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Virus Infection and Type I Interferon in Endometriosis

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1. Introduction

Endometriosis is a chronic disease in which endometrium-like lesions are located ectopically, frequently in the pelvic cavity but also in more distant regions. It has been estimated that 5 to 10% of fertile women are suffering from the disease, and in a population of women with dysmenorrhoea (painful periods), around 50% have endometriosis (Faquhar, 2007). The symptoms include chronic pelvic pain, dysmenorrhoea, dyspareunia (pain during intercourse), and subfertility. The pathogenesis of endometriosis is unclear. Endometriosis is hormonal-dependent and therefore mainly found in women in the fertile age, although rare cases have been found in men and postmenopausal women. The risk is increased seven- to nine-fold for women who have a close relative (mother and/or sister) with endometriosis, indicating some genetic involvement (Simpson et al., 1980). Endometriosis displays malignant-like features, such as invasiveness and metastasis, and DNA viruses might play a role in endometriosis, like human papillomavirus (HPV) is part of the pathogenesis of cervix cancer (zur Hausen, 2009). Signs of inflammation are the key findings in endometriosis. From the above, some evidence points towards a possible involvement of the type I interferons (IFNs). This chapter will discuss whether DNA-viruses and the innate immune system might be involved in the pathogenesis of endometriosis.

1.1 Immunology and inflammation in endometriosis

Inflammation, characterized by activated lymphocytes, neutrophils, and macrophages, is a key feature of endometriosis tissue, associated with the overproduction of prostaglandins, metalloproteinases, cytokines, and chemokines (Bulun, 2009). It is debated whether the peritoneal inflammation is a consequence or a cause of endometriosis, or both. The immune system is involved in the pathogenesis of endometriosis on multiple levels, of which important features will be presented in this section.

It is believed that defective immunosurveillance in women with endometriosis contributes to the attachment, persistence and progression of the endometriosis tissue (Kyama et al., 2003; Osuga et al., 2011). Following retrograde menstruation, endometrial cells in the

peritoneal cavity should be cleared by the immune system, and a number of mechanisms contribute to the failure of this in endometriosis. These mechanisms are summarized in figure 1 and will be addressed here in the order of disease progression.

1.1.1 Viable endometrial cells in the peritoneal cavity

The persistent endometriosis-associated presence of viable peritoneal endometrial cells is partially ascribed to augmented retrograde menstruation, which can directly induce endometriosis, probably by overcoming the immune systems by simple outnumbering (Kyama et al., 2003). Endometrial cell clearance is presumably also reduced by altered phagocytotic properties of peritoneal macrophages. Moreover, decreased cytotoxicity towards autologous endometrial cells by decreased natural killer (NK) cell activity is thought to be an immune defect in endometriosis and is correlated with disease severity (Osuga et al., 2011). The tightly regulated balance between T helper cells type 1 (Th1) and 2 (Th2) is altered towards higher Th2 activity in women with endometriosis. These cells secrete interleukin 4 (IL-4) and IL-10, which are speculated to reduce NK and T-cell cytotoxicity, and thus further reducing immune surveillance.

The endometrial cells themselves also contribute to their own enhanced survival. The transcriptional expression of intercellular adhesion molecule 1 (ICAM-1) is up-regulated in endometriosis lesions, a fact that might contribute to early implantation of peritoneal endometriosis (Wu et al., 2004). The inflammatory cytokines interferon γ (IFNγ) and IL-1β induce ICAM-1 protein expression and the secretion of the soluble form of ICAM-1, which competitively inhibits ICAM-mediated cytotoxicity thus increasing survival. Furthermore, the peritoneal endometrial cells also have a decreased ability to undergo apoptosis.

1.1.2 Endometrial-peritoneal adhesion

The immune system is believed to assist the adhesion of the viable endometrial cells (figure 1). Increased peritoneal infiltration of leukocytes, especially macrophages, and an increased proportion of activated macrophages, is found in endometriosis patients (Haider & Knöfler, 2009). Peritoneal macrophage depletion has been shown to effectively inhibit the initiation and growth of endometriosis implants in rats (Haber et al., 2009), pointing towards a pathogenic effect of macrophages. This inflammation is associated with elevated levels of inflammatory cytokines, growth factors and chemokines.

The pleiotropic pro-inflammatory cytokine tumor necrosis factor α (TNFα) is mainly produced by monocytes and macrophages and stimulates the secretion of the interleukins IL-1, IL-6 and IL-8 by endometriotic cells (Haider & Knöfler, 2009). TNFα can either promote cell regeneration (through the Nuclear Factor kB (NF-κB) pathway) or cell destruction (by initiating the caspase cascade), depending on its local concentration, expression pattern of receptors, and abundance of inhibitors. Macrophages in the peritoneal fluid from women with endometriosis secrete higher levels of TNFα than those from healthy women, and elevated peritoneal fluid levels of TNFα has been detected in women with endometriosis. The adherence of endometrial stromal cells to mesothelial cells is significantly increased by pretreatment of mesothelial cells with TNF-α *in vitro*, suggesting a role in facilitating pelvic adhesion.

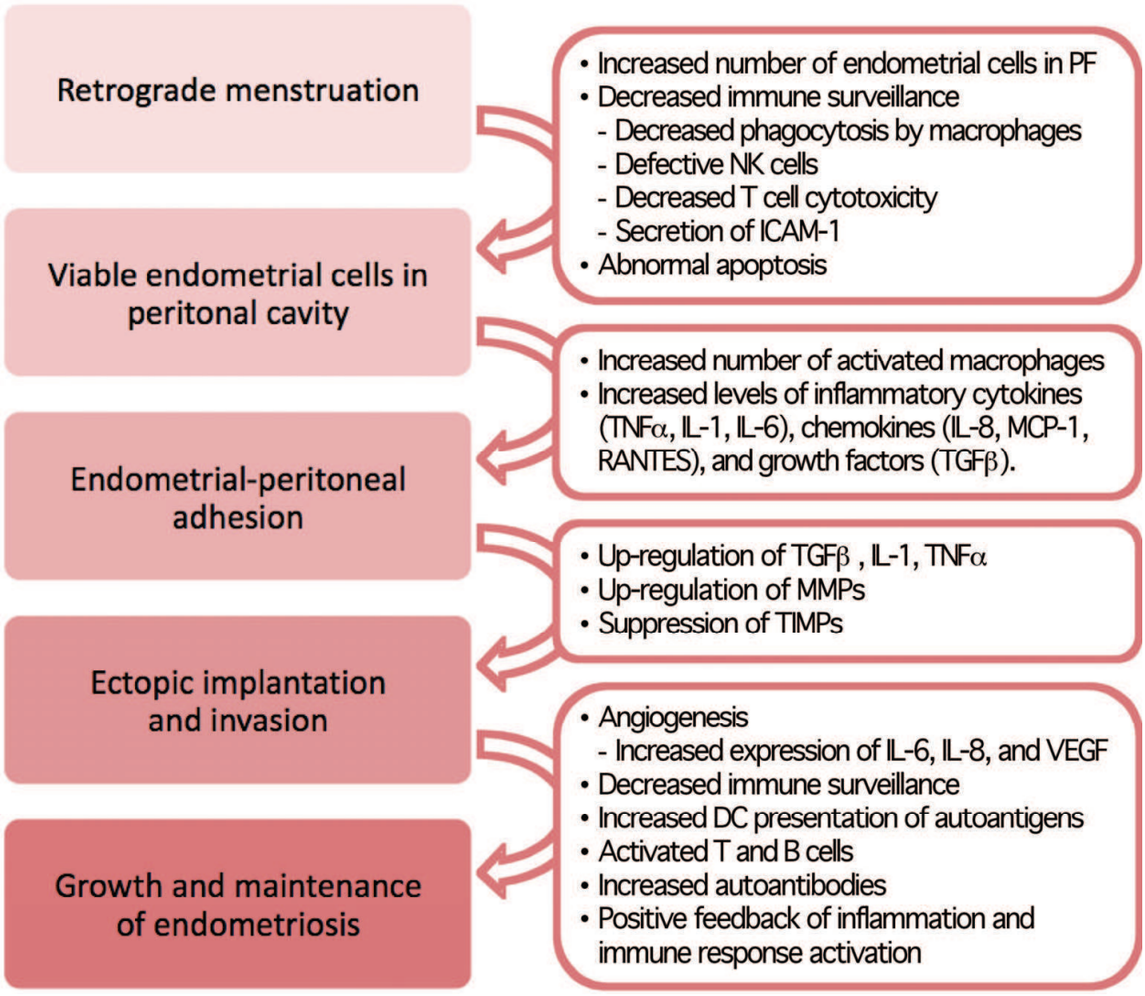

Fig. 1. The role of the immune system in the development and maintenance of endometriosis. Following retrograde menstruation, multiple altered actions of the immune system influence step-wise disease progression. PF, peritoneal fluid; NK cells, natural killer cells; ICAM, Intercellular Adhesion Molecule; TNF, Tumour Necrosis Factor; IL, interleukin; MCP, Monocyte Chemotactic protein; RANTES, Regulated upon Activation, Normal T-cell Expressed and Secreted; TGF, Transforming Growth Factor; MMP, Matrix Metalloproteinase; TIMPs, Tissue Inhibitor of Matrix Metalloproteinases; VEGF, Vascular Endothelial Growth Factor; DC, dendritic cells. Simplified overview inspired by (Kyama et al., 2003).

TNFα also stimulates the growth of both endometrial and endometriotic stromal cells from women with endometriosis, whereas it inhibits the growth of endometrial cells from healthy women (Haider & Knöfler, 2009). The peritoneal fluid level of TNFα is correlated with disease severity. In addition, anti-TNFα therapy effectively reduces endometriosis lesions in rats and baboons (Zulfikaroglu et al., 2010) revealing a potential future medical treatment for endometriosis. However, a placebo-controlled clinical trial of anti-TNFα treatment failed to demonstrate a significant decrease of pain associated with deep endometriosis (see Haider & Knöfler, 2009).

IL-1β and IL-6 are also a pro-inflammatory cytokine found elevated in the peritoneal fluid of women with endometriosis, and macrophages in the peritoneal fluid produce IL-1β *in vitro* in the absence of stimulants (Bondza et al., 2009). Both IL-1α and IL-1β as well as IL-6 are involved in the activation of T-lymphocytes and the differentiation of B-lymphocytes, thereby contributing to the increased peritoneal active immune state in endometriosis.

In addition to these cytokines, a number of chemokines have been implicated in the pathogenesis of endometriosis, believed to contribute by recruiting and activating leukocytes to the inflammatory sites, thereby assisting the adhesion process. Yet another member of the interleukin family, namely the chemokine IL-8 (also named CXCL-8) has been implicated in endometriosis (Bondza et al., 2009). Macrophages in the peritoneal fluid from women with endometriosis secrete higher levels of IL-8 than those from healthy women, which stimulate the growth of endometrial and endometriotic stromal cells. Elevated levels of IL-8 were found in the peritoneal fluid of women with endometriosis, and concentrations correlated with the severity of the disease. IL-8 stimulates the adhesion of endometrial stromal cells to fibronectin (see Kyama et al., 2003) and is therefore likely to contribute to the adhesion of endometrial cells in the pelvic cavity.

Monocyte chemotactic protein-1 (MCP-1, also named CCL2) is one of the most potent chemokines in attracting monocytes. MCP-1 has been found elevated in both peritoneal fluid and serum from women with endometriosis, and in the endometrial glands the level varies according to disease stage (Bondza et al., 2009). Moreover, estrogen up-regulates IL-1-induced MCP-1 expression, which may occur locally in the inflammatory site and contribute to peritoneal macrophage recruitment and activation.

The chemokine RANTES (regulated upon activation, normal T cell expressed and secreted, also named CCL5) plays an important role in recruiting several types of lymphocytes into the endometrium (Fang et al., 2009). It is secreted by stromal cells at the endometriosis lesions upon stimulation with TNFα, IFNγ, or IL-1β. IL-1β treatment yields a higher level of RANTES in endometriotic compared with endometrial cells from endometriosis patients, which in turn display higher levels than normal endometrial cells, entailing increased monocyte chemotactic activity. This is presumed to be a major contributor to leukocyte recruitment and inflammation in the pathogenesis of endometriosis.

It should be noted that the results on whether cytokines and chemokines are involved are equivocal. However, accumulating evidence predominantly reports that both cytokines and chemokines through enhanced local inflammation and cellular adhesion contribute to the pathogenesis of endometriosis.

Transforming growth factor β (TGFβ) peritoneal fluid levels are significantly higher in women with endometriosis compared with healthy control women, and the TGFβ level increases with the severity of the disease (Liu et al., 2009). The adhesion of human endometrial cells to murine peritoneum is increased by treatment with TGFβ (as well as with IL-1β, IL-6, and TNFα) (Omwandho et al., 2009). The same could not be shown for human peritoneal mesothelial cells, but TGFβ enhances trans-mesothelial invasion by primary and immortalized endometrial epithelial cell lines *in vitro* (Liu et al., 2009).

1.1.3 Ectopic implantation and invasion

TGF-β might not only facilitate adhesion but also implantation in endometriosis (figure 1). Its expression in the endometrium varies with the menstrual cycle, peaking at the time of menstruation (Omwandho et al., 2009). This expression pattern is roughly opposite to that of TNFα, and co-expression with progesterone occurs in the secretory phase (figure 2). TGFβ has been shown to mediate progesterone-associated suppression of matrix metalloproteinase (MMP) expression in endometrial tissue, and it is required for progesterone action in the prevention of experimental endometriosis (Bondza et al., 2009).

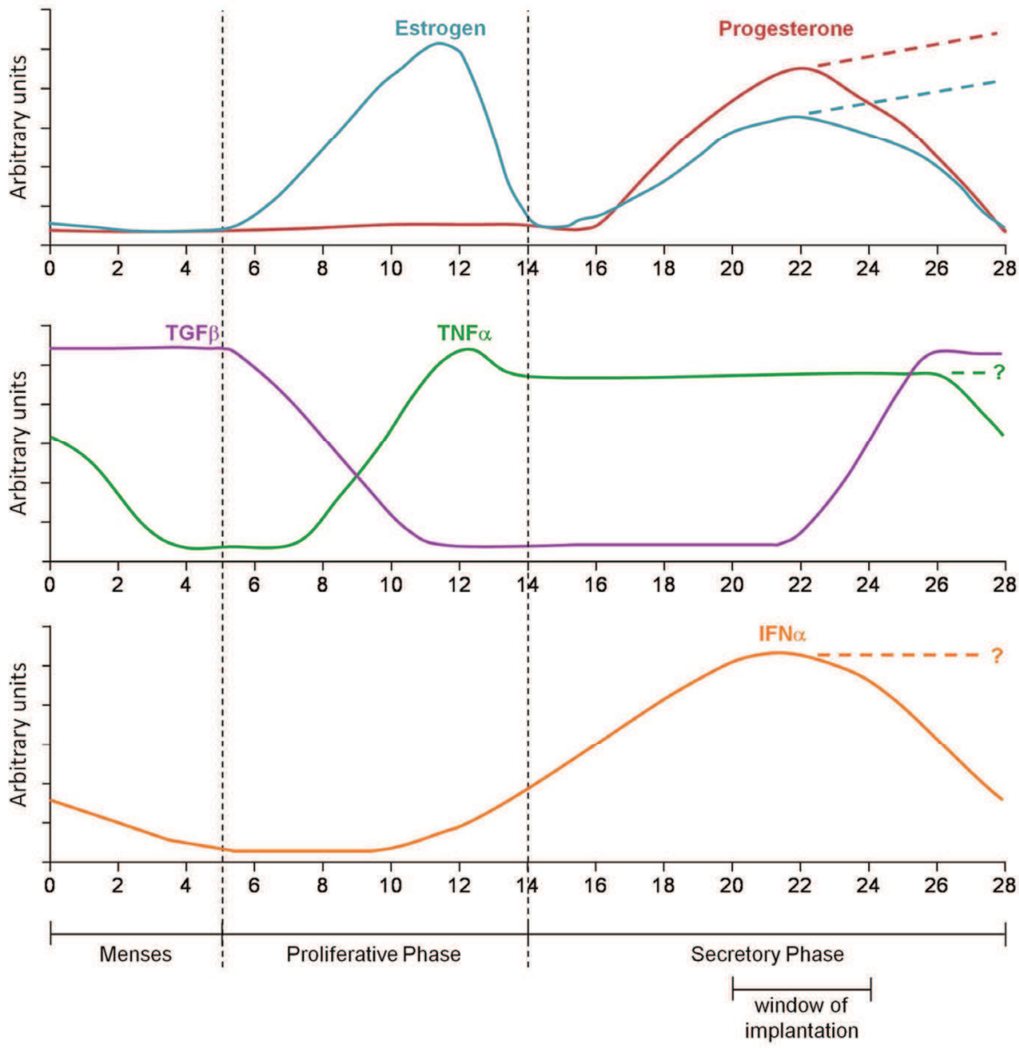

Fig. 2. Levels of estrogen, progesterone, TGFβ, TNFα, and IFNα in the human endometrium during menstrual cycling. Numbers denote days after the first menstrual day. Dashed lines show levels following implantation. There are indications that TNFα and IFNα levels are up-regulated in early pregnancy. TGF, Transforming Growth Factor; TNF, Tumour Necrosis Factor; IFN, interferon. Schematic representation based on (Li et al., 2001; Haider & Knöfler, 2009; Omwandho et al., 2009).

MMPs are important for the control of extracellular matrix turnover, and they are believed to influence the implantation of adhered endometriotic cells in endometriosis (Kyama et al., 2003). The level of MMPs is in up-regulated and tissue inhibitors of matrix metalloproteinases (TIMPs) down-regulated in the peritoneal fluid from women with endometriosis compared with controls (Bondza et al., 2009). MMPs are upregulated in response to the inflammatory cytokines TNFα and IL-1 and are expressed during the proliferative and menstrual phases but suppressed by progesterone in the secretory phase. In the absence of a normal progesterone response of the endometriosis lesions, sensitivity to TGFβ may be altered, and this may result in a failure to down-regulate MMPs, thus contributing to the implantation failure.

1.1.4 Growth and maintenance of endometriosis

Following implantation, the growth and maintenance of endometriosis lesions is influenced by a number of factors (figure 1). First of all, the implanted tissue is in need of vascularization and an increase in the concentration of several angiogenic factors has been reported. IL-6 and IL-8, which as stated above are up-regulated in endometriosis, both promote angiogenesis (Bondza et al., 2009). IL-1β, which is also elevated in the peritoneal fluid of endometriosis patients, induces IL-6, the vascular endothelial growth factor (VEGF), and an angiogenic phenotype in endometriotic but not endometrial stromal cells. Women with moderate and severe endometriosis have elevated levels of both IL-6 and VEGF in the peritoneal fluid compared with healthy controls (Kyama et al., 2003), underlining that angiogenesis is a feature of disease exacerbation. Furthermore, high proliferative activity of endometriosis lesions is associated with increased levels of VEGF and its receptor as well as increased microvessel density (Bondza et al., 2009).

A positive feedback of activated immune response contributes further to the maintenance of endometriosis. The reduced NK cell activity has been suggested to result in less effective killing of autologous dendritic cells (DCs) loaded with endometrial self-antigens. This would facilitate self-antigen presentation to autoreactive T cells and the subsequent production of autoantibodies (Kyama et al., 2003), pointing towards a pathogenic contribution by an autoimmune disease mechanism. Indeed, the frequency of autoantibodies towards endometrial antigens is elevated in both serum and peritoneal fluid of women with endometriosis compared with healthy women (Osuga et al., 2011). Activation of Th2 immune response in endometriosis and elevated B cell levels has been reported both systemically and locally in the endometriosis lesions, supposedly causing sustained autoantibody production and continuous infiltration of immune cells, thus maintaining the immunological contribution to the pathogenesis of endometriosis.

2. Virus and endometriosis

Pathogenic DNA virus infection has been associated with the aetiology of several human diseases. The double-stranded (ds) DNA viruses include herpesviruses, polyomaviruses, papillomaviruses, hepadnaviruses (e.g. hepatitis B virus (HBV)), adenoviruses and poxviruses. With the exception of poxviruses, these viruses often establish persistent or latent infections and can be reactivated in both healthy and immunosuppressed persons.

Only a few studies have addressed the possible involvement of a pathogenic virus in the aetiology of endometriosis (Oppelt et al., 2010; Vestergaard et al., 2010). The presence of specific human herpes viruses, human polyomaviruses, and human papillomaviruses was analysed. Also, the presence of human endogenous retroviruses have been investigated in endometriosis lesions (Hu et al., 2006; Oppelt et al., 2009), and these studies will be discussed in the following section.

2.1 Herpes viruses

The *Herpesviridae* are a large, diverse family of double-stranded, enveloped viruses. Eight human herpesviruses (HHVs) have been identified: Epstein-Barr virus (EBV, of the subfamily *Gammaherpesvirinae*), cytomegalovirus (CMV or HHV5 of the subfamily *Betaherpesvirinae*), herpes simplex virus type 1 and 2 (HSV-1 and HSV-2 of the subfamily *Alphaherpesvirinae*), varicella zoster (VZV), HHV6, HHV7, and the Karposi's sarcoma herpes virus (KHSV or HHV8). The seroprevalence of each of these herpes viruses varies according to a number of demographic factors (Prober, 2011). For EBV, CMV, and HSV-1 the prevalences range from 50 to 75%, whereas the prevalence for HSV-2 is about 25%. For VZV, HHV6 and HHV7 the seroprevalence is almost 100%, whereas the prevalence for HHV8 is less than 10%. The *Herpesviridae* family are found in many human tissue types, including the fallopian tubes (salpingitis) and the endometrium (see Vestergaard et al., 2010). These viruses are commonly distributed as asymptomatic infectious agents but are also all associated with diseases, such as genital sores or a variety of malignancies. EBV can cause infectious mononucleosis and has been associated with lymphomas of B, T and NK cell origin, but also cancers of epithelial origin like nasopharyngeal carcinomas and gastric adenocarcinomas (Dolcetti & Masucci, 2003). EBV maintains latency in B cells. CMV can cause pneumonitis and delayed neurological complications like sensory neural hearing deficits and learning disabilities (Brown & Abernathy, 1998). CMV maintains latency in monocytes and macrophages. HSV-1 and HSV-2 can cause encephalitis and genital ulcerative disease (Wilson et al., 2009). The HSVs maintain latency in neuronal cells. VSV cause chickenpox (varicella) as primary infection following which it becomes latent in neuronal cells (Kennedy & Cohrs, 2010). VZV can be reactivated and thus cause herpes zoster (shingles) and extremely painful vesicular eruption. HHV6 cause roseola during the primary infection (Prober, 2011). HHV6 becomes latent in salivary glands, the brain and in mononuclear cells or macrophages. Most reactived HHV6 infections are asymptomatic.

The presence of most of the herpes viruses in endometriosis has been analysed., Endometriosis samples from 32 patients were analysed for the presence of EBV, CMV, HSV-1 and HSV-2 by multiplex PCRs (Vestergaard et al., 2010). None of the clinical samples were positive for any of these four herpes viruses. In another study, 66 endometriosis samples from 56 patients were tested for the presence of EBV, CMV, HSV-1, HSV-2, VZV, and HHV6 by a PCR based analysis (Oppelt et al., 2010). Also this study failed to detect the presence of any of these six herpes viruses. Apart from HHV7 and HHV8, which has not yet been analysed, the six herpes viruses EBV, CMV, HSV-1, HSV-2, VZV, and HHV6 do not seem to be involved in the pathology of endometriosis.

2.2 Polyomaviruses

The well-described human polyomaviruses of the *Polyomaviridae* family, JC (JCV), BK (BKV) and simian virus 40 (SV40) are widely distributed in the general population (Moens & Johannessen, 2008). Thus antibodies against JCV and BKV have been found in more than 75% of the human adult population, and up to 15% of healthy humans are seropositive for SV40. These three polyomaviruses have been found in many different tissues (see Vestergaard et al., 2010). BKV has been found in the kidney tubule epithelium, urethral epithelium, the uterine cervix, and in the spleen. JCV has been found in tongue squamous cell epithelium, urethral epithelium, and in the spleen, whereas SV40 has been found in the liver and the mesothelium. Furthermore, these three polyomaviruses induce tumours in animal models and are able to transform cultured human cells (Moens & Johannessen, 2008). In 2007, two new human polyomaviruses, WU polyomavirus (WUV) and KI polyomavirus (KIV) were discovered in respiratory tract secretions and have subsequently been detected in faeces, blood, and lymphoid tissue (see Vestergaard et al., 2010). The full spectrum of their tissue tropism and their role in disease has yet to be elucidated. More recently, yet another human member of the *Polyomaviridae* family, the Merkel cell polyomavirus (MCV) was described in apparent association with Merkel cell carcinoma, an aggressive form of skin cancer (Moens & Johannessen, 2008). When analysed, none of these polyomaviruses were detected either in the endometrium or in endometriosis, suggesting an infrequent presence of polyomaviruses in the endometrium (Vestergaard et al., 2010).

2.3 Human papillomaviruses

Closely related to the polyomavirus family, the papillomavirus family is a very significant example of the large impact of a broad spectrum of pathogenic DNA viruses, which have emerged during the last two decades. More than 118 papillomaviruses have been fully described, and new virus types are constantly emerging (zur Hausen, 2009). The prevalence of human papillomavirus (HPV, of the family *Papillomaviridae*) in cervical carcinomas is 99.7%. Specific high-risk HPV types have been shown to cause the vast majority of cervical cancers as well as a substantial proportion of other anogenital and head and neck cancers as well as certain cutaneous cancers. HPV has not yet been detected in intraperitoneal tissues, but HPV has been detected in blood, including on the surface of peripheral blood mononuclear cells, suggesting a potential alternative route of transmission (see Vestergaard et al., 2010).

The prevalence of HPV in endometriosis has been analysed (Oppelt et al., 2010; Vestergaard et al., 2010). In one study, no HPV DNA was found in the endometriosis samples from 32 patients (Vestergaard et al., 2010). In another study, certain high-risk HPV types was found in endometriosis lesions (Oppelt et al., 2010). However, due to previous cervical HPV infections in the analysed patients, it was concluded that the detected HPV in the endometriosis samples possibly originated from these associated malignant transformations and might not have any association with the endometriosis *per se*.

In the endometrium, a HPV prevalence of less than 10% was found in samples from both women with endometriosis as well as controls, which is remarkably low compared with the well-known high frequencies of infection with these viruses (Vestergaard et al., 2010). It is

estimated that 75% to 80% of sexually active individuals are infected with HPV during their lifetime, with the highest rates in women younger than 25 years of age. However, as the mean age of the women enrolled in this particular study were between 32 and 36 years of age, most HPV infections would have been cleared by the immune system, correlating well with the fact that none of these women had genital HPV infection in their cervical smears at the time of analysis. However, it is possible that transient HPV infections could cause the initiation of malignant-like processes by a "hit-and-run" mechanism and in this way contribute to the initiation of endometriosis. This theory is yet to be investigated further.

2.4 Endogenous retroviruses

Retroviruses are RNA viruses, which integrate into the genome of the host cell in the form of a DNA copy, which is denominated the provirus. Endogenous retroviruses refer to proviruses integrated into germ line cells, which are transmitted from one generation to the next. Approximately 5% of the human genome consists of complete and partial sequences from human endogenous retroviruses (HERVs) (Muir et al., 2004). The significance and consequences of the presence of HERVs in the human genome have been the subject of intensive investigation. HERVs have been implicated in autoimmune diseases and neoplasia as well as in placental function and protection from exogenous retroviral infection. Considering the placental function, it is now believed that syncytin, a retroviral envelope protein encoded by the endogenous retrovirus HERV-W, is involved in the fusion of the cytotrophoblast cells to form the syncytial layer of the placenta, and the envelope protein encoded by ERV3 has been associated with cytotrophoblast differentiation. Other endogenous retroviruses like HERV-E also seem to be involved in placental function (reviewed in Muir et al., 2004).

Expression of HERVs in endometriotic tissues has been detected, indicating that endogenous retrovirus expression might be involved in endometriosis (Hu et al., 2006; Oppelt et al., 2009). Endometriosis samples from 14 women were analysed for the presence of HERV-E HERV-W, HERV-I/T, and HERV-H mRNA by PCR analysis (Hu et al., 2006). It was found that HERV-E was expressed at higher levels in the endometriosis samples than in normal endometrium control samples. In another study, 15 endometriosis samples showed low levels of RNA encoding the HERV-W encoded envelope protein syncytin, as analysed by reverse transcriptase PCR (Oppelt et al., 2009). It was also found in this study that the endometriosis samples did not show an elevated expression of the HERV-W encoded envelope protein syncytin. Interestingly though, samples from the endometrium of women with endometriosis showed an increased expression of syncytin RNA compared with endometrial samples from controls, suggesting that this protein might be involved in the pathology of endometriosis. At this point, the involvement of endogenous virus expression in the pathology of endometriosis is not clarified. Further studies are required, analysing larger groups of endometriosis samples and including tissue samples from the endometrium of women with and without endometriosis.

3. Type I interferon and endometriosis

We will now focus on the putative implication of the important immune modulating cytokine family type I interferons in endometriosis, as a line of circumstantial evidence

indicates that these cytokines could be involved. A key constituent of the immune system that has been only slightly investigated in relation to endometriosis is the family of interferons (IFNs). IFNs are cytokines secreted from cells in response to viral challenge and various other stimuli. The human interferons are by structural homology classified into type I, which consists of IFNα (counting 13 subtypes), IFNβ, IFNε, IFNκ, and IFNω, type II, which is only IFNγ, and type III, the more recently discovered IFNλ (comprising 3 subtypes) (Platanias, 2005; Hall & Rosen, 2010). Secreted IFNs bind to specific plasma membrane receptors, which initiate intracellular pathways leading to the transcription of hundreds of interferon stimulated genes (ISGs) (Platanias, 2005; Hall & Rosen, 2010). In spite of their common signaling pathway, IFNα and IFNβ display specific activity profiles.

Main biological activities resulting from IFN signaling are primarily antiviral but also anti-proliferative, anti-angiogenic, and antigen-presenting effects as well as regulation of pro- and anti-apoptotic genes and proteins (Platanias, 2005). The current state of the cells and complex balances of feedback mechanisms determine the overall outcome of the IFN response, *e.g.* survival vs. apoptosis. Recombinant human IFNs (mainly IFNα and IFNβ) are used as therapeutic agents against a variety of cancers, multiple sclerosis, and viral diseases, such as hepatitis B and C.

Type II IFN is produced only by activated T or NK cells and its primary role is to modulate the adaptive immune response, *e.g.* by contributing to the activation of macrophages and T cell development (Hall & Rosen, 2010). Expression of the receptors of type III IFN is primarily limited to epithelial and DCs, thus restricting their scope of action. We will only focus on type I IFNs and their possible role in endometriosis.

High levels of type I IFNs are secreted rapidly by most cell types upon stimuli (Hall & Rosen, 2010). Type 1 IFNs have immunostimulatory effects on NK cells, macrophages and DCs, which all are essential effector cells in the innate immune system. Among the downstream targets of type I IFN signaling are the IFNs themselves as well as associated receptors, signal transducers and transcription factors enabling a strong feed-forward mechanism. It is strongly believed that type I IFNs are directly involved in the pathogenesis of autoimmune disease by enhancing the self-amplification of systemic autoimmunity (Hall & Rosen, 2010). The aberrant antigen-presentation of debris from apoptotic cells and the resulting production of autoantibodies in endometriosis could be speculated to give rise to a type I IFN-amplified autoimmune pathogenic mechanism. In autoimmunity, self antigens elicit an immune response that includes substantial production of type I IFNs. Upon DC presentation of endometrial autoantigens, type I IFN may then promote monocyte differentiation, DC survival and cytotoxic T cell activity, which may enhance killing of endometrial cells presenting autoantigens. Debris from dying cells is then taken up by DCs and presented for recognition by T cells in a self-amplifying loop. Type I IFNs also induce the differentiation of B cells, which would promote autoantibody production. Immune complexes of endometrial self antigens and autoantibodies would further amplify the IFN production, constituting yet another positive feedback loop.

The approved drug Intron A (human recombinant IFNα-2b) has been proposed as a possible immunomodulatory therapeutic against reoccurrence of endometriosis cysts after surgery. The hypothesis was that this could enhance the cytotoxic activity of macrophages and

natural killer cells and thus reduce the growth of endometriosis tissue (Acién et al., 2002). Rats with surgically induced endometriosis showed persistent significant reductions in the implant sizes upon intraperitoneal or subcutaneous administration of human IFNα-2b. However, a clinical study showed that intraperitoneal administration of IFNα-2b following surgical treatment for endometriosis increased the cyst reoccurrence rate significantly after 21 months. Hence, the normal anti-proliferative effects of type I IFNs appear to be challenged in endometriosis.

The endometrial level of IFNα mRNA is increased in the mid-secretory phase of the human menstrual cycle (Li et al., 2001), which is the putative window of implantation (figure 2). It has been found that the transcription of IFNα and the interferon α receptor 2 (IFNAR2) is highly up-regulated in the endometrium of women suffering from endometriosis compared with healthy women (Kao et al., 2003). Moreover, *JAK1*, which has an important function in type I IFN signaling, is up-regulated in endometriosis stromal cells compared with the endometrial cells from endometriosis patients (Matsuzaki et al., 2006). The sum of these findings suggests a role of type I IFN in the human endometrium and a possible dysregulation in endometriosis.

The possible involvement of type I IFNs in endometriosis has recently been investigated directly (Vestergaard et al., 2011). A type I interferon-specific PCR Array indicated significantly down-regulated transcription of the genes *HOXB2* and *ISG20* in endometriosis lesions compared with endometrium from endometriosis patients and healthy controls, but no difference in the expression of any other interferon stimulated genes were observed. These results were independent of the menstrual phase. As only two out of 84 genes of the type I IFN response was significantly dysregulated in endometriosis, the type I IFNs do not appear to be generally involved in the pathogenesis of endometriosis. Yet, specific gene regulation involving type I IFNs could still play distinct roles in endometriosis. The putative involvement of *ISG20, HOXB2* and *HOXA10* in the pathogenesis will now be discussed.

3.1 The ISG20 protein

The transcriptional expression of *ISG20* (interferon-stimulated gene product of 20 kDa, also called *HEM45*) was shown by validated qRT-PCR to be highly down-regulated in endometriosis lesions (Vestergaard et al., 2011). *ISG20* transcription is induced synergistically by type I and II IFNs, induced by estrogen, and regulated in a progesterone-dependent manner in mice, especially in the mouse endometrium. This gene encodes a 3′ to 5′ exonuclease with specificity for single-stranded RNA (and to a lesser extent for DNA), which is localized in the nucleus and is associated with promyelocytic leukemia (PML) protein nuclear bodies. The exonuclease has been proposed to down-regulate the estrogen-dependent transcriptional response by degrading estrogen-induced mRNAs within PML oncogenic domains (PODs). The subcellular localization of ISG20 in the nucleus argues for its involvement in the maturation rather than in the degradation of mRNAs.

ISG20 has also been implicated in the anti-angiogenic properties of interferon (Taylor et al., 2008). In an *in vitro* angiogenic assay system, ISG20 was found to be up-regulated in endothelial cells treated with interferon, however, overexpression of ISG20 did not lead to

reduced angiogenesis *per se*. However, overexpression of the enzymatically inactive, dominant-negative ISG20 mutant inhibited angiogenesis in this system and potentiated the anti-angiogenic properties of interferon. How ISG20 might be involved in angiogenesis is currently not clear.

The ISG20 protein mediates antiviral effects of interferons by inhibiting the replication of several RNA viruses, like vesicular stomatitis virus, influenza virus, encephalomyocarditis virus, West Nile virus, Dengue virus, hepatitis A and C viruses, yellow fever virus, and bovine viral diarrhea virus (Zhou et al., 2011). The antiviral activity of ISG20 is only observed with enzymatically active ISG20 expression, since expression of an ISG20 mutant without enzymatic activity, did not possess the same antiviral activities.

Importantly, ISG20 mRNA was found to be up-regulated in the uterine epithelium during the implantation window in mouse (Pan et al., 2006). Whether abolished ISG20 enzyme activity is implicated in the pathogenesis of endometriosis or a marker of altered hormonal expression, or both, needs to be further investigated.

3.2 The HOX proteins

The HOX proteins encoded by the homeobox genes are DNA binding transcription factors known to regulate embryonic development. In addition, the *HOX* genes are dynamically expressed in the endometrium during the menstrual cycle, where they are necessary for endometrial growth, differentiation, and implantation (Cakmak & Taylor, 2010; Zanatta et al., 2010). Furthermore, HOX proteins are molecular mediators of the steroid hormones during endometrial cell development.

It has been suggested that a HOX gene-related defect in endometrial development exists in patients with endometriosis (Zanatta et al., 2010). According to this theory, endometriosis might originate from estrogen stimulated metaplasia of mesenchymal embryonic cells distributed in the pelvis during organogenesis. However, transcriptional dysregulation of the HOX genes in the adult endometrium compatible with the more well-supported retrograde menstruation pathogenesis model is also well substantiated. Recently, a systematic dysregulation of *HOX* genes in the endometrium from healthy women compared with the endometrium in women with endometriosis has been demonstrated (Borghese et al., 2008). A down-regulation of *HOXA* and *HOXB* genes and an up-regulation of HOXC genes were found in endometrium from women with endometriosis compared with endometrium from healthy women.

The HOXA10 protein is up-regulated in response to circulating estrogen and progesterone in the healthy endometrium, thus indicating a role in endometrial maturation, implantation and maintenance of pregnancy implantation (Cakmak & Taylor, 2010; Zanatta et al., 2010). *HOXA10* transcription is normally up-regulated in the endometrium during the window of implantation but this up-regulation is abolished in women with endometriosis. Low levels of HOXA10 could explain the lower fertility of women with endometriosis, and this is further supported by studies of the HOXA10 knock-out mice, in which the targeted disruption of the *HOXA10* gene generated uterine factor infertility. Also in the endometriotic tissue, the HOXA10 transcriptional levels were found to be low

(Langendonckt et al., 2010). The endometrial down-regulation of HOXA10 protein in women with endometriosis seems to be due to increased methylation of the *HOXA10* genomic enhancer region in the endometrium leading to epigenetic silencing of this gene (reviewed in Cakmak & Taylor, 2010). In conclusion, low levels of HOXA10 may result in resistance to progesterone action in the endometriotic tissue.

HOXB2 is part of the *HOX* gene family involved mainly in embryonic development. A very solid down-regulation of *HOXB2* in endometriotic lesions compared with endometrium from both endometriosis and healthy women has been observed (Vestergaard et al., 2011). Little is known about *HOXB2* expression in the endometrium, but several studies have demonstrated that *HOXB2* expression is altered in tumours. In a xenograft breast tumour mouse model, HOXB2 acts as a negative tumour growth regulator, since *HOXB2* expression decreases proliferation of tumour cells (Boimel et al., 2011). Other results have shown that overexpression of HOXB2 in pancreatic, lung and cervical cancer was associated with malignancy. However, a more in-depth analysis correlated lower HOXB2 expression with higher grades of tumours. Finally, it has been reported that the HOXB2 protein binds the interferon-induced protein p205, involved in the growth inhibitory activities of interferon (see Vestergaard et al., 2011). Whether HOXB2 interaction with p205 modifies the growth inhibitory activities of p205 has not yet been investigated. Further studies are needed to determine the mechanism and implications of the abolished *HOXB2* expression in endometriosis lesions.

4. Conclusion

The pathogenesis and the similarity to cancer invasiveness suggested that a viral background could be part of the pathogenesis of endometriosis, but so far no investigations have demonstrated this connection in the aetiology. The prevalence of pathogenic dsDNA viruses in the human endometrium was found to be generally low (0-10%), and nothing points towards any evidence that endometriosis is caused by currently known DNA viruses (Oppelt et al., 2010; Vestergaard et al., 2010). It can be speculated that the endometrium and endometriotic tissue is difficult to access or simply an unfavorable environment for virus progression, leading to a generally low prevalence in these deeper tissues. It is possible that viruses can infect the endometrium transiently but subsequently be either shed with the endometrial tissue during menstruation or be rapidly cleared by an efficient immune response. Thus stable infections of the endometrium would not be frequent. However, a pathogenic virus could theoretically initiate a malignant cell process during a shorter infectious period and then flee the scene. This “hit-and-run” strategy has been previously shown *e.g.* for CMV *in vitro* and indicated in clinical studies of both polyomaviruses and papillomaviruses (see Vestergaard et al., 2010). This could explain why no virus DNA so far has been found associated with endometriosis lesions.

To address the viral “hit-and-run” strategy hypothesis, one could analyse for an elevated level of serum antibodies against the viruses, which would then show previous viral infections. Even though a broad selection of the most common pathogenic DNA viruses have been tested for, other more rare or even undiscovered viruses or bacteria might still be involved. Conclusively, the prevalence of pathogenic DNA viruses in the endometrium

and endometriosis lesions is very low and does not indicate a virological cause of endometriosis. However, the existence of a causative infectious agent can still not be ruled out at this point.

The altered expression of a large number of genes has been reported in endometriosis (Kao et al., 2003; Matsuzaki et al., 2006), an imbalance mediated by altered levels of a number of signaling molecules as well as by epigenetic alterations. Dysregulation of a large number of hormones, cytokines, chemokines, and growth factors is a feature of endometriosis. The abolished expression of *HOXA10*, *HOXB2* and *ISG20* in the endometriosis tissue seems to be influenced by a number of these factors, which regulate gene transcription via complex, overlapping mechanisms, possibly in combination with the action of estrogen and progesterone. The observed abolishment of *ISG20* and *HOXB2* expression in endometriosis lesions indicates that they fail to be induced by estrogen. The altered ERα/ERβ ratio in endometriosis could be important in this mechanism. The lack of *HOXA10* expression in the endometrium as well as in endometriotic lesions is probably due to epigenetic changes in the gene promoter, resulting in abolished response to estrogen and progesterone. Moreover, the expression of other *HOX* genes in endometriosis might shed a light on the function of this family of proteins in the development of this disease.

As the aetiology of endometriosis is unknown, and as several factors are thought to influence the course of the disease, the pathogenesis of endometriosis is still a challenge. One of the main problems is the lack of a non-invasive test to diagnose endometriosis. Several markers have been investigated. The best known is CA125, which is not specific to endometriosis but frequently also elevated in cancer. CA125 is often elevated in endometriosis, and the level is to some extend related to the degree of the infiltration in active endometriosis. However, the CA125 levels are individual, and change in intensity of pain or other symptoms related to endometriosis are not automatically reflected in the level. Therefore, the identification of a non-invasive marker able to diagnose endometriosis would be of great importance. Furthermore, if this marker could indicate changes in disease intensity this would be a great help both for the patient and the doctor. The success of different treatments could also be measured more objectively e.g. by an independent marker. So far hormonal treatment is the first line of treatment, and if pain cannot be resolved by medication, surgery may be performed. However, the endometriosis often reoccurs and more operations are needed, thus bringing the risk of complications.

As clarified in this chapter, many possibilities for causative or aetiological mechanism for the pathogenesis of endometriosis are in focus. If the aetiology of endometriosis is to be found related to virus, we can probably in the future prevent the disease or at least weaken the intensity. A marker for the disease based on virus aetiology would most likely be possible to develop, and the development of a specific treatment could be feasible. If HOX genes and/or ISG20 are involved in the aetiology of endometriosis, this would also give possibilities for new diagnostic tools and medical treatments.

In conclusion, the possible involvement of viruses, type I IFNs, and the innate immune system in the pathogenesis of endometriosis is yet unclear. More investigations are needed in order to resolve the riddle of endometriosis.

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This book provides an insight into the emerging trends in pathogenesis, diagnosis and management of endometriosis. Key features of the book include overviews of endometriosis; endometrial angiogenesis, stem cells involvement, immunological and hormonal aspects related to the disease pathogenesis; recent research reports on infertility, endometrial receptivity, ovarian cancer and altered gene expression associated with endometriosis; various predictive markers, and imaging modalities including MRI and ultrasound for efficient diagnosis; as well as current non-hormonal and hormonal treatment strategies This book is expected to be a valuable resource for clinicians, scientists and students who would like to have an improved understanding of endometriosis and also appreciate recent research trends associated with this disease.

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