recur in 75% of cases within 2 years (Candiani *et al.*, 1991; Kuohung *et al.*, 2002) and in about 10% of women even after hysterectomy or bilateral salpingo-oopherectomy (Namnoum *et al.*, 1995).

Pelvic endometriosis, the most common form of the disease, is associated with increased secretion of pro-inflammatory cytokines, impaired cell-mediated immunity, neo-angiogenesis and anomalies of refluxed endometrium. To date many cytokines suspected to be involved in endometriosis have been analyzed (Nezhat *et al.*, 2008). In this review we concentrate on transforming growth factor-beta (TGF-βs), because we suspect that they may play a major role in the biological processes leading to establishment and maintenance of endometriosis.

TGF-βs are implicated in the gene expression, cell motility, proliferation, apoptosis, differentiation, immune responses and tumorigenesis (Derynck *et al.*, 2001). In mammals, three TGF-βs, TGF-β1, TGF-β2 and TGF-β3, have been cloned and shown to have overlapping

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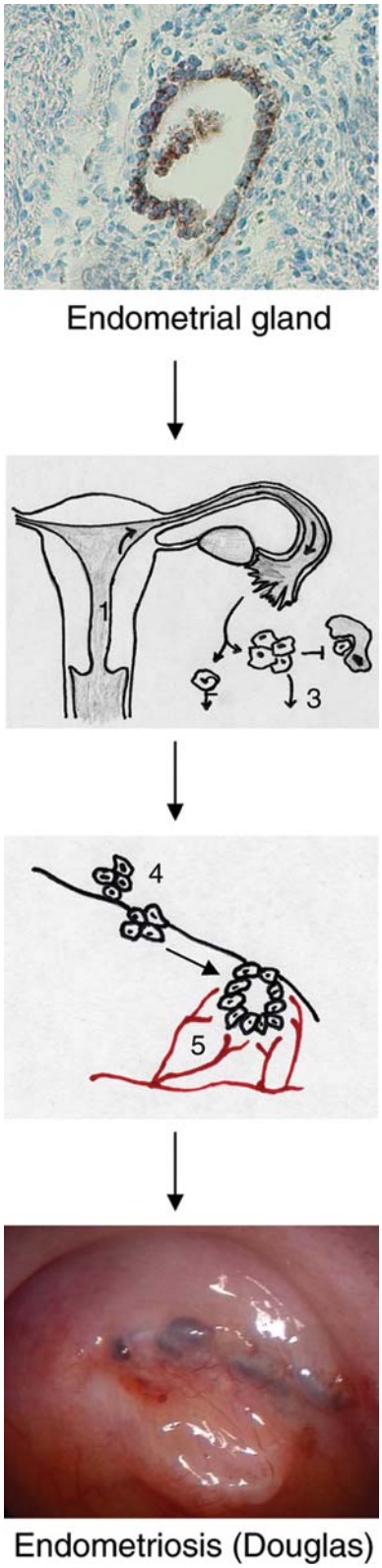

Figure 1 Schematic view of endometriosis. Starting with an endometrial gland labeled with mucin-1, endometrial cells are shed from the endometrium (1), travel through the fallopian tube (2), some cells are eliminated by the immune system (3), whereas few cells survive, adhere and invade the peritoneum (4) and after building new endometriotic structures finally induce neoangiogenesis (5).

functions *in vitro*. However, although studies on isoform-specific knockout mice have revealed non-redundant and non-overlapping phenotypes, only TGF-β1 and 2 knockout mice exhibit gonadal defects in both females and males (Memon *et al.*, 2008).

Inactivation and secretion of the TGF-βs are regulated by latency-associated peptides and latent TGF-βs binding proteins (LTBP1–4) in tissues (Koli *et al.*, 2001), whereas in blood TGF-βs are associated mostly with α2-macroglobulin (Arandjelovic *et al.*, 2006). Increasing evidence supports the thesis that activation of the TGF-βs by proteolytic enzymes is also an important regulatory mechanism for the different biological functions *in vivo* (Jenkins, 2008). After activation, the TGF-βs bind with high affinity to TGF-β receptor (TβR)II that phosphorylates TβRI. However, TGF-β2 binds to the accessory receptor TβRIII (betaglycan) to achieve high-affinity binding to TβRII. Interestingly, on endothelial cells TGF-β1 and 3 bind to the accessory receptor endoglin. The TβRI/TβRII complex propagates the signal downstream to Smad2 and/or Smad3, which together with Smad4 regulate gene expression (Lutz and Knaus, 2001). Also, Smad-independent TGF-β pathways and cross-talks to other signaling pathways have been described (Derynck and Zhang, 2003).

TGF-βs are abundantly and differentially expressed in the endometrium and are secreted by endometrial cells and macrophages into the uterine fluid where interaction with the preimplantation embryo is suspected (Jones *et al.*, 2006). Secretion of TGF-βs into peritoneal fluid of women suffering from endometriosis suggests that they may be crucial in establishment and/or maintenance of endometriosis. This review examines the role of TGF-βs in the human endometrium and in the pathophysiology of endometriosis.

Localization of the TGF-βs and their high-affinity receptors in the endometrium

All TGF-βs and their high-affinity receptors are stage-specifically expressed in the human endometrium with highest levels around menstruation (Fig. 2). Many researchers have reported staining of TGF-β1 and 3 in stromal and glandular cells (Chegini *et al.*, 1994; Gold *et al.*,

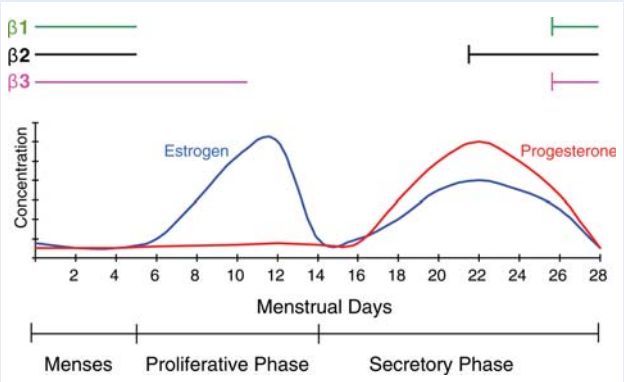

Figure 2 Levels of progesterone, estrogens and TGF-β in the human menstrual cycling. For TGF-β only the start and end-points of strongest protein expression are given according to the report from Gaide Chevronnay *et al.* (2008).

1994; Johnson *et al*., 2005; Komiyama *et al*., 2007; Gaide Chevronnay *et al*., 2008) and for TGF-β1 also in nerve fibres (Tamburro *et al*., 2003) and inflammatory cells especially in macrophages (Chegini *et al*., 1994; Tamura *et al*., 1999; Komiyama *et al*., 2007). TGF-β2 is more strongly expressed in stromal compared with glandular cells (Gold *et al*., 1994; Bruner *et al*., 1995; Gaide Chevronnay *et al*., 2008), although opposite staining intensity has been reported (Chegini *et al*., 1994). Localization of TβRII and RI was observed in both cellular compartments of the endometrium (Chegini *et al*., 1994; Gaide Chevronnay *et al*., 2008) with stronger expression of TβRII than TβRI (Gaide Chevronnay *et al*., 2008) suggesting that TβRI might be a limiting factor for signal transduction in the endometrium or during endometriosis.

TGF-β1 was found in the stromal cells (Johnson *et al*., 2005) and expression increased in the epithelial cells of endometriotic cysts (Tamura *et al*., 1999) and endometriotic nerve fibers (Tamburro *et al*., 2003). The TGF-β signal transducers Smad3, pSmad3, Smad4 and the inhibitory Smad7 proteins were also observed in the endometrial stromal and epithelial cells (Luo *et al*., 2003a). These observations suggest a role of the TGF-βs in the normal function of the human endometrium.

Hormonal regulation of TGF-β expression in the endometrium

Although most biological functions in the endometrium are under hormonal control, locally produced paracrine factors mediate cell–cell communication. For example endometrial expression of matrix metalloproteinase (MMP)-3, MMP-7 and MMP-11 occurs during menstrual breakdown and subsequent estrogen-mediated growth, but not during the secretory phase (Osteen *et al*., 1999; Zhou and Nothnick, 2005). Thus, MMPs are supposedly suppressed by progesterone via paracrine factors including TGF-βs and retinoic acid (Bruner-Tran *et al*., 2002). Although further experiments confirmed the concept (Osteen *et al*., 1999; Bruner-Tran *et al*., 2002; 2006), TGF-βs are still not used in therapeutic management of endometriosis.

In a classic experiment, Luo *et al*. (2003b) demonstrated that the GnRH analog leuprolide increases expression of inhibitory Smad7 mRNA, moderately increases Smad4 and Smad7 protein levels in endometrial surface epithelial cells, decreases rate of Smad3 activation (pSmad3) and alters cellular distribution of Smad3 in endometrial stromal and epithelial cells in a dose- and time-dependent manner. Pretreatment with Antide® (GnRH antagonist) resulted in further suppression of Smad3 in endometrial stromal cells but co-treatment with GnRH and TGF-β1 or pretreatment with TβRII antisense partially inhibited TGF-β1-activated Smad3. Taken collectively, these observations suggest that GnRH may prevent endometriosis by altering expression and activation of Smads and interrupting TGF-β receptor signaling.

Estradiol

Studies into influence of androgen receptor on TGF-β signaling have identified Smad3 as the crucial protein of the cross-talk (Danielpour, 2005). Similarly, transcriptional activity of Smad3 is suppressed by the estrogen receptor (ER) in an estradiol-dependent manner, and ER-mediated transcription increases after activation of TGF-β signaling (Matsuda *et al*., 2001; Cherlet and Murphy, 2007). In human endometrial cells, the TGF-β1 gene is activated by ER in the presence of estrogen metabolites or antagonists (Kanzaki *et al*., 1995; Yang *et al*., 1996) and a combination of estradiol and progesterone stimulated TGF-β1 mRNA expression (Casslen *et al*., 1998). However, in explant cultures of human endometrium, estradiol counteracted progesterone-dependent suppression of TGF-β1 expression (Gaide Chevronnay *et al*., 2008). Notably, TGF-β2 secretion by human endometrial stromal cells was inhibited by estradiol, whereas TGF-β1 secretion was only slightly increased (Kanzaki *et al*., 1995).

Progesterone

Although both progesterone and TGF-βs have been shown to repress MMPs (Bruner *et al*., 1995; Bruner-Tran *et al*., 2002), no direct link between the two pathways has been established. In cultured explants of human endometrium, it was clearly demonstrated that progesterone alone and in combination with estradiol inhibited TGF-β2 and 3 mRNA and protein expression (Gaide Chevronnay *et al*., 2008). In contrast, progesterone inhibited TGF-β1 mRNA and protein expression in explants but did not show any influence in microdissected tissues (Gaide Chevronnay *et al*., 2008). Similarly, progesterone inhibited TGF-β2 secretion in human endometrial stromal cells (Kanzaki *et al*., 1995).

These data contradict earlier reports showing stimulatory effects of progesterone on TGF-β2 mRNA (Bruner *et al*., 1995), of combined action of progesterone and estradiol on TGF-β1 (Casslen *et al*., 1998), and data from Arici *et al*. (1996) showing an increase in TGF-β1 and a decrease in TGF-β3 mRNA in stromal cells. Although it was hypothesized that the discrepancy might be due to a biphasic effect of progesterone (Gaide Chevronnay *et al*., 2008), it is important to note that in three studies (Bruner *et al*., 1995; Arici *et al*., 1996; Gaide Chevronnay *et al*., 2008), different concentrations of the hormones were used, which might also explain the diverse results.

Genetic predisposition to endometriosis

Genetic predisposition is suggested by reports that first-degree relatives of women with severe endometriosis were six times more likely to develop endometriosis than relatives of unaffected women (Simpson *et al*., 1980). Familial aggregation has also been shown in clinical (Simpson *et al*., 1980; Kennedy *et al*., 1995), population based (Stefansson *et al*., 2002) and twin studies (Hadfield *et al*., 1997). Although polymorphism (509C/T) in the TGF-β1 gene was observed by several groups, a recent meta-analysis does not find a consistent link of endometriosis to polymorphisms in the TGF-β1 gene (Tempfer *et al*., 2008).

Biological picture of endometriosis

Although endometriosis was reported to comprise five developmental processes (Yanez and Gonzalez, 2007), we present a more inclusive six-stage biological picture, namely cell shedding, cell survival, suppression of immune system, cell adhesion and invasion, angiogenesis and bleeding and analyzed each stage for direct or indirect involvement

of TGF-βs (Fig. 3). Most of these biological stages resemble the process of metastasis (Starzinski-Powitz *et al*., 1999).

Menstruation

Following the withdrawal of E2 and progesterone, ante-grade menstruation occurs in the upper two-thirds of the endometrial mucosa. Early events start with tissue shrinkage due to loss of hyaluronic acid and fluid absorption, destruction of ECM by up-regulation of MMPs 1/3/9, episodic vasoconstriction and relaxation of spiral arterioles, leakage of blood vessels, fibrinolysis, influx of macrophages and lymphocytes resulting in tissue apoptosis and necrosis. An increase in E2 terminates loss of blood and tissue fluid and allows regeneration of the endometrium (Jabbour *et al*., 2006).

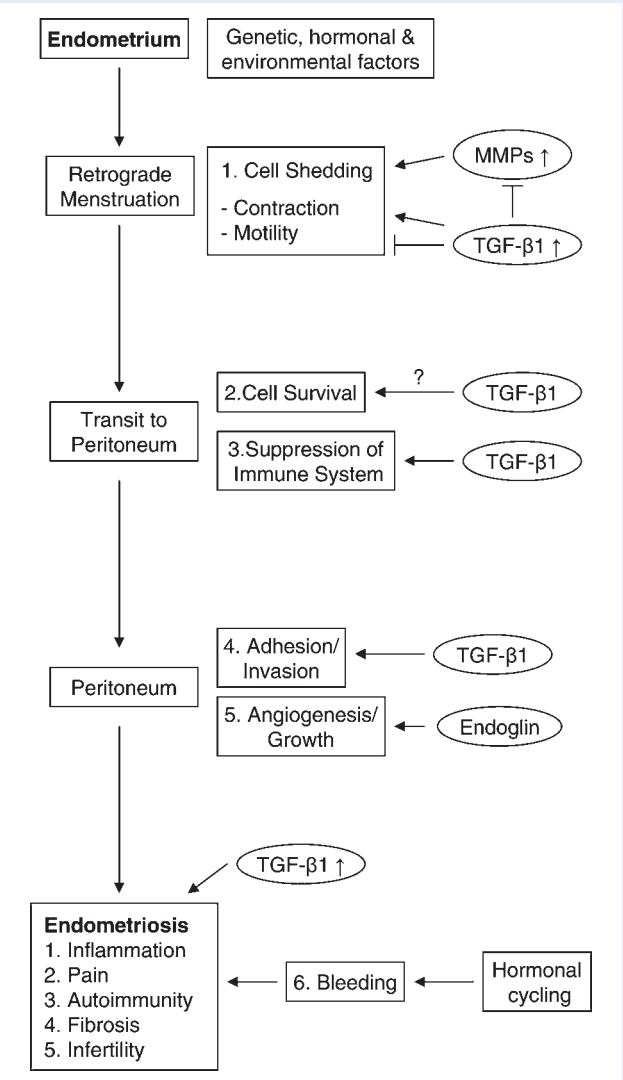

Figure 3 A schematic view of the six biological stages (numbered 1–6) leading to endometriosis with particular emphasis on the role of TGF-β. Sequence of events is shown starting with retrograde menstruation and ending with the clinical manifestation of endometriosis. ↑ indicates an increase, ⊥ indicates inhibition. MMP, matrix metalloproteinases.

Although mRNA and protein expression of the three TGF-βs is increased at menstruation, the rise in TGF-β2 starts at mid-late secretory phase and declines after menstruation. TGF-β3 increases at menstruation but in contrast TGF-β1 and 2 remains high during the proliferative phase (Gaide Chevronnay *et al*., 2008). Consequently, we hypothesize that high levels of TGF-β3 and low levels of TGF-β1 and 2 may facilitate scar-less post-menstrual endometrial repair as reported previously for fetuses (Shah *et al*., 1995; Samuels and Tan, 1999). However, high expression of TGF-βs around menstruation does not support the thesis (Osteen *et al*., 2003) that TGF-βs are critical transducers of progesterone action.

High TGF-βs levels around menstruation might increase production of endothelin-1, a potent vasoconstrictor involved in endometrial bleeding and cessation thereof, by endometrial epithelial cells (Salamonsen *et al*., 1999) suggesting that TGF-βs may indirectly induce menses via vasoconstriction. Elevated levels of TGF-βs at menstruation may also result from infiltration of the endometrium (Lea and Clark, 1991) or increased secretion of TGF-βs by immune cells (Zhou and Nothnick, 2005).

TGF-βs maintain integrity of the ECM and prevent breakdown of the endometrial tissue (Tabibzadeh, 2002). This assumption is based on the observation that Lefty-2/EBAF (endometrial bleeding associated factor), a member of the TGF-β family, is dramatically up-regulated during endometriosis (Kothapalli *et al*., 1997) and antagonized TGF-β signaling by inhibiting phosphorylation of Smad2 downstream of the TβRI (Ulloa and Tabibzadeh, 2001). Lefty-2 suppressed TGF-β-dependent down-regulation of MMPs in the human endometrium (Bruner *et al*., 1995; Tabibzadeh, 2002) and stimulated expression of MMP3 and MMP7 (Cornet *et al*., 2002). That Lefty-2 was noticeably more abundant in patients with endometriosis who did not conceive compared with those who became pregnant, suggested a role in implantation (Tabibzadeh *et al*., 2000). This thesis was further corroborated by the observation that Lefty-2 knockout mice were infertile due to implantation failure (Tang *et al*., 2005).

Stage 1: Shedding of cells/aggregates into peritoneal cavity

There is evidence that menstrual effluent contains viable single cells, cell aggregates and gland-like structures that can be cultured (Koks *et al*., 1997; Bulletti *et al*., 2002). Whereas estradiol causes retrograde contractions in the sub-endometrial layers of the myometrium (Bulletti *et al*., 2004), progesterone is responsible for uteroquiescence characterized by low amplitude of bilateral contraction that precedes proper positioning of the embryo for implantation and pregnancy. During menses, antegrade contractions take place due to progesterone withdrawal and involve all muscle layers of the uterus. It is therefore not surprising that progesterone or tamoxifen in vaginal suppositories inhibit uterine contractions (Pierzynski *et al*., 2006; Ruddock *et al*., 2008). However, abnormal myometrial contractions with higher frequency, amplitude and basal pressure tones have been described in women with endometriosis (Bulletti *et al*., 2002). Although involvement of TGF-βs in uterine contractions still needs to be investigated, it has been shown that TGF-β1 also induces contractions of decidual stromal cells (Kimatrai *et al*., 2003) and inhibits motility of stromal endometrial cells (Nasu *et al*., 2005).

Stage 2: Cell survival during transit to peritoneal cavity

Although emerging evidence suggests that TGF-β induce a mitochondrial pathway to trigger apoptosis, direct interaction between TGF-β signaling and the apoptotic pathway remains elusive. Numerous studies indicate that TGF-β are important regulators of cell survival, stimulate proliferation of stromal cells and inhibit proliferation of epithelial cells (Rahimi and Leof, 2007). However, in the human endometrium, TGF-β1 stimulated DNA synthesis in epithelial cells at low concentrations, but inhibited DNA synthesis at higher concentrations in women with and without endometriosis (Meresman *et al*., 2003). Proliferation of isolated stromal cells was not influenced by any of the TGF-β isoforms at low concentrations, but DNA synthesis was induced and metabolic activity inhibited (Tang *et al*. 1994). Similarly, all three TGF-β isoforms inhibited metabolic activity of normal human endometrial stromal cells dose dependently (Nasu *et al*., 2005). Nasu *et al*. (2005) and Meresman *et al*. (2003) used metabolic assays and ^{3}H-thymidine incorporation assays respectively and reported an influence of TGF-βs on cell proliferation but did not standardize their data by directly counting the cells.

Additional evidence showed that TGF-β1 induces expression of FasL mRNA and protein in endometrial stromal cells (Garci-Velasco *et al*., 1999), possibly preventing apoptosis during transit to the peritoneal cavity.

Stage 3: Suppression of the immune system

That TGF-β1 represses the immune system was demonstrated in knockout mice that died of multiorgan inflammation (Shull *et al*., 1992). Target cells included lymphocytes, especially regulatory T cells (Treg), cytolytic T cells, natural killer cells (NK) and macrophages (Pardali and Moustakas, 2007). Additional studies have demonstrated that TGF-β1 inhibits IFN-γ and IL-10 secretion by uterine NK cells (uNK) in the human endometrium, and that blocking TGF-β1 in human endometrial cells increases secretion of IFN-γ by uNK (Eriksson *et al*., 2004), possibly by increased production of Toll-like receptor agonist (Eriksson *et al*., 2006). Suppression of immune surveillance by enhanced secretion of TGF-β1 by endometrial cells resembles closely the process of metastasis (Jakowlew, 2006) and might trigger inflammation in the peritoneum as was observed in endometriosis (Agic *et al*., 2006). Escape from immune surveillance is also important for adhesion of endometriotic cells in the peritoneum.

Stage 4: Adhesion to peritoneum and invasion

Cell–cell-interactions are mainly mediated by integrins, which in the case of TGF-βs also activate latent TGF-β1 (Wipff and Hinz, 2008). The importance of TGF-β1 activation is supported by the recent observation that a mutation in the RGD amino acid sequence in TGF-β1, which mediates binding to integrins, resulted in similar knockout phenotypes as TGF-β1 (Yang *et al*., 2007). *In vitro* experiments have shown that TGF-β1 increases adhesion of normal human endometrial stromal cells to mouse peritoneum (Beliard *et al*., 2003), but not to human peritoneal mesothelial cells (Liu *et al*., 2009). Interestingly, a ferric hyaluronate gel inhibited pro-adhesive effects of TGF-β1. These barriers are commonly used in clinics to limit peritoneal adhesions which are often induced by surgical injuries and are a leading cause of pelvic pain, bowel obstruction and infertility (Chegini, 2008). A recent report indicated that TGF-β1 enhanced trans-mesothelial invasion of primary and immortalized endometrial epithelial cells *in vitro* (Liu *et al*., 2009).

Stage 5: Angiogenesis and growth of implants

Although the role of TGF-βs in angiogenesis in the peritoneum is not well defined at present, mutations in endoglin (CD105), an accessory TGF-β1/3 receptor, is responsible for the autosomal disorder hereditary hemorrhagic telangiectasia-1 (HHT-1; Abdalla and Letarte, 2006). Endoglin is expressed mainly on proliferating endothelial cells and tumor-associated endothelium and is involved in numerous diseases with vascular abnormalities (ten Dijke *et al*., 2008).

Endoglin, a marker of active neo-angiogenesis and activated endothelium, was found on endometrial cells or pericytes in the human uterus (Zhang *et al*. 2002; Hayrabedyan *et al*., 2005) with a preferential localization in the human myometrium (Hayrabedyan *et al*., 2005). Furthermore endoglin staining was found in the microvessels of eutopic endometrium from endometriosis cases (Hayrabedyan *et al*., 2005) but was only increased significantly in the late secretory phase (Kim *et al*., 2001). We conclude that endoglin and TGF-β1 may be involved in the vascular remodelling in endometriotic angiogenesis and thus maintain growth of endometriotic implants in the peritoneum.

Stage 6: Bleeding in pelvic peritoneum, manifestation of endometriosis and clinical consequences

Monthly hormonal cycling leads to menstrual-like bleeding in the pelvic peritoneum resulting in inflammation, adhesion and pain (Matarese *et al*., 2003). Presentation of autoantigens by macrophages and dendritic cells to auto-reactive T cells in the context of their major histocompatibility complex leads to formation of autoantibodies and autoimmunity (Matarese *et al*., 2003). Arguably, combined effects of inflammation, autoimmunity and adhesion are more likely the cause of a clinical picture, typically presenting with pain, fibrosis and infertility. Taken together, we have highlighted that TGF-β and their receptors are involved in most of the biological processes leading to endometriosis, although they are not the sole factors.

Are TGF-βs potent markers for diagnosis of endometriosis?

That retrograde menstruation occurs in almost all women (Liu and Hitchcock, 1986; Eskenazi and Warner, 1997) yet not as many women develop endometriosis is intriguing. Based on previous observations that blind biopsies of normal appearing peritoneum have shown 13–56% occult endometriotic lesions (Murphy *et al*., 1989; Nisolle *et al*., 1990; Balasch *et al*., 1996), Evers *et al*. (2005) asserted that if by extrapolation these researchers had taken 8–16 biopsies in each of these patients, all women with normal peritoneum would have shown evidence of endometriosis. In one study, Machino *et al*. (2005) obtained histological confirmation of endometriotic disease in only 64.5% of classic lesions and in 41.7% of atypical lesions demonstrating

that laparoscopic assessment though currently the gold standard for diagnosis of pelvic disease is fallible. According to Buchweitz *et al.* (2006), a better sensitivity and specificity in detecting endometriotic lesions during laparoscopy is achieved with 5-aminolevulinic acid combined with fluorescent detection. However, it is still unclear whether the recurrence rate after surgery is also improved.

These observations underscore the need for identification of reliable molecular markers or reporter molecules for early diagnosis of endometriosis before investigative surgery. Although many proteins have been described as potential new markers, to date CA-125 (Cancer antigen 125; Mucin 16) is the only validated serum marker used in the non-invasive diagnosis of endometriosis, but with several restrictions. Thus, CA-125 is present in 80% of non-mucinous ovarian carcinomas and is used as a progression marker for human epithelial ovarian cancer, but has a low sensitivity of 27%, despite a high specificity of 97% (Bedaiwy and Falcone, 2004) and therefore it is not advisable to use CA-125 as the only marker for diagnosis of endometriosis. A recent report indicated that CA-125 together with macrophage chemotactic protein-1 (MCP-1), leptin and macrophage migration inhibitory factor (MIF) achieved 93% accuracy in 48% of the patients described (Seeber *et al.*, 2008). Similarly, serum measurements of CA-125 combined with MCP-1 achieved a sensitivity of 92.2%, a specificity of 81.6%, a positive predictive value of 92.3% and a negative predictive value of 83.3% (Agic *et al.*, 2008).

This new approach might be interesting for TGF-β1, because only two conflicting reports have been published to date; one showing no association of higher TGF-β1 levels with higher stages (D'Hooghe *et al.*, 2001), whereas in another study, higher TGF-β1 levels were associated with higher stage-specificity in endometriosis (Pizzo *et al.*, 2002). Similarly, except for one study (Hao *et al.*, 2000), two other studies indicated that subjects with endometriosis exhibit higher levels of TGF-β1 in peritoneal fluid (Oosterlynck *et al.*, 1994; Küpker *et al.*, 1998). However, it is important to stress that in these studies, different enzyme-linked immunosorbent assay (ELISA) protocols were used and, with the exception of one study (Oosterlynck *et al.*, 1994), the authors did not indicate whether or not the total or bioactive levels of TGF-β1 were analyzed. Consequently, we suggest that further studies with higher subject numbers and standardized ELISA protocols are needed before a final conclusion can be reached regarding specificity of the TGF-βs as potent diagnostic markers for endometriosis. Future studies should also take into account that impaired TGF-β1 levels have been demonstrated also in some cancers, autoimmune diseases, artherosclerosis, osteoporosis and fibrosis and that aspirin, tamoxifen or hepatectomy also modulate plasma TGF-β1 levels (Grainger *et al.*, 2000). Although there is no consensus on absolute plasma TGF-β1 levels, many groups have reported highest levels for TGF-β1, moderate and low levels for TGF-β3 and 2, respectively (Grainger *et al.*, 2000).

Conclusions

Despite the recent advances in medical sciences, endometriosis continues to impact negatively on quality of life among affected women. Timely and non-invasive diagnosis is elusive due to unreliability of markers, and the fact that laparoscopic examination, the gold standard for diagnosis of pelvic disease, is fallible.

Stage-specific expression of all TGF-βs and their high-affinity receptors in the human endometrium indicate that they are under negative hormonal control although conclusive evidence is still lacking. We suggest that TGF-βs participate in the initiation of menstruation via vasoconstriction, in menstrual tissue repair and in endometriosis. Consequently, we propose that TGF-βs might be potent factors involved in pathogenesis of endometriosis.

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