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Peritoneal endometriosis is an inflammatory disease

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1. ABSTRACT

Peritoneal endometriosis is a chronic inflammatory disease characterized by increased numbers of peritoneal macrophages and their secreted products. Inflammation plays a major role in pain and infertility associated with endometriosis, but is also extensively involved in the molecular processes that lead to peritoneal lesion development. Peritoneal oxidative stress is currently thought to be a major constituent of the endometriosis-associated inflammatory response. Excessive production of reactive oxygen species, secondary to peritoneal influx of pro-oxidants such as heme and iron during retrograde menstruation, may induce cellular damage and increased proinflammatory gene expression through nuclear factor-kappa B activation. In particular, prostaglandin biosynthetic enzyme expression is regulated by this transcriptional factor, and increased peritoneal prostaglandin concentrations have been demonstrated in endometriosis. In the light of available data collected from patient biopsies, as well as *in vitro* and *in vivo* studies, the respective involvement and potential molecular interactions of iron, nuclear factor-kappa B and prostaglandins in the pathogenesis of endometriosis are explored and discussed. The key role of peritoneal macrophages is emphasized and potential therapeutic targets are examined.

2. INTRODUCTION

Endometriosis is a common, benign gynecological disorder affecting at least 10% of all reproductive-age women and is associated with pain and infertility (1). It is characterized by the presence of endometrial tissue outside the uterine cavity, mainly on the pelvic peritoneum, but also on the ovaries and in the rectovaginal septum (2). Despite being one of the most frequently recognized gynecological diseases, the etiology of endometriosis remains unclear due, in part, to its multifactorial characteristics. Indeed, a growing body of evidence indicates that a combination of genetic, hormonal, environmental, immunological and anatomical factors may play a role in the pathogenesis of this disorder (3).

In most menstruating women, menstrual effluent containing endometrial cells and blood is transported into the abdominal cavity through the fallopian tubes (4). This phenomenon, known as retrograde menstruation, was first described in 1927 by Sampson (5), who suggested that it may be the origin of peritoneal endometriosis. Although retrograde menstruation occurs in most cycling women with patent tubes, clinical endometriosis develops in only 10 to 15% of women during their reproductive life. Additional factors that increase susceptibility to

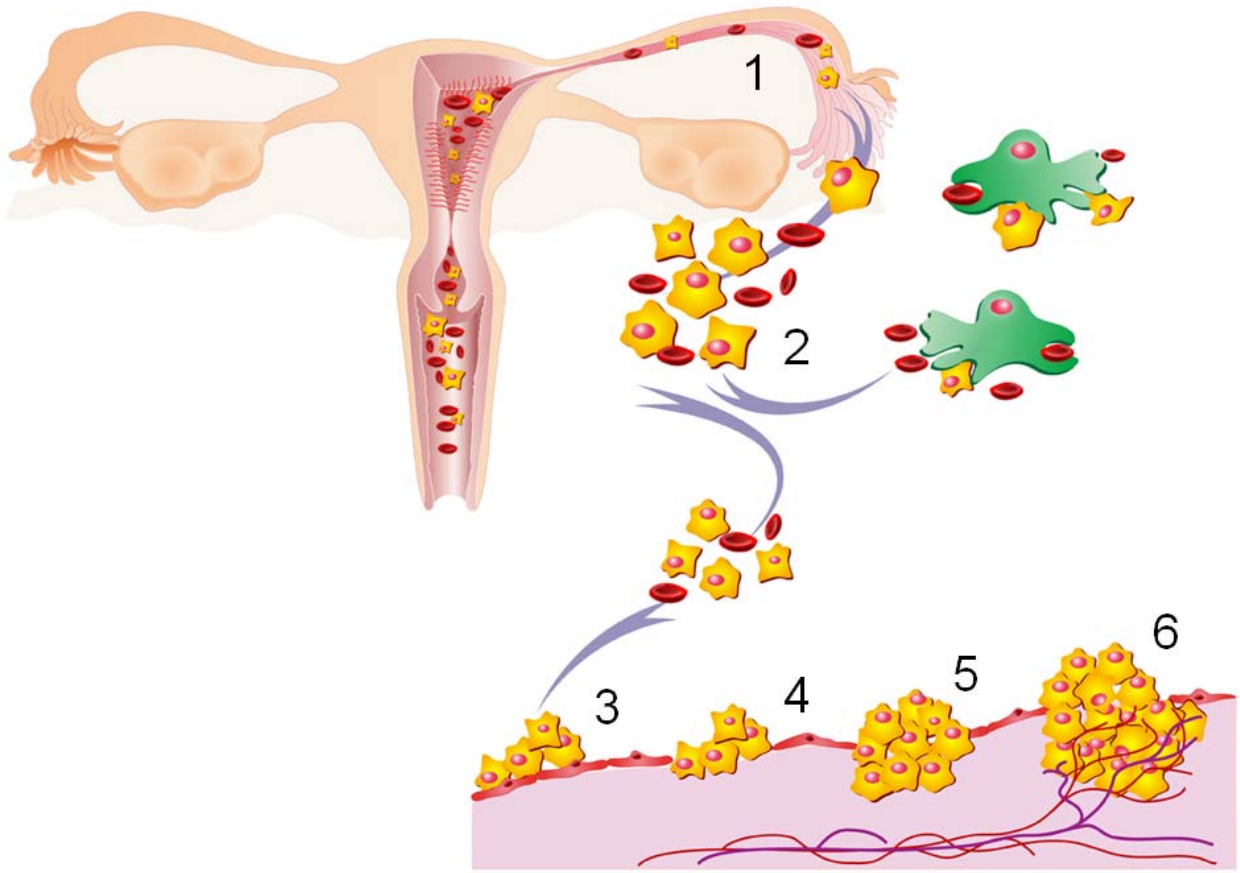

Figure 1. The retrograde transplantation theory. During menses, endometrial tissue and erythrocytes are retrogradely shed through the fallopian tubes into the peritoneal cavity (1). Endometrial tissue fragments evade the immune surveillance system, particularly peritoneal macrophages (2), adhere to the peritoneum (3), invade the peritoneal mesothelial lining (4), proliferate (5) and acquire a blood supply (6), leading to macroscopic peritoneal endometriotic lesion development.

endometriosis must therefore exist and remain to be identified. The development of peritoneal endometriotic lesions involves a whole series of events, starting with the survival of refluxed endometrial cells and evasion of the immune surveillance system, adhesion of these cells to the peritoneum, invasion of the mesothelial lining and degradation of the underlying extracellular matrix, proliferation, resistance to apoptosis and, finally, generation of neovascularization (2,6) (Figure 1).

Among the multiple factors known to be involved in endometriosis pathogenesis, an immunological/inflammatory etiology has been suggested. Endometriosis is now considered to be a chronic local pelvic inflammatory process, with this local inflammation playing a major role in both disease development and associated pain and infertility. Supporting this concept are numerous studies reporting that peritoneal fluid (PF) of women with endometriosis contains increased numbers of activated macrophages and other immune cells that secrete various local products, such as cytokines, growth and angiogenic factors (7). Peritoneal oxidative stress is thought to be a major component of endometriosis-associated inflammation, as it may regulate expression of numerous genes encoding immunoregulators, cytokines and cell adhesion molecules (8).

2.1. Peritoneal fluid and environment in peritoneal endometriosis

Changes in PF volume and the presence of various cells, hormones and other compounds have been studied during normal menstrual cycles and in pathologic conditions. Syrop and Halme (9) analyzed PF volume in patients and found that women with endometriosis showed greater fluid volume than fertile controls, patients with adhesive disease, or those with unexplained infertility. Moreover, functional changes were also identified in several cellular immunological components of PF, or inflammatory mediators, such as prostaglandins (PGs), cytokines, growth and angiogenic factors.

PF contains various free-floating cells, including macrophages, mesothelial cells, lymphocytes, eosinophils and mast cells. It normally contains leukocytes in concentrations of 0.5 to 2.0 x 10^6/ml, approximately 85% of which are macrophages (9). Peritoneal concentrations of macrophages appear to fluctuate during the menstrual cycle, and are highest during menses (10). As well as

increasing in number, peritoneal macrophages are more activated in case of endometriosis, and may release products like PGs, cytokines, growth factors, complement components and hydrolytic enzymes (7). These products may affect other cells present in the peritoneal cavity, such as ectopic endometrial cells, and it has been postulated that peritoneal macrophage activation might be a central contributor to the pathogenesis of endometriosis, as these cells might play an active role in the initiation, maintenance and progression of the disease. Increased PF concentrations of cytokines that lead to migration, proliferation and activation of macrophages have been reported in patients with endometriosis. Monocyte chemotactic protein (MCP)-1, secreted by cytokine-stimulated endometriotic and mesothelial cells (11,12), regulated on activation and normally T-cell expressed and secreted (RANTES), which has potent chemotactic activity for human monocytes and T lymphocytes, and interleukin (IL)-8 are increased in the PF of women with endometriosis (13).

The main source of peritoneal inflammatory factors in endometriosis is thought to be macrophages, although other peritoneal cells, like lymphocytes, mesothelial cells (14) or ectopic endometrial implants (15), may also be involved. PG and cytokine activities are diverse and include the following: proliferation and differentiation of immune cells; induction of release of hormones, enzymes and acute-phase proteins; enhancement of various cytotoxic activities; regulation of immunoglobulin secretion; and chemotaxis. Cytokines exert biological effects on a variety of cell types. They may regulate the actions of leukocytes in PF or impact directly on ectopic endometrium, where they may play various roles in the pathogenesis of endometriosis (7).

2.2. Oxidative stress and peritoneal endometriosis

A number of studies suggest that oxidative stress is a constituent of endometriosis-associated inflammation. Indeed, retrograde menstruation is likely to carry highly pro-oxidant factors, such as heme and iron, into the peritoneal cavity, as well as apoptotic endometrial cells, which are well known inducers of oxidative stress. Peritoneal production of reactive oxygen species (ROS) may be involved in endometriosis-associated inflammation by regulating the expression of numerous inflammatory genes (8,16).

ROS are intermediaries produced by normal oxygen metabolism and have been implicated in the pathogenesis of many human diseases and aging (17). To protect themselves from the deleterious effects of ROS, cells have developed a wide range of antioxidant systems to limit ROS production, inactivate them, and repair cell damage. However, oxidative stress may occur when the balance between ROS production and antioxidant defense is disrupted. The growing number of diseases associated with oxidative stress suggests that oxidative balance may be precarious (18). Almost all major classes of biomolecules, including lipids, proteins and nucleic acids, are potential targets for ROS. Oxidative destruction of polyunsaturated fatty acids, known as lipid peroxidation, is particularly damaging because it is a self-perpetuating chain reaction that may alter the integrity of cell membranes. Cells have evolved enzymatic antioxidants, such as superoxide dismutase, catalase and glutathione peroxidase, and nonenzymatic antioxidants, including vitamin E, vitamin C, taurine and glutathione (19).

Zeller *et al.* (20) showed that production of ROS by PF mononuclear cells was increased in endometriosis. Expression of xanthine oxidase, an enzyme which produces ROS, remained high in ectopic and eutopic endometrium throughout the menstrual cycle in women with endometriosis; by contrast, cyclic variations in its expression were seen in controls (21). A study by Portz *et al.* (22) reported that injection of the antioxidant enzymes superoxide dismutase and catalase into the peritoneal cavity prevented formation of intraperitoneal adhesions in endometriosis sites in rabbits. Moreover, several studies appear to indicate that, in women with endometriosis, the endometrium shows altered expression of enzymes involved in defense against oxidative stress (21). However, other studies based on direct measurement of ROS production failed to show an obvious oxidant or antioxidant imbalance in the peritoneal cavity of patients with endometriosis (16,23-25). This apparent discrepancy between results may be due to the fact that only persistent markers of oxidative stress, such as enzymes or stable by-products of oxidative reactions, can still be detected when endometriosis is diagnosed. Another possible explanation is that oxidative stress occurs only locally (in lesions) and does not result in an increase in total PF concentrations (8).

Erythrocytes, apoptotic endometrial tissue and cell debris transplanted into the peritoneal cavity by menstrual reflux and pelvic macrophages have been implicated as potential inducers of oxidative stress (8) (Figure 2). Erythrocytes are likely to release pro-oxidant and proinflammatory factors, such as hemoglobin and its highly toxic by-products heme and iron, into the peritoneal cavity. As in most tissue, activated macrophages probably play an important role in the degradation of erythrocytes. Iron and heme are essential to living cells. However, unless they are appropriately chelated, free iron and, to a lesser extent, heme, play a key role in the formation of deleterious ROS, and iron-induced oxidative stress has been implicated in numerous pathologic processes (17).

In other tissues, iron is known to induce oxidative stress, leading to macromolecular oxidative damage, tissue injury and chronic inflammation (17). Oxidative stress was suggested to be responsible for local destruction of the peritoneal mesothelium, thereby creating adhesion sites for ectopic endometrial cells (26). It may also promote apoptosis (27), some studies indicating that apoptosis and oxidative stress are related (28). ROS could alter cell function by regulating protein activity and gene expression. In particular, ROS appear to play an essential role in the regulation of the transcription factors nuclear factor-kappa B (NF-kappa B) and activator protein-1, which control genes involved in immunologic response, cellular defense and expression of cytokines and cellular adhesion molecules (29). Many factors that are overexpressed in patients with endometriosis have NF-kappa B-binding sites in their genes (27,30), and involvement of the NF-kappa B

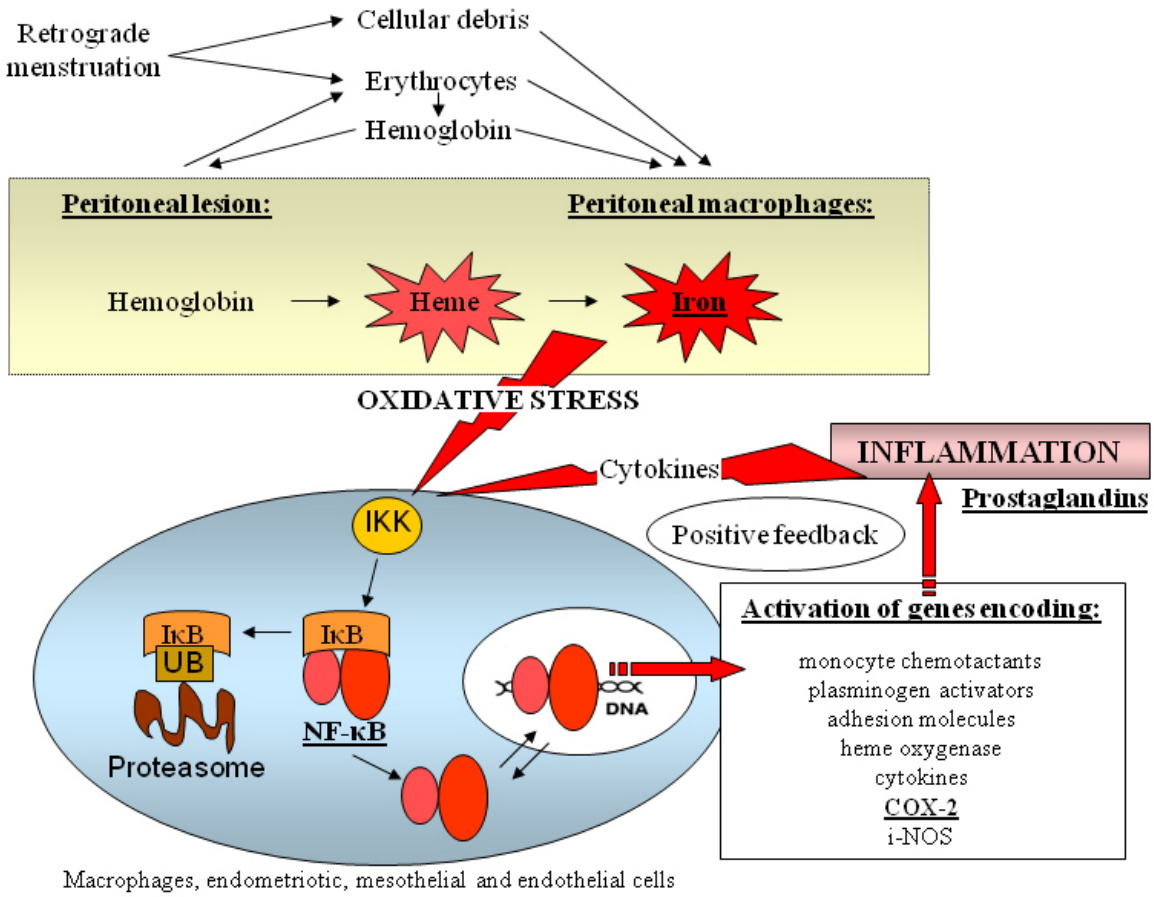

Figure 2. Iron, NF-kappa B and PGs in endometriosis. Iron overload in the peritoneal cavity in case of endometriosis may induce oxidative stress, involving peritoneal macrophages and endometriotic cells. Triggers of oxidative stress, such as proinflammatory cytokines, are potent activators of the NF-kappa B pathway. Induced NF-kappa B activation leads to expression of numerous genes implicated in inflammation, cellular adhesion, invasion and proliferation, and angiogenesis. Many of these genes have been implicated in endometriosis pathogenesis, PG biosynthetic enzymes in particular.

pathway in endometriosis will be discussed further. Important NF-kappa B-regulated molecules are PG biosynthesis enzymes, and cyclooxygenase (COX)-2 in particular. Increased concentrations of PGs have been evidenced in the PF of endometriosis patients and COX-2 inhibitors have proved to be effective in *in vitro* and *in vivo* experimental models (31).

In this manuscript, the respective involvement and potential molecular interactions of iron, NF-kappa B and PGs in the pathogenesis of endometriosis are discussed in the light of our personal observations and available data from the literature. The key role of peritoneal macrophages is emphasized and potential therapeutic targets are examined.

3. INVOLVEMENT OF IRON IN THE PATHOGENESIS OF ENDOMETRIOSIS

3.1. Iron overload in the peritoneal cavity of endometriosis patients

Iron is an essential metal for almost all living organisms because of its involvement in a large number of iron-containing enzymes and proteins. However, excess iron accumulation within cells and tissues can result in toxicity (17) and is associated with the pathogenesis of a variety of diseases, such as thalassemia, hemochromatosis and neurodegenerative disorders (32).

As reported in Table 1, studies have demonstrated the presence of iron overload in the different components of the peritoneal cavity of endometriosis patients (PF, ectopic endometrial tissue, peritoneum adjacent to lesions and peritoneal macrophages). However, it remains strongly localized in the pelvic cavity and does not affect body iron content (33). Higher levels of iron (26,33-35), ferritin (33,35), transferrin (36) and hemoglobin (37) have been detected in the PF of women with endometriosis than control subjects. Transferrin saturation is also higher in the PF of endometriosis patients (35). Hepcidin is a recently discovered peptide that appears to be the homeostatic regulator of iron metabolism by macrophages, acting by inhibiting iron efflux through ferroportin, thereby inducing cellular iron sequestration (38). Hepcidin synthesis is upregulated by inflammation and iron overload, and occurs predominantly in the liver.

Table 1. Presence of iron overload in different compartments of the peritoneal cavity in case of endometriosis

Peritoneal fluid	Peritoneal macrophages	Peritoneal lesions	Peritoneum
Higher iron levels (26,34)	Presence of iron-laden macrophages (43)	Iron-laden macrophages in lesions (43) Iron deposits in lesions (43)	More iron deposits in peritoneum adjacent to lesions than in healthy peritoneum (33)
Higher transferrin levels (36)	Higher transferrin receptor expression (46)	Iron deposits in lesions (41)	
Higher hemoglobin levels (37)	Haptoglobin-saturated macrophages (47)	Haptoglobin secreted by lesions (44,45)	
Higher ferritin and iron levels (33)	Higher ferritin levels in macrophages (35)	Heme oxygenase expression (37)	
Higher transferrin saturation, ferritin and iron levels (35)		Iron deposits in the stroma and macrophages of peritoneal lesions (33)	

However, inflammatory cells, particularly macrophages and neutrophils, can also directly express this peptide (39,40). In a recent study, no statistically significant difference was found in peritoneal hepcidin concentrations between patients with and without endometriosis, suggesting that peritoneal macrophage iron mobilization is regulated by other pathways (35).

In the stroma of endometriotic lesions and peritoneum, cytologic and histochemical data revealed the presence of iron conglomerates (41,42) and macrophages heavily laden with ferric pigment (43). Sharpe-Timms *et al.* showed that endometriotic lesions were also able to synthesize and secrete haptoglobin (44,45).

As in most tissues, pelvic macrophages have two important functions: first, in the regulation of the inflammatory response; and second, in iron homeostasis. Iron metabolism by macrophages appears to be enhanced in case of endometriosis. This is supported by the fact that endometriosis is characterized by the presence of siderophages (iron-storing macrophages) heavily laden with hemosiderin inside the pelvic cavity (43). Furthermore, after setting up a simple and reproducible technique of peritoneal macrophage isolation, we recently demonstrated increased iron storage (ferritin load) in peritoneal macrophages of endometriosis patients compared to healthy subjects, correlating with iron overload in PF (35). Bilirubin pigment, which is a normal metabolite of hemoglobin, was also identified inside macrophages (43). Finally, in case of endometriosis, peritoneal macrophages were found to express more transferrin receptors (46) and to be haptoglobin-saturated (47).

3.2. Origin of iron in the pelvic cavity

In case of endometriosis, iron overload may originate from lysis of pelvic erythrocytes (42). Retrograde menstruation, transporting menstrual endometrial tissue through the fallopian tubes into the peritoneal cavity, is a common physiologic event in all menstruating women with patent tubes (4). Peritoneal iron overload in endometriosis may be a consequence of increased influx caused by erythrocyte degradation, resulting either from more abundant menstrual reflux or bleeding lesions, or be due to a deficiency in the peritoneal iron metabolism system (33).

Retrograde menstruation may be increased in endometriosis patients by certain anatomical dispositions or uterine malformations, and menstrual periods are also frequently longer and heavier than in controls. Some experimental studies mimicking conditions of retrograde menstruation in mouse models have confirmed the origin of iron in the pelvic cavity in the context of endometriosis pathology (42,48).

3.3. Iron metabolism in the pelvic cavity

Studies with experimental models and analysis of patient biopsies (Table 1) have yielded further information on iron metabolism in the pelvic cavity in case of endometriosis, which has been interpreted in the light of data on erythrocyte metabolism from the literature. As in most tissue, activated macrophages recruited within the pelvic cavity of women play an important role in the degradation of erythrocytes, as suggested by the presence of numerous iron-loaded macrophages observed in the PF of endometriosis patients (35,43). Macrophages usually phagocytose senescent erythrocytes or endocytose the hemoglobin-haptoglobin complex formed after lysis of pelvic erythrocytes (49). Metabolism of hemoglobin and heme by heme oxygenase releases iron, which is then incorporated into ferritin in macrophages or returned to the iron transporter transferrin in PF. Transferrin may then be assimilated by ectopic endometrial cells. Indeed, endometrial cells express transferrin receptor (50), and *in vitro* studies have shown that endometrial stromal and epithelial cells are able to incorporate transferrin and metabolize it into ferritin (51).

Iron is sequestrated within tissue and bound to proteins such as ferritin in a soluble, non-toxic and bioavailable form (52). However, iron conglomerates have also been observed in endometriotic lesions in patients (41,42) and in a murine endometriosis model (48). These conglomerates consist of hemosiderin, another iron storage form, which is found in conditions of iron overload, usually associated with toxic pathological states in humans (52). The presence of hemosiderin in ectopic endometrial tissue and macrophages strongly suggests that peritoneal protective mechanisms might be overwhelmed in case of endometriosis.

3.4. Effect of iron overload on endometriosis development

Peritoneal iron overload could impair the functionality of different cell types involved in endometriosis pathophysiology, thereby contributing to the development of the disease. We recently showed iron storage levels (ferritin load) to be significantly higher in peritoneal macrophages of endometriosis patients than controls (35). Cellular iron storage within ferritin limits the capacity of iron to generate free radicals (53). However,

continued delivery of iron to macrophages can overwhelm the capacity of ferritin to store and sequester the metal, inducing oxidative injury to cells. Indeed, iron can act as a catalyst in the Fenton reaction to potentiate oxygen and nitrogen toxicity by generation of highly reactive free radical species (such as the hydroxyl radical). When the balance between ROS production and antioxidant defense is disrupted, marginally higher levels of ROS are generated and oxidative stress may occur, leading to macromolecular oxidative damage, tissue injury and chronic inflammation (17).

In case of endometriosis, iron overload in the peritoneal cavity may induce oxidative stress, particularly involving peritoneal macrophages, but also endometriotic, mesothelial and endothelial cells (51). Indeed, iron accumulation in these cells may severely compromise their function as a result of excessively increased production of ROS and subsequently enhanced activation of the proinflammatory transcriptional factor NF-kappa B (54). Induced NF-kappa B activation by cellular oxidative stress leads to expression of numerous genes implicated in inflammation, cellular adhesion, invasion and proliferation, and angiogenesis (30).

Peritoneal mesothelium is a fragile barrier, which can be damaged by ectopic menstrual endometrium or inflammatory cells creating adhesion sites on its surface, facilitating the development of endometriosis (55). Oxidative stress has been suggested to be responsible for local destruction of the peritoneal mesothelium, producing adhesion sites for ectopic endometrial cells (8,26). This hypothesis is supported by the fact that the iron-binding protein hemoglobin has been identified as one of the menstrual effluent factors harmful to mesothelium (55).

Iron is an absolute requirement for proliferation, as iron-containing proteins catalyze key reactions involved in oxygen sensing, energy metabolism, respiration, folate metabolism and deoxyribonucleic acid (DNA) synthesis (e.g. ribonucleotide reductase that catalyzes the conversion of ribonucleotides into deoxyribonucleotides for DNA synthesis). In a murine endometriosis model, erythrocyte injection was shown to increase the proliferative activity of epithelial cells in endometriotic lesions, while desferrioxamine administration, a common iron chelator, engendered a significant decrease, suggesting that iron overload may contribute to the further growth of endometriosis by promoting epithelial cell proliferation (48).

3.5. Iron chelators as endometriosis treatment

Treatment with desferrioxamine has proved beneficial and is currently used for pathologies characterized by iron overload, such as beta-thalassemia and hereditary hemochromatosis (56). In a murine endometriosis model, desferrioxamine was found to decrease the number of lesions with iron deposits, iron concentrations in PF and the percentage of iron-loaded pelvic macrophages (48). Moreover, desferrioxamine treatment effectively reduced cellular proliferation of lesions. Treatment with an iron chelator like desferrioxamine could thus be beneficial in case of endometriosis to prevent iron overload in the pelvic cavity, thereby diminishing its possible deleterious effects. In women suffering from endometriosis, menstrual periods are often longer and heavier than the norm (57-60), and cycles tend to be shorter (61). Therefore, iron overload observed in these patients is localized in the pelvic cavity, while body iron content may actually be decreased due to abundant menstruation. For this reason, iron chelator treatment should only be applied locally, only inside the peritoneal cavity.

4. INVOLVEMENT OF NUCLEAR FACTOR-KAPPA B IN THE PATHOGENESIS OF ENDOMETRIOSIS

NF-kappa B is a transcriptional factor that plays a crucial role in the immune and inflammatory response and modulates proliferation, apoptosis, adhesion, invasion and angiogenesis in many cell types (30). These cell processes are involved in the development of endometriosis and other diseases. Activation of NF-kappa B has been implicated in the early development of endometriotic lesions (62,63).

4.1. The NF-kappa B signaling system

NF-kappa B peptides are assembled through the dimerization of five existing subunits: p50/p105 (NF-kappa B1), p52/p100 (NF-kappa B2), p65 (RelA), c-Rel and RelB, the most extensively studied dimer being p50/p65 (64). NF-kappa B dimers are located mostly in the cytoplasm in an inactive form, binding to specific NF-kappa B inhibitors, I-kappa B proteins, which prevent NF-kappa B-DNA binding (Figure 2).

NF-kappa B is activated by diverse proinflammatory stimuli like cytokines (e.g. IL-1 beta, tumor necrosis factor (TNF)-alpha), lipopolysaccharide and oxidative stress, which may activate the I-kappa B kinase complex. This complex phosphorylates NF-kappa B-coupled I-kappa B peptides, inducing their polyubiquitination and rapid degradation by the 26S proteasome. Thus, liberated NF-kappa B dimers capable of binding to DNA translocate to the nucleus, activating the transcription of several target inflammatory genes (65,66) (Figure 2). A complete list of NF-kappa B-regulated genes is available at *http://www.nf-kb.org*.

Regulation of NF-kappa B involves positive feedback stimulation through NF-kappa B-mediated synthesis of IL-1 beta and TNF-alpha, and negative feedback inhibition through NF-kappa B-modulated transcription of I-kappa B alpha, p100, and/or p105 (67).

4.2. NF-kappa B activation and endometriosis

Human endometrial epithelial and stromal cells have been shown to express NF-kappa B subunits (68,69), and King *et al.* (70) demonstrated activation of this transcriptional factor in the glandular epithelium and endothelium of the endometrium during menstruation. NF-kappa B activity in human eutopic endometrium supports its important role in endometrial cell physiology and pathophysiology. Although NF-kappa B activation status in endometrium appears to be similar in patients with and

without endometriosis (71), further studies are required to assess the activation status of the NF-kappa B pathway in endometrium during the menstrual cycle in both healthy and pathologic conditions.

In vitro and *in vivo* studies have both found the NF-kappa B pathway to be activated in endometriotic cells, implicating this inflammatory pathway in endometriosis. *In vitro*, basal and stimulated NF-kappa B activation have been demonstrated in endometriotic stromal cells, with endometriotic cells showing IL-1 beta, TNF-alpha or lipopolysaccharide-dependent NF-kappa B activation, positively modulating the proinflammatory cytokines RANTES, macrophage migration inhibitory factor (MIF), IL-8, IL-6, TNF-alpha, intercellular adhesion molecule (ICAM)-1, granulocyte macrophage colony-stimulating factor and MCP-1 (67). *In vivo*, constitutive activation of NF-kappa B has been evidenced in endometriotic lesions in endometriosis patients (62). Concentrations of active p65-containing dimers and ICAM-1 expression were found to be significantly higher in red endometriotic lesions than black lesions, while expression of the NF-kappa B inhibitor I-kappa B alpha was similar in red and black lesions, confirming the more extensive inflammatory pattern of red lesions. We recently demonstrated increased NF-kappa B activation in peritoneal macrophages originating from endometriosis patients compared to healthy women (63).

NF-kappa B-dependent activation of proinflammatory genes, such as RANTES, ICAM-1, IL-1 or TNF-alpha, may provide positive feedback to the pathway, thus self-perpetuating macrophage recruitment and the inflammatory response in the peritoneal cavity of endometriosis patients.

4.3. Effect of NF-kappa B activation on endometriosis development

Iron overload in peritoneal macrophages of endometriosis patients (35) may induce cellular oxidative stress (8), and excessive production of ROS has been shown to abnormally activate the transcriptional factor NF-kappa B in macrophages (54). NF-kappa B-activated macrophages release proinflammatory, growth and angiogenic factors (such as inducible nitric oxide synthase, COX-2, IL-1, IL-6, IL-8, TNF-alpha and vascular endothelial growth factor (VEGF)), which contribute to endometriosis pathogenesis and may activate NF-kappa B in endometriotic cells, consequently promoting cell survival and proinflammatory cytokine production, self-perpetuating the inflammatory reaction in endometriotic lesions.

Adhesion of endometrial cells involves expression of mediators of cell-cell and cell-matrix adhesion, such as integrins and cadherins (72). There is a lack of literature on the regulation of these molecules at the transcriptional level, specifically by NF-kappa B in endometrial and endometriotic cells. It has been hypothesized that cytokines may induce expression of cellular adhesion molecules in endometrial and/or mesothelial cells (73). However, there is no consensus on the impact of proinflammatory cytokines on endometrial cell adhesion to peritoneum, with some *in vitro* studies reporting a promoting effect, and others not (74). Matrix metalloproteinases (MMPs), urokinase-type plasminogen activator (uPA) and tissue plasminogen activator have all been implicated in remodeling the extracellular matrix, which could lead to endometrial invasion of the submesothelial space of the peritoneum, and have been found to be positively regulated by NF-kappa B. While uPA is known to be transcriptionally induced by IL-1 or TNF-alpha-dependent NF-kappa B activation (75), these pathways have not been studied in endometrial or endometriotic cells. Expression of MMP-1, -2, -3, -9 and -13 is modulated by NF-kappa B in different cell types (76-78); MMP-9 is upregulated by NF-kappa B in endometriotic epithelial cells, and invasion of these cells is inhibited by an NF-kappa B inhibitor (79).

Proliferation and resistance of endometriotic cells to apoptosis contribute to endometriosis development. The role of NF-kappa B as a proliferative and antiapoptotic factor has been evidenced in many studies (75). *In vitro*, NF-kappa B blocking has been shown to inhibit cell proliferation and stimulate apoptosis of endometriotic cells (67). *In vivo*, NF-kappa B inhibition in early-stage endometriotic lesions induced in nude mice was found to decrease endometriotic epithelial cell proliferation and stimulate both endometriotic stromal and epithelial cell apoptosis (80).

Angiogenesis is an essential process for endometriotic lesion formation, and VEGF facilitates the development of these lesions (81). NF-kappa B upregulates angiogenic factors like IL-8 and MIF in endometriotic stromal cells, and endometriotic cells release MIF that stimulates endothelial cell proliferation *in vitro* (82,83). In a rat endometriosis model, a significant reduction in microvessel density was achieved by inhibiting the NF-kappa B pathway (84). All this indicates that NF-kappa B-mediated transcription of proangiogenic proteins stimulates angiogenesis in endometriotic lesions.

4.4. NF-κB inhibitors as endometriosis treatment

Studying the effect of NF-kappa B inhibitors in endometrial and endometriotic cells *in vitro* and *in vivo* has shown NF-kappa B inhibition to reduce endometriosis development and maintenance (Table 2). In the first *in vivo* experimental model studying the involvement of NF-kappa B in endometriosis, González Ramos *et al.* (80) demonstrated that NF-kappa B inhibition by BAY 11-7085 and SN-50 was able to diminish initial development of endometriosis in a murine model. NF-kappa B activation and ICAM-1 expression in induced endometriotic lesions were found to be significantly lower in treated mice, while cell proliferation was significantly reduced in BAY 11-7085-treated mice. Both inhibitors produced a significant increase in apoptosis, as evidenced by active caspase-3 immunostaining and the TUNEL method.

Since NF-kappa B is involved in a wide range of important cell processes, clinical application of the majority of NF-kappa B inhibitors is not possible at this stage, and systemic use of this type of drug could well prove

Table 2. NF-kappa B inhibitors and endometriosis

NF-kappa B inhibitor	Experimental model	Effects
MG-132 (68,137,138)	*In vitro*, EEC *In vitro*, bovine ESC	↓ IL-6 and LIF ↓ PTGS-2 and COX-2
Parthenolide (137)	*In vitro*, bovine ESC	↓ PTGS-2
SN-50 (68,80)	*In vitro*, EEC *In vivo*, nude mouse	↓ IL-6 and LIF ↓ lesion development, ↓ ICAM-1 and ↑ apoptosis
Curcumin (139,140)	*In vitro*, ESC and EcSC *In vivo*, human	↓ MIF Unknown, ↓ symptoms?
Sulindac (141)	*In vitro*, ESC	↓ RANTES
Thalidomide (142)	*In vitro*, EcSC	↓ IL-8
TPCK (82)	*In vitro*, EcSC	↓ IL-8
GnRH-a (82)	*In vitro*, EcSC *In vivo*, human	↓ IL-8
Progesterone, danazol, dienogest (143)	*In vitro*, EcSC	↓ IL-8
IKK-2 inhibitor (79)	*In vitro*, EcEC line	↓ IL-8, IL6, MCP-1, GM-CSF, ICAM-1 and MMP-9, ↓ cell invasion
Pioglitazone (83)	*In vitro*, EcSC	↓ IL-8 and cell proliferation
IL-10 (144)	*In vitro*, EcSC	↓ IL-6
NF-kappa B decoy ODNs (145)	*In vitro*, EcSC	↓ RANTES ↓ monocyte chemotactic activity
BAY-11-7085 (80,146)	*In vitro*, EcSC *In vivo*, nude mouse	↓ Bcl-2 and Bcl-XLn ↑ caspase-3, -9, and -8 activation ↓ lesion development, ↓ cell proliferation and ↑ apoptosis, ↓ ICAM-1
PDTC and bortezomib (84)	*In vivo*, rat	↓ lesion development ↓ cell proliferation and angiogenesis
CAPE (147)	*In vivo*, rat	↓ lesion development ↓ oxidative stress
Eicosapentanoic acid (148)	*In vivo*, rat	↓ inflammation ↓ PGES, MMP-13 and NF-kappa B
Glycine (149)	*In vivo*, hamster	No effect
hCG (150,151)	*In vitro*, EcSC *In vivo*, human	↓ IL-1 beta and TNF-alpha ↓ symptoms
ER beta agonist (145)	*In vitro*, peritoneal macrophages	↓ iNOS

Abbreviations: Bcl: B-cell lymphoma/leukemia; CAPE: caffeic acid phenethyl ester; EcEC: endometriotic epithelial cells; EcSC: endometriotic stromal cells; EEC: endometrial epithelial cells; ER: estrogen receptor; ESC: endometrial stromal cells; GM-CSF: granulocyte macrophage–colony-stimulating factor; GnRH-a: gonadotropin-releasing hormone agonist; hCG: human chorionic gonadotropin; IKK: IkB kinase; iNOS: inducible nitric oxide synthase; LIF: leukemia inhibitory factor; ODNs: decoy oligonucleotides; PDTC: pyrrolidine dithiocarbamate; PGES: prostaglandin E synthase; PTGS: prostaglandin-endoperoxide synthase; TPCK: N-tosyl-l-phenylalanine chloromethyl ketone

dangerous. Further research is needed to evaluate the safety and side effects of these drugs before they can be considered suitable for use in humans. Local treatments inhibiting NF-kappa B could be an attractive proposition and should be evaluated in humans (6,62).

5. INVOLVEMENT OF PROSTAGLANDINS IN THE PATHOGENESIS OF PERITONEAL ENDOMETRIOSIS

Increased concentrations of PGs (85,86) and leukotrienes (87) have been found in the PF of endometriosis patients. These are the major constituents of a group of biologically active oxygenated fatty acids known as eicosanoids, and have been implicated in various inflammatory diseases such as asthma, psoriasis, rheumatoid arthritis and inflammatory bowel disease (88,89).

Recent studies in *in vitro* models have shown that enhanced synthesis of PGs plays a role in enhancing proliferation while inhibiting apoptosis (90), promoting angiogenesis (91) and increasing the metastatic potential of epithelial cells (92), as well as immunosuppression, by inhibiting T- and B-cell proliferation and differentiation and accessory monocyte-macrophage function (93). These inflammatory mediators may therefore be implicated in the pain and infertility associated with endometriosis, but also in the molecular and cellular processes that result to peritoneal endometriotic lesion development.

5.1. Eicosanoid biosynthesis

Unlike many other biologically active molecules, the final products of eicosanoids are not stored preformed, but are synthesized *de novo* through a cascade of enzymes (Figure 3) that are mainly regulated by transcriptional control. Arachidonic acid, which is the common precursor of eicosanoids, is released from membrane phospholipids by phospholipase A2 enzymatic activity (94). The COX pathway leads to the formation of PGs and involves COX and PG synthase activities. Each of these reactions can be rate-limiting and catalyzed by several enzymatic isoforms that are differentially regulated (94).

In contrast to constitutive isoforms, inducible isoforms are typically undetectable under normal

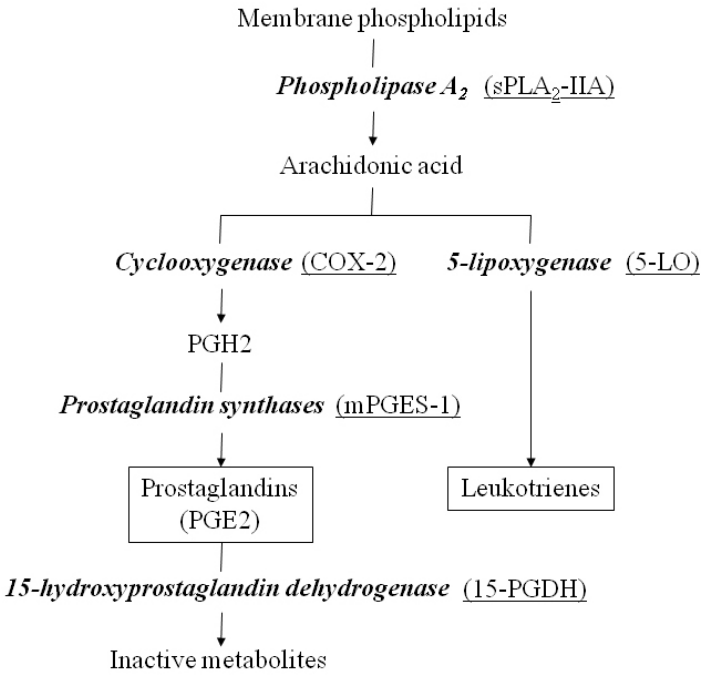

Figure 3. The eicosanoid enzymatic pathway. Arachidonic acid, which is the common precursor of eicosanoids, is liberated from membrane phospholipids by phospholipase A_2 enzymatic activity. The COX pathway leads to the formation of PGs and involves COX and PG synthase activities. PGE2 is the most biologically active prostanoid and is metabolically inactivated by the 15-PGDH enzyme. In contrast to constitutive isoforms, inducible isoforms are typically undetectable under normal physiological conditions, but can be expressed at high levels following stimution by proinflammatory cytokines, growth factors and phorbol esters. The most relevant inducible isoforms in the COX pathway are $sPLA_2$-IIA, COX-2 and mPGES-1. The lipoxygenase pathway, responsible for the synthesis of leukotrienes, involves a catalytic complex consisting of 5-LO.

physiological conditions, but may be expressed at high levels following stimulation by proinflammatory cytokines (95). This is particularly well known in case of COX enzymatic activity: COX-1 and -3 isoforms are constitutively expressed, while COX-2 is only expressed following stimulation (95). COX-2 expression is mainly regulated by the transcriptional NF-kappa B pathway, similarly to other inducible PG biosynthesis enzymes, such as type IIA secretory phospholipase A2 (sPLA2-IIA) and microsomal prostaglandin E synthase-1 (mPGES-1) (95). Prostaglandin E2 (PGE2) is the most biologically active prostanoid and is metabolically inactivated by the enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH) (94). The lipoxygenase pathway, responsible for the synthesis of leukotrienes, involves a catalytic complex consisting of 5-lipoxygenase (5-LO) (88).

5.2. Prostaglandin receptors

After biosynthesis, PGs are transported out of the cell by a PG transporter (96), where they exert their biological function through G-protein receptor-mediated interaction. There are eight types and subtypes of prostanoid receptor, which are encoded by different genes. Separate receptors, showing selective ligand binding specificity, have been described for PGD2, PGE2, PGF2α, thromboxane A2 and prostacyclin (97).

PGE2 elicits its autocrine-paracrine effects on target cells after interaction with four subtypes of PGE2 receptors, which are pharmacologically divided into EP1, EP2, EP3 and EP4. These receptors use alternate and in some cases opposing intracellular pathways (98). Activation of EP2 and EP4 receptors results in a cellular increase in cyclic adenosine monophosphate (97).

Functional roles for prostanoid receptors were determined by studies in knockout mouse model systems, deficient for each of the receptors. The most startling observations were made with EP2 and PGF2α receptor knockouts, showing these receptors to be indispensable in female reproduction (99,100). Indeed, loss of EP2 receptor function by gene ablation in a mouse model resulted in impaired ovulation and a marked reduction in litter size (99).

5.3. Prostaglandin biosynthesis in endometriosis

Increased concentrations of PGs have been reported in the PF of endometriosis patients (86) and may be involved in the progression of the disease by inhibiting macrophage scavenger function, while increasing cellular proliferation and angiogenesis (31). The cellular origin of peritoneal PGs in endometriosis remains unclear. Since PGs are unstable factors with a very short half-life (101), it is generally believed that they must be produced and function locally. Hence, peritoneal macrophages and ectopic endometriotic tissue represent two of the most likely candidates contributing to the elevated levels of peritoneal PGs. According to Wu *et al.* (86), peritoneal

macrophages from endometriosis patients are known to have greater PG synthetic capability than those from endometriosis-free women. Using quantitative RT-PCR and Western blot analyses, they found that expression of COX-2 was markedly increased in peritoneal macrophages isolated from endometriosis patients (86).

We recently evaluated expression levels of five main eicosanoid biosynthetic and catabolic enzymes in peritoneal macrophages and endometriotic lesions, and demonstrated a significant increase in messenger ribonucleic acid (mRNA) expression for sPLA2-IIA, COX-2 and mPGES-1 in peritoneal macrophages from women with endometriosis compared to controls (102). In our study, higher macrophage COX-2 mRNA expression was found in endometriosis patients with red as opposed to black peritoneal lesions, which is consistent with the more extensive inflammatory pattern of these lesions (102).

PGs may also be synthesized by pelvic endometriotic implants because eutopic endometrium is able to produce PGs during the normal menstrual cycle (103). Consistent with this hypothesis, several studies have reported increased expression of COX-2 in endometriosis patients. The most cited studies compare COX-2 expression in ovarian endometriotic tissue with that of eutopic endometrium of disease-free women, either by immunohistochemistry (104) or polymerase chain reaction (105,106). However, only a few studies have been conducted in relation to peritoneal endometriosis, showing contradictory results. In a small series of peritoneal lesion biopsies, mRNA expression of COX-2 was found to be higher than in the eutopic endometrium of endometriosis-free women (107). The same authors also postulated that elevated COX-2 expression in endometriotic tissues might result from increased sensitivity to proinflammatory cytokines such as IL-1 beta (107). However, COX-2 mRNA expression was recently compared between peritoneal implants, eutopic endometrium of women with endometriosis and control endometrium, but differences among these groups did not reach levels of significance (108). In our study, we also compared mRNA expression of sPLA2-IIA, COX-2, mPGES-1, 15-PGDH and 5-LO between peritoneal lesions and matched eutopic endometrium in a series of 40 endometriosis patients (102). While sPLA2-IIA, mPGES-1 and 15-PGDH mRNA expression levels were found to be increased in lesions, no difference was found for COX-2 expression.

Transcriptional regulation of the COX-2 gene is very complex as it can involve numerous signaling pathways, and the mechanism varies depending on the specific stimulus and cell type (109). PGs are reported to feedback-regulate COX-2 gene expression and have been implicated as positive and negative regulators, depending on cell type (110). It has recently been suggested that positive COX-2 feedback occurs in macrophages (110). By contrast, PGs may inhibit COX-2 gene expression in endometrial epithelial cells (111), and such distinct regulation may explain our findings of COX-2 overexpression in peritoneal macrophages of endometriosis patients, but no difference between peritoneal lesions and eutopic endometrium.

Expression of 15-PGDH, which deactivates PGE2 in its inactive form, was found to slowly increase in peritoneal endometriosis compared to matched eutopic endometrium, constituting a protective, but probably insufficient, mechanism against PG overproduction in peritoneal ectopic tissues. Interestingly, in a comparison of eutopic endometrium from endometriosis patients versus disease-free women, 15-PGDH mRNA expression was found to be lower in endometriosis sufferers (102). Such a decrease in expression of this deactivating enzyme may explain the increased levels of PGs detected in the eutopic endometrium of endometriosis patients, compared to controls (112).

By immunohistochemistry, we demonstrated that PG biosynthetic enzymes were mainly localized in the glandular epithelium of eutopic and ectopic endometrium (102), consistent with epithelial COX-2 expression evidenced in endometriosis (104) and cancer (113).

5.4. Effect of prostaglandins on endometriosis development

This section outlines some of the cellular mechanisms whereby PG biosynthetic enzymes and PGs are able to mediate their role in endometriosis. High concentrations of PGs present in endometriotic tissues may inhibit B- and T-cell proliferation and accessory monocyte-macrophage function, thereby allowing defective cells to proliferate undetected by the immune system (114). It has been postulated that PGs are able to reduce phagocytic efficiency and attenuate scavenger function in peritoneal macrophages of endometriosis patients by decreasing MMP-9 activity (31). Moreover, it has recently been demonstrated that PGE2 inhibits expression of CD36 in peritoneal macrophages via the EP2 receptor-dependent signaling pathway, resulting in reduced phagocytic ability (115).

COX enzymes and PGs have been reported to promote invasion of surrounding tissues and may be important in diseases such as endometriosis (92). In a recent study, Banu *et al.* (116) demonstrated the promoting effect of COX-2/PGE2 in the migration and invasion of endometriotic cells. By contrast, inhibition of COX-2 decreased endometriotic epithelial and stromal cell invasion (116).

In various model systems of epithelial cell lines overexpressing COX-1 or COX-2, COX enzymes have been found to play a role in cell proliferation and inhibition of apoptosis in response to growth factor, mitogen and cytokine stimuli (96). Selective inhibition of COX-2 in these models resulted in a decrease in cell proliferation and restoration of the apoptosis rate (117). PGs have been shown to directly enhance the proliferation rate of endometrial epithelial cells (118), suggesting a role for these compounds and biosynthetic enzymes in endometrial pathologies associated with proliferation and apoptosis. In both endometriotic epithelial and stromal cells, inhibition

of COX-2 decreased cell proliferation while increasing cellular apoptosis (116,119). It has also been demonstrated that selective inhibition of PGE2 receptors EP2 and EP4 induces apoptosis of endometriotic cells, notably through suppression of the NF-kappa B pathway (120).

In epithelial cell lines transfected with COX-1 or COX-2, overexpression of either or both results in a concomitant increase in the production of PGE2. This PGE2 then acts in an autocrine-paracrine manner on EP receptors to trigger intracellular signal transduction cascades and transcription of proangiogenic factors such as VEGF or angiopoietin-1 and -2 (96), or to downregulate expression of antiangiogenic genes like cathepsin-D (121). In endometrial epithelial cells too, elevated PGE2-EP2 receptor interaction promotes expression of proangiogenic genes such as VEGF (122). In animal models of endometriosis, selective COX-2 inhibition has been shown to reduce endometriosis growth through an antiangiogenic effect (123,124).

5.5. Targeting prostaglandin biosynthesis as endometriosis treatment

The involvement of PGs and impact of COX-2 inhibitors have been assessed by several *in vitro* and *in vivo* studies. Banu *et al.* (116) demonstrated that inhibition of COX-2 decreased endometriotic epithelial and stromal cell survival, migration and invasion in an *in vitro* study. Another recent study suggested a direct effect of celecoxib, a selective COX-2 inhibitor, on the reduction of endometrial epithelial cell proliferation (111). Animal studies have shown treatment with COX-2 inhibitors to prevent implantation of endometrium in ectopic sites in rats (125) and to induce regression of endometrial explants in rats (126), mice (127) and Syrian golden hamsters (123). The effectiveness of COX-2 inhibitors was also assessed in endometriosis patients (128), although little is known about the mechanisms of such inhibitors. However, ablation of the COX-2 gene resulted in multiple reproductive failures in mice (129), and therapeutic use of COX-2 inhibitors in humans led to undesirable cardiovascular side effects (130), underlining the need for novel anti-inflammatory compounds.

In our study, sPLA2-IIA was found to be dramatically increased in peritoneal macrophages and lesions of endometriosis patients (102). In addition to being a key regulator of eicosanoid biosynthesis, by liberating the precursor for subsequent production of PGs and leukotrienes, sPLA2 may activate inflammatory cells through mechanisms unrelated to its enzymatic activity (131). Furthermore, sPLA2-IIA has been shown to stimulate vascular endothelial cell migration (132) and may therefore be involved in angiogenesis in endometriosis. As PLA2-IIA can upregulate its own expression (133), establishing an inflammatory positive feedback loop, it could explain the considerable increase in mRNA expression observed in peritoneal endometriotic lesions in our study. Targeting phospholipase A2 activity could be particularly valuable in the medical treatment of endometriosis, as it initiates biosynthesis of both PGs and leukotrienes, and should therefore constitute an effective anti-inflammatory approach (134). However, an inhibitor selective for the production of inflammatory metabolites, but not inhibiting the beneficial properties of phospholipase A2, still needs to be developed (134).

6. SUMMARY AND PERSPECTIVES

The involvement of inflammation in peritoneal endometriosis has been evidenced by numerous studies performed on patient biopsies or using *in vitro* or *in vivo* experimental models. The inflammatory process plays a major role in the clinical symptoms of pain and infertility associated with endometriosis, but also interacts in the different molecular and cellular events that lead to peritoneal endometriotic lesion development (15). Indeed, it has been demonstrated that several inflammatory mediators inhibit the scavenger function of macrophages (31), while increasing endometrial cell proliferation (31) and angiogenesis (31). As in most tissues, peritoneal macrophages are widely implicated in inflammation, and their involvement in the pathogenesis of endometriosis has been well documented (7). Identification of the inflammatory mediators involved in endometriosis is therefore very important to determine potential therapeutic targets for future treatment of the disease. In this article, we focus on three such mediators that have been clearly implicated in peritoneal endometriosis and may be linked.

Iron overload has been demonstrated in different compartments of the peritoneal cavity in case of endometriosis (33,35). As excess iron exposure can induce oxidative stress leading to inflammation (17), iron metabolism and distribution in the peritoneal cavity play an essential role in preventing iron-associated generation of oxidative stress (35). Cellular iron storage within ferritin, particularly in macrophages but also in endometriotic and endothelial cells, limits the capacity of iron to generate free radicals and confers an antioxidant effect (53). However, excess delivery of iron to macrophages and endometriotic cells can overwhelm the capacity of ferritin to store and sequester the metal, causing oxidative injury to cells (35).

Oxidative stress has been shown to be a potent activator of the NF-kappa B pathway, as well as proinflammatory cytokines and lipopolysaccharide (Figure 2). Constitutive activation of this transcriptional pathway has been demonstrated in endometriotic lesions (62), and increased activation has been evidenced in peritoneal macrophages of endometriosis patients compared to controls (63). Activation of NF-kappa B leads to expression of multiple genes encoding proinflammatory cytokines, chemokines, adhesion, growth and angiogenic factors and inducible enzymes such as PG biosynthetic enzymes (62). All these factors have been found to be expressed in peritoneal macrophages of endometriosis patients and endometriotic peritoneal lesions, and to be involved in cell adhesion, invasion, proliferation and angiogenic processes. Oxidative stress is also associated with activation of other transcription factors, such as activator protein (AP)-1, hypoxia-inducible factor (HIF)-1, signal transducer and activator of transcription and CCAAT/enhancer binding protein (135). AP-1 activation was shown to be critical for

TNF-alpha-induced IL-6 expression in endometriotic cells (136), while mRNA and protein levels of HIF-1 alpha were greater in ectopic endometriotic tissue than its eutopic counterpart (31). However, the involvement of these factors in endometriosis needs to be better elucidated in the future.

PGs are synthesized by the enzymatic COX pathway and have also been implicated in the pathogenesis of endometriosis and its associated pain and infertility. As PGs promote angiogenesis, inhibit apoptosis, enhance proliferation and modulate immune responses such as macrophage function (96), they also represent interesting therapeutic targets for endometriosis treatment. Enhanced concentrations have been reported in the PF of endometriosis patients (86), as well as increased expression of the inducible COX-2 isoform in peritoneal macrophages (86). Involvement of other inducible enzymes of the PG biosynthetic pathway should also be further investigated. Increased expression of $sPLA_2$-IIA and mPGES-1 in peritoneal macrophages and lesions was recently demonstrated in endometriosis (102).

Endometriosis is a multifactorial disease associated with abnormal chronic pelvic inflammation, but also other hormonal, genetic and environmental deregulations (3). We therefore believe that future medical therapy should associate different targets in order to optimize its efficacy. Identification of the pathological pathways involved in endometriosis-associated inflammation, and in macrophage dysfunction in particular, could well prove useful in the treatment of this disease, as observed in many other inflammatory pathologies. As previously discussed, iron metabolism, NF-kappa B activation and PG synthesis are all valid targets.

The beneficial effects of inhibitors of these pathways in endometriosis have also been reviewed, and our data highlight potential new therapeutic targets such as $sPLA_2$-IIA, which was found to be markedly increased in peritoneal macrophages and lesions of endometriosis patients. However, since iron, NF-kappa B and PGs are involved in a wide range of important cell processes, systemic use of inhibitors could result in adverse effects. Further research is therefore needed to evaluate the safety of these drugs before they can be considered suitable for use in humans. By targeting different inflammatory and hormonal mediators at the same time and/or acting more locally, we aim to reduce doses to achieve optimal efficacy without toxicity.

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8. REFERENCES

1. J. Donnez, F. Chantraine and M. Nisolle: The efficacy of medical and surgical treatment of endometriosis-associated infertility: arguments in favour of a medico-surgical aproach. *Hum Reprod Update* 8, 89-94 (2002)

2. M. Nisolle and J. Donnez: Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three ifferent entities. *Fertil Steril* 68, 585-596 (1997)

3. L.C. Giudice and L.C. Kao: Endometriosis. *Lancet* 364, 1789-1799 (2004)

4. J. Halme, M.G. Hammond, J.F. Hulka, S.G. Raj and L.M. Talbert: Retrograde menstruation in healthy women and in patients with endometriosis. *Obstet Gynecol* 64, 151-154 (1984)

5. J.A. Sampson: Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol* 14, 422-469 (1927)

6. J.C. Lousse, S. Defrère, R. González Ramos, A. Van Langendonckt, S. Colette and J. Donnez: Involvement of iron, nuclear factor-kappa B (NF-kappa B) and prostaglandins in the pathogenesis of peritoneal endometriosis-associated inflammation: a review. *J Endometr* 1, 19-29 (2009)

7. R. Gazvani and A. Templeton: Peritoneal environment, cytokines and angiogenesis in the pathophysiology of endometriosis. *Reproduction* 123, 217-226 (2002)

8. A. Van Langendonckt, F. Casanas-Roux and J. Donnez: Oxidative stress and peritoneal endometriosis. Fertil Steril 77, 861-870 (2002)

9. C.H. Syrop and J. Halme: Cyclic changes of peritoneal fluid parameters in normal and infertile patients. *Obstet Gynecol* 69, 416-418 (1984)

10. E. Oral, D.L. Olive, A. Arici: The peritoneal environment in endometriosis. *Hum Reprod Update* 2, 385-398 (1996)

11. A. Akoum, A. Lemay, C. Brunet and J. Hébert: Secretion of monocyte chemotactic protein-1 by cytokine-stimulated endometrial cells of women with endometriosis. Le groupe d'investigation en gynécologie. *Fertil Steril* 63, 322-328 (1995)

12. A. Arici, E. Oral, O. Bukulmez, S. Buradagunta, O. Bahtiyar and E.E. Jones: Monocyte chemotactic protein-1 expression in human preovulatory follicles and ovarian cells. *J Reprod Immunol* 32, 201-219 (1997)

13. D. Hornung, I.P. Ryan, V.A. Chao, J.L. Vigne, E.D. Schriock and R.N. Taylor: Immunolocalization and regulation of the chemokine RANTES in human endometrial and endometriosis tissues and cells. *J Clin Endocrinol Metab* 82, 1621-1628 (1997)

14. C.M. Kyama, L. Overbergh, S. Debrock, D. Valckx, S. Vander Perre, C. Meuleman, A. Mihalyi, J.M. Mwenda, C. Mathieu and T.M. D'Hooghe: Increased peritoneal and endometrial gene expression of biologically relevant cytokines and growth factors during the menstrual phase in

women with endometriosis. *Fertil Steril* 85, 1667-1675 (2006)

15. T. Harada, T. Iwabe and N. Terakawa: Role of cytokines in endometriosis. *Fertil Steril* 76, 1-10 (2001)

16. A. Agarwal, S. Gupta and S. Sikka: The role of oxidative stress in endometriosis. *Curr Opin Obstet Gynecol* 18, 325-332 (2006)

17. S. Hippeli and E.F. Elstner: Transitional metal ion-catalyzed oxygen activation during pathogenic processes. *FEBS Lett* 443, 1-7 (1999)

18. B. Halliwell and J.M. Gutteridge: Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 186, 1-85 (1990)

19. B.P. Yu: Cellular defenses against damage from reactive oxygen species. *Physiol Rev* 74, 139-159 (1994)

20. J.M. Zeller, I. Henig, E. Radwanska and W.P. Dmowski: Enhancement of human monocyte and peritoneal macrophage chemiluminescence activities in women with endometriosis. *Am J Reprod Immunol Microbiol* 13,78-82 (1987)

21. H. Ota, S. Igarashi and T. Tanaka: Xanthine oxidase in eutopic and ectopic endometrium in endometriosis and adenomyosis. *Fertil Steril* 75, 785-790 (2001)

22. D.M. Portz, T.E. Elkins, R. White, J. Warren, S. Adadevoh and J. Randolph: Oxygen free radicals and pelvic adhesion formation: blocking oxygen free radical toxicity to prevent adhesion formation in an endometriosis model. *Int J Fertil* 36, 39-42 (1991)

23. Y. Wang, R.K. Sharma, T. Falcone, J. Goldberg and A. Agarwal: Importance of reactive oxygen species in the peritoneal fluid of women with endometriosis or idiopathic infertility. *Fertil Steril* 68, 826-830 (1997)

24. H.N. Ho, M.Y. Wu, S.U. Chen, K.H. Chao, C.D. Chen and Y.S. Yang: Total antioxidant status and nitric oxide do not increase in peritoneal fluids from women with endometriosis. *Hum Reprod* 12, 2810-2815 (1997)

25. G. Polak, M. Koziol-Montewka, M. Gogacz, I. Blaszkowska and J. Kotarski: Total antioxidant status of the peritoneal fluid in infertile women. *Eur J Obstet Gynecol Reprod Biol* 94, 261-263 (2001)

26. K. Arumugam and Y.C. Yip: De novo formation of adhesions in endometriosis: the role of iron and free radical reactions. *Fertil Steril* 64, 62-64 (1995)

27. A.A. Murphy, N. Santanam and S. Parthasarathy: Endometriosis: a disease of oxidative stress? *Semin Reprod Endocrinol* 16, 263-73 (1998)

28. T.M. Buttke and P.A. Sandstrom: Oxidative stress as a mediator of apoptosis. *Immunol Today* 15, 7-10 (1994)

29. T.P. Dalton, H.G. Shertzer and A. Puga: Regulation of gene expression by reactive oxygen. *Annu Rev Pharmacol Toxicol* 39, 67-101 (1999)

30. P. Viatour, M.P. Merville, V. Bours and A. Chariot: Phosphorylation of NF-kappa B and I-kappa B proteins: implications in cancer and inflammation. *Trends Biochem Sci* 30, 43-52 (2005)

31. M.H. Wu, Y. Shoji, P. Chuang and S. Tsai: Endometriosis: disease pathophysiology and the role of prostaglandins. *Expert Rev Mol Med* 9, 1-20 (2007)

32. R. Crichton, S. Wilmet, R. Legssyer and R.J. Ward: Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells. *J Inorg Biochem* 91, 9-18 (2002)

33. A. Van Langendonckt, F. Casanas-Roux and J. Donnez: Iron overload in the peritoneal cavity of women with pelvic endometriosis. *Fertil Steril* 78, 712-718 (2002)

34. K. Arumugam: Endometriosis and infertility: raised iron concentration in the peritoneal fluid and its effect on the acrosome reaction. *Hum Reprod* 9, 1153-1157 (1994)

35. J.C. Louse, S. Defrère, A. Van Langendonckt, J. Gras, R. González Ramos, S. Colette and J. Donnez: Iron storage is significantly increased in peritoneal macrophages of endometriosis patients and correlates with iron overload in peritoneal fluid. *Fertil Steril* 91, 1668-1675 (2009)

36. S.P. Mathur, J.H. Lee, H. Jiang, P. Arnaud and P.F. Rust: Levels of transferrin and alpha 2-HS glycoprotein in women with and without endometriosis. *Autoimmunity* 29, 121-127 (1999)

37. A. Van Langendonckt, F. Casanas-Roux, M.M. Dolmans and J. Donnez: Potential involvement of hemoglobin and heme in the pathogenesis of peritoneal endometriosis. *Fertil Steril* 77, 561-70 (2002)

38. T. Ganz: Hepcidin-a regulator of intestinal iron absorption and iron recycling by macrophages. *Best Pract Res Clin Haematol* 18, 171-182 (2005)

39. C. Peyssonnaux, A.S. Zinkernagel, V. Datta, X. Lauth, R.S. Johnson and V. Nizet: TLR4-dependent hepcidin expression by myeloid cells in response to bacterial pathogens. *Blood* 107, 3727-3732 (2006)

40. N.B. Nguyen, K.D. Callaghan, A.J. Ghio, D.J. Haile and F. Yang: Hepcidin expression and iron transport in alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 291, 417-425 (2006)

41. V. Petrozza, F.M. Magliocca, S. Pulvirenti, G. Massimi, C. de Salazar and F. Carpino: Asymptomatic peritoneal endometriosis following cesarean section. *Minerva Ginecol* 45, 349-353 (1993)

42. A. Van Langendonckt, F. Casanas-Roux, J. Eggermont and J. Donnez: Characterization of iron deposition in

endometriotic lesions induced in the nude mouse model. *Hum Reprod* 19, 1265-1271 (2004)

43. A. Gaulier, A. Jouret-Mourin, C. Marsan: Peritoneal endometriosis: report of a case with cytologic, cytochemical and histopathologic study. *Acta Cytol* 27, 446-449 (1983)

44. K.L. Sharpe-Timms, E.A. Ricke, M. Piva and G.M. Horowitz: Differential expression and localization of de-novo synthesized endometriotic haptoglobin in endometrium and endometriotic lesions. *Hum Reprod* 15, 2180-2185 (2000)

45. M. Piva, G.M. Horowitz and K.L. Sharpe-Timms: Interleukin-6 differentially stimulates haptoglobin production by peritoneal and endometriotic cells *in vitro*: a model for endometrial-peritoneal interaction in endometriosis. *J Clin Endocrinol Metab* 86, 2553-2561 (2001)

46. S. Martínez-Román, J. Balasch, M. Creus, F. Fábregues, F. Carmona, R. Vilella and J.A. Vanrell: Transferrin receptor (CD71) expression in peritoneal macrophages from fertile and infertile women with and without endometriosis. *Am J Reprod Immunol* 38, 413-417 (1997)

47. K.L. Sharpe-Timms, R.L. Zimmer, E.A. Ricke, M. Piva, G.M. Horowitz: Endometriotic haptoglobin binds to peritoneal macrophages and alters their function in women with endometriosis. *Fertil Steril* 78, 810-819 (2002)

48. S. Defrère, A. Van Langendonckt, S. Vaesen, M. Jouret, R. González Ramos, D. Gonzalez and J. Donnez: Iron overload enhances epithelial cell proliferation in endometriotic lesions induced in a murine model. *Hum Reprod* 21, 2810-2816 (2006)

49. M. Knutson and M. Wessling-Resnick: Iron metabolism in the reticuloendothelial system. *Crit Rev Biochem Mol Biol* 38, 61-88 (2003)

50. H. Mizuuchi, R. Kudo, H. Tamura, K. Tsukahara, N. Tsumura, K. Kumai and K. Sato: Identification of transferrin receptor in cervical and endometrial tissues. *Gynecol Oncol* 31, 292-300 (1988)

51. S. Defrère, J.C. Lousse, R. González Ramos, S. Colette, J. Donnez and A. Van Langendonckt: Potential involvement of iron in the pathogenesis of peritoneal endometriosis. *Mol Hum Reprod* 14, 377-385 (2008)

52. R. Crichton: Inorganic biochemistry of iron metabolism: from molecular mechanisms to clinical consequences. *John Wiley and Sons*, Chichester (2001)

53. G. Balla, H.S. Jacob, J. Balla, M. Rosenberg, K. Nath, F. Apple, J.W. Eaton and G.M. Vercellotti: Ferritin: a cytoprotective antioxidant stratagem of endothelium. *J Biol Chem* 267, 18148-18153 (1992)

54. R.J. Ward, S. Wilmet, R. Legssyer and R.R. Crichton: The influence of iron homoeostasis on macrophage function. *Biochem Soc Trans* 30, 762-765 (2002)

55. A.Y. Demir, P.G. Groothuis, A.W. Nap, C. Punyadeera, A.F. de Goeij, J.L. Evers and G.A. Dunselman: Menstrual effluent induces epithelial-mesenchymal transitions in mesothelial cells. *Hum Reprod* 19, 21-29 (2004)

56. T.F. Tam, R. Leung-Toung, W. Li, Y. Wang, K. Karimian and M. Spino: Iron chelator research: past, present and future. Curr Med Chem 10, 983-995 (2003)

57. J.S. Sanfilippo, N.G. Wakim, K.N. Schikler, M.A. Yussman: Endometriosis in association with uterine anomaly. *Am J Obstet Gynecol* 154, 39-43 (1986)

58. S.L. Darrow, J.E. Vena, R.E. Batt, M.A. Zielezny, A.M. Michalek, S. Selman: Menstrual cycle characteristics and the risk of endometriosis. *Epidemiology* 4, 135-142 (1993)

59. P. Vercellini, O. De Giorgi, G. Aimi, S. Panazza, A. Uglietti and P.G. Crosignani: Menstrual characteristics in women with and without endometriosis. *Obstet Gynecol* 90, 264-268 (1997)

60. D. Vinatier, G. Orzi, M. Cosson and P. Dufour: Theories of endometriosis. *Eur J Obstet Gynecol Reprod Biol* 96, 21-34 (2001)

61. K. Arumugam and J.M. Lim: Menstrual characteristics associated with endometriosis. *Br J Obstet Gynaecol* 104, 948-950 (1997)

62. R. González Ramos, J. Donnez, S. Defrère, I. Leclerq, J. Squifflet, J.C. Lousse and A. Van Langendonckt: Nuclear factor-kappa B is constitutively activated in peritoneal endometriosis. *Mol Hum Reprod* 13, 503-509 (2007)

63. J.C. Lousse, A. Van Langendonckt, R. González Ramos, S. Defrère, E. Renkin and J. Donnez: Increased activation of nuclear factor-kappa B (NF-kappa B) in isolated peritoneal macrophages of patients with endometriosis. *Fertil Steril* 90, 217-220 (2008)

64. M. Karin: Nuclear factor-kappa B in cancer development and progression. *Nature* 441, 431-436 (2006)

65. M. Karin, Y. Yamamoto and Q.M. Wang: The IKK NF-kappa B system: a treasure trove for drug development. *Nat Rev Drug Discov* 3, 17-26 (2004)

66. A. Hoffmann and D. Baltimore: Circuitry of nuclear factor κB signaling. *Immunol Rev* 210, 171-186 (2006)

67. R. González Ramos, A. Van Langendonckt, S. Defrère, J.C. Lousse, S. Colette, L. Devoto and J. Donnez: Involvement of the nuclear factor-kappaB (NF-kappa B) pathway in the pathogenesis of endometriosis. *Fertil Steril* online Feb 24 (2010)

68. S.M. Laird, E.M. Tuckerman, B.A. Cork and T.C. Li: Expression of nuclear factor kappa B in human endometrium; role in the control of interleukin 6 and leukaemia inhibitory factor production. *Mol Hum Reprod* 6, 34-40 (2000)

69. M. Page, E.M. Tuckerman, T.C. Li and S.M. Laird: Expression of nuclear factor kappa-B components in human endometrium. *J Reprod Immunol* 54, 1-13 (2002)

70. A.E. King, H. Critchley and R.W. Kelly: The NF-kappa B pathway in human endometrium and first trimester decidua. *Mol Hum Reprod* 7, 175-183 (2001)

71. C. Ponce, M. Torres, C. Galleguillos, H. Sovino, M.A. Boric, A. Fuentes and M.C. Johnson: Nuclear factor-kappa B pathway and interleukin-6 are affected in eutopic endometrium of women with endometriosis. *Reproduction* 137, 727-737 (2009)

72. C.A. Witz: Cell adhesion molecules and endometriosis. *Semin Reprod Med* 21, 173-182 (2003)

73. R. Zhang, R.A. Wild and J.M. Ojago: Effect of tumor necrosis factor-alpha on adhesion of human endometrial stromal cells to peritoneal mesothelial cells: an *in vitro* system. *Fertil Steril* 59, 1196-1201 (1993)

74. J.S. Griffith, A.K. Rodgers, R.S. Schenken: Reviews: *In vitro* models to study the pathogenesis of endometriosis. *Reprod Sci* 17, 5-12 (2010)

75. B.B. Aggarwal: Nuclear factor-kappa B: the enemy within. *Cancer Cell* 6, 203-208 (2004)

76. A.R. Farina, A. Tacconelli, A. Vacca, M. Maroder, A. Gulino and A.R. Mackay: Transcriptional up-regulation of matrix metalloproteinase-9 expression during spontaneous epithelial to neuroblast phenotype conversion by SK-N-SH neuroblastoma cells, involved in enhanced invasivity, depends upon GT-box and nuclear factor kappa B elements. *Cell Growth Differ* 10, 353-367 (1999)

77. A.S. Baldwin: Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappa B. *J Clin Invest* 107, 241-246 (2001)

78. E. Andreakos, C. Smith, S. Kiriakidis, C. Monaco, R. de Martin, F.M. Brennan, E. Paleolog, M. Feldmann and B.M. Foxwell: Heterogeneous requirement of IkappaB kinase 2 for inflammatory cytokine and matrix metalloproteinase production in rheumatoid arthritis: implications for therapy. *Arthritis Rheum* 48, 1901-1912 (2003)

79. E.M. Grund, D. Kagan, C.A. Tran, A. Zeitvogel, A. Starzinski-Powitz, S. Nataraja and S.S. Palmer: Tumor necrosis factor-alpha regulates inflammatory and mesenchymal responses via mitogen-activated protein kinase kinase, p38, and nuclear factor kappa B in human endometriotic epithelial cells. *Mol Pharmacol* 73, 1394-1404 (2008)

80. R. González Ramos, A. Van Langendonckt, S. Defrère, J.C. Lousse, M. Mettlen, A. Guillet and J. Donnez: Agents blocking the nuclear factor-kappa B (NF-kappa B) pathway are effective inhibitors of endometriosis in an *in vivo* experimental model. *Gynecol Obstet Invest* 65, 174-186 (2008)

81. J. McLaren, A. Prentice, D.S. Charnock-Jones, S.K. Smith: Vascular endothelial growth factor (VEGF) concentrations are elevated in peritoneal fluid of women with endometriosis. *Hum Reprod* 11, 220-223 (1996)

82. Y. Sakamoto, T. Harada, S. Horie, Y. Iba, F. Taniguchi, S. Yoshida, T. Iwabe and N. Terakawa: Tumor necrosis factor-alpha-induced interleukin-8 (IL-8) expression in endometriotic stromal cells, probably through nuclear factor-kappa B activation: gonadotropin-releasing hormone agonist treatment reduced IL-8 expression. *J Clin Endocrinol Metab* 88, 730-735 (2003)

83. Y. Ohama, T. Harada, T. Iwabe, F. Taniguchi, Y. Takenaka and N. Terakawa: Peroxisome proliferator-activated receptor-gamma ligand reduced tumor necrosis factor-alpha-induced interleukin-8 production and growth in endometriotic stromal cells. *Fertil Steril* 89, 311-317 (2008)

84. O. Celik, S. Hascalik, K. Elter, M.E. Tagluk, B. Gurates and N.E. Aydin: Combating endometriosis by blocking proteasome and nuclear factor-kappa B pathways. *Hum Reprod* 23, 2458-2465 (2008)

85. F.D. De Leon, R. Vijayakumar, M. Brown, C.V. Rao, M.A. Yussman and G. Schultz: Peritoneal fluid volume, estrogen, progesterone, prostaglandin, and epidermal growth factor concentrations in patients with and without endometriosis. *Obstet Gynecol* 68, 189-194 (1986)

86. M.H. Wu, H.S. Sun, C.C. Lin, K.Y. Hsiao, P.C. Chuang, H.A. Pan and S.J. Tsai: Distinct mechanisms regulate cyclooxygenase-1 and -2 in peritoneal macrophages of women with and without endometriosis. *Mol Hum Reprod* 8, 1103-1110 (2002)

87. M. Yamaguchi and N. Mori: Prostaglandin and leukotriene concentration of the peritoneal fluid of endometriosis and other gynecologic disorders in the secretory phase. *Prostaglandins Leukot Essent Fatty Acids* 39, 43-45 (1990)

88. M. Peters-Golden and W. Henderson: Leukotrienes. *New Engl J Med* 357, 1841-1854 (2007)

89. E.M. Smyth, T. Grosser, M. Wang, Y. Yu and G.A. FitzGerald: Prostanoids in health and disease. *J Lipid Res* 50, S423-S428 (2009)

90. M. Tsujii and R.N. DuBois: Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 83, 493-501 (1995)

91. M. Tsujii, S. Kawano, S. Tsuji, H. Sawaoka, M. Hori and R.N. DuBois: Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* 93, 705-716 (1998)

92. M. Tsujii, S. Kawano, R.N. DuBois: Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci USA* 94, 3336-3340 (1997)

93. D.L. DeWitt: Prostaglandin endoperoxide synthase: regulation of enzyme expression. *Biochim Biophys Acta* 1083, 121-134 (1991)

94. M. Murakami and I. Kudo: Recent advances in molecular biology and physiology of the prostaglandin E_2-biosynthetic pathway. *Prog Lipid Res* 43, 3-35 (2004)

95. T. Lindstrom and P. Bennett: Transcriptional regulation of genes for enzymes of the prostaglandin biosynthetic pathway. *Prostaglandins Leukot Essent Fatty Acids* 70, 115-135 (2004)

96. K. Sales and H. Jabbour: Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium. *Reproduction* 126, 559-567 (2003)

97. S. Narumiya, Y. Sugimoto and F. Ushikubi: Prostanoid receptors structures, properties, and functions. *Physiol Rev* 79, 1193-226 (1999)

98. B. Ashby: Co-expression of prostaglandin receptors with opposite effects: a model for homeostatic control of autocrine and paracrine signalling. *Biochem Pharmacol* 55, 239-246 (1998)

99. C.R. Kennedy, Y. Zhang, S. Brandon, Y. Guan, K. Coffee, C.D. Funk, M.A. Magnuson, J.A. Oates, M.D. Breyer and R.M. Breyer: Salt-sensitive hypertension and reduced fertility in mice lacking the prostaglandin EP2 receptor. *Nat Med* 5,217-220 (1999)

100. S. Narumiya and G.A. FitzGerald: Genetic and pharmacological analysis of prostanoid receptor function. *J Clin Invest* 108, 25-30 (2001)

101. S. Ferreira and J. Vane: Half-lives of peptides and amines in the circulation. *Nature* 215, 1237-1240 (1967)

102. J.C. Lousse, S. Defrère, S. Colette, A. Van Langendonckt, J. Donnez: Expression of eicosanoid biosynthetic and catabolic enzymes in peritoneal endometriosis. *Hum Reprod* 25, 734-741 (2010)

103. S. Smith: Prostaglandins and growth factors in the endometrium. *Baillieres Clin Obstet Gynaecol* 3, 249-270 (1989)

104. H. Ota, S. Igarashi, M. Sasaki and T. Tanaka: Distribution of cyclooxygenase-2 in eutopic and ectopic endometrium in endometriosis and adenomyosis. *Hum Reprod* 16, 561-566 (2001)

105. F. Chishima, S. Hayakawa, K. Sugita, N. Kinukawa, S. Aleemuzzaman, N. Nemoto, T. Yamamoto and M. Honda: Increased expression of cyclooxygenase-2 in local lesions of endometriosis patients. *Am J Reprod Immunol* 48, 50-56 (2002)

106. F. Chishima, S. Hayakawa, T. Yamamoto, M. Sugitani, M. Karasaki-Suzuki, K. Sugita and N. Nemoto: Expression of inducible microsomal prostaglandin E synthase in local lesions of endometriosis patients. *Am J Reprod Immunol* 57, 218-226 (2007)

107. M.H. Wu, C. Wang, C. Lin, L. Chen, W. Chang and S. Tsai: Distinct regulation of cyclooxygenase-2 by interleukin-1 beta in normal and endometriotic stromal cells. *J Clin Endocrinol Metab* 90, 286-295 (2005)

108. O. Bukulmez, D. Hardy, B. Carr, A. Word and C. Mendelson: Inflammatory status influences aromatase and steroid receptor expression in endometriosis. *Endocrinology* 149, 1190-1204 (2008)

109. Y.J. Kang, U.R. Mbonye, C.J. DeLong, M. Wada and W.L. Smith: Regulation of intracellular cyclooxygenase levels by gene transcription and protein degradation. *Prog Lipid Res* 46, 108-125 (2007)

110. T.O. Ishikawa, N. Jain, H.R. Herschman: Feedback regulation of cyclooxygenase-2 transcription *ex vivo* and *in vivo*. *Biochem Biophys Res Commun* 378, 534-538 (2009)

111. C. Olivares, M. Bilotas, R. Buquet, M. Borghi, C. Sueldo, M. Tesone and G. Meresman: Effects of a selective cyclooxygenase-2 inhibitor on endometrial epithelial cells from patients with endometriosis. *Hum Reprod* 23, 2701-2708 (2008)

112. E.A. Willman, W.P. Collins and S.G. Clayton: Studies in the involvement of prostaglandins in uterine symptomatology and pathology. *Br J Obstet Gynaecol* 83, 337-341 (1976)

113. A.T. Koki, N.K. Khan, B.M. Woerner, K. Seibert, J.L. Harmon, A.J. Dannenberg, R.A. Soslow and J.L. Masferrer: Characterization of cyclooxygenase-2 (COX-2) during tumorigenesis in human epithelial cancers: evidence for potential clinical utility of COX-2 inhibitors in epithelial cancers. *Prostaglandins Leukot Essent Fatty Acids* 66, 13-18 (2002)

114. D.L. DeWitt: Prostaglandin endoperoxide synthase: regulation of enzyme expression. Biochim Biophys Acta 1991;1083:121-34.

115. P.C. Chuang, Y.J. Lin, M.H. Wu, L.Y. Wing, Y. Shoji and S.J. Tsai: Inhibition of CD36-dependent phagocytosis by prostaglandin E2 contributes to the development of endometriosis. *Am J Pathol* 176, 850-60 (2010)

116. S.K. Banu, J. Lee, V.O. Speights, A. Starzinski-Powitz and J.A. Arosh: Cyclooxygenase-2 regulates survival, migration, and invasion of human endometriotic cells through multiple mechanisms. *Endocrinology* 149, 1180-1189 (2008)

117. B.A. Erickson, W.E. Longo, N. Panesar, J.E. Mazuski and D.L. Kaminiski: The effect of selective cyclooxygenase inhibitors on intestinal epithelial cell

mitogenesis. *Journal of Surgery Research* 81, 101-107 (1999)

118. S.A. Milne and H.N. Jabbour: Prostaglandin (PG) F(2alpha) receptor expression and signaling in human endometrium: role of PGF(2alpha) in epithelial cell proliferation. *J Clin Endocrinol Metab* 88, 1825-1832 (2003)

119. J. Lee, S.K. Banu, R. Rodriguez, A. Starzinski-Powitz and J.A. Arosh: Selective blockade of prostaglandin E2 receptors EP2 and EP4 signaling inhibits proliferation of human endometriotic epithelial cells and stromal cells through distinct cell cycle arrest. *Fertil Steril* 93, 2498-2506 (2010)

120. S.K. Banu, J. Lee, V.O. Speights, A. Starzinski-Powitz and J.A. Arosh: Selective inhibition of prostaglandin E2 receptors EP2 and EP4 induces apoptosis of human endometriotic cells through suppression of ERK1/2, AKT, NF-kappa B, and beta-catenin pathways and activation of intrinsic apoptotic mechanisms. *Mol Endocrinol* 23, 1291-1305 (2009)

121. G.B. Perchick and H.N. Jabbour: Cyclooxygenase-2 overexpression inhibits cathepsin D-mediated cleavage of plasminogen to the potent antiangiogenic factor angiostatin. *Endocrinology* 144, 5322-5328 (2003)

122. K.J. Sales, S. Maudsley and H.N. Jabbour. Elevated prostaglandin EP2 receptor in endometrial adenocarcinoma cells promotes vascular endothelial growth factor expression via cyclic 3',5'-adenosine monophosphate-mediated transactivation of the epidermal growth factor receptor and extracellular signal-regulated kinase 1/2 signaling pathways. *Mol Endocrinol* 18, 1533-1545 (2004)

123. M. Laschke, A. Elitzsch, C. Scheuer, B. Vollmar and M. Menger: Selective cyclo-oxygenase-2 inhibition induces regression of autologous endometrial grafts by down-regulation of vascular endothelial growth factor-mediated angiogenesis and stimulation of caspase-3-dependent apoptosis. *Fertil Steril* 87, 163-171 (2007)

124. D.E. Machado, P.T. Berardo, R.G. Landgraf, P.D. Fernandes, C. Palmero, L.M. Alves, M.S. Abrao and L.E. Nasciutti: A selective cyclooxygenase-2 inhibitor suppresses the growth of endometriosis with an antiangiogenic effect in a rat model. *Fertil Steril* 93, 2674-2679 (2010)

125. S. Matsuzaki, M. Canis, C. Darcha, R. Dallel, K. Okamura, G. Mage: Cyclooxygenase-2 selective inhibitor prevents implantation of eutopic endometrium to ectopic sites in rats. *Fertil Steril* 82, 1609-1615 (2004)

126. E. Dogan, U. Sayigili, C. Posaci, B. Tuna, S. Caliskan, S. Altunyurt and B. Saatli: Regression of endometrial explants in rats treated with the cyclooxygenase-2 inhibitor rofecoxib. *Fertil Steril* 82, 1115-1120 (2004)

127. Y. Ozawa, T. Murakami, M. Tamura, Y. Terada, N. Yaegashi and K. Okamura: A selective cyclooxygenase-2 inhibitor suppresses the growth of endometriosis xenografts via antiangiogenic activity in severe combined immunodeficiency mice. *Fertil Steril* 86, 1146-1151 (2006)

128. L. Cobellis, S. Razzi, S. De Simone, A. Sartini, A. Fava, S. Danero, W. Gioffrè, M. Mazzini and F. Petraglia: The treatment with a COX-2 specific inhibitor is effective in the management of pain related to endometriosis. Eur *J Obstet Gynecol Reprod Biol* 116, 100-102 (2004)

129. H. Lim, B.C. Paria, S.K. Das, J.E. Dinchuk, R. Langenbach, J.M. Trzaskos and S.K. Dey: Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91, 197-208 (1997)

130. Y.I. Cha and R.N. DuBois: NSAIDs and cancer prevention: targets down-stream of COX-2. *Annu Rev Med* 58, 239-252 (2007)

131. M. Triggiani, F. Granata, A. Frattini and G. Marone: Activation of human inflammatory cells by secreted phospholipases A2. *Biochim Biophys Acta* 1761, 1289-1300 (2006)

132. M.T. Rizzo, E. Nguyen, M. Aldo-Benson, G. Lambeau: Secreted phospholipase A(2) induces vascular endothelial cell migration. *Blood* 96, 3809-3815 (2000)

133. S. Beck, G. Lambeau, K. Scholz-Pedretti, M.H. Gelb, M.J. Janssen, S.H. Edwards, D.C. Wilton, J. Pfeilschifter and M. Kaszkin: Potential of tumor necrosis factor alpha-induced secreted phospholipase A2 (sPLA2)-IIA expression in mesangial cells by an autocrine loop involving sPLA2 and peroxisome proliferator-activated receptor alpha activation. *J Biol Chem* 278, 29799-29812 (2003)

134. M.C. Meyer, P. Rastogi, C.S. Beckett and J. McHowat: Phospholipase A2 inhibitors as potential anti-inflammatory agents. *Curr Pharm Des* 11, 1301-1312 (2005)

135. H. Kobayashi, Y. Yamada, S. Kanayama, N. Furukawa, T. Noguchi, S. Haruta, S. Yoshida, M. Sakata, T. Sado and H. Oi: The role of iron in the pathogenesis of endometriosis. *Gynecol Endocrinol* 25, 39-52 (2009)

136. N. Yamauchi, T. Harada, F. Taniguchi, S. Yoshida, T. Iwabe and N. Terakawa: Tumor necrosis factor-α induced the release of interleukin-6 from endometriotic stromal cells by the nuclear factor-kappa B and mitogen-activated protein kinase pathways. *Fertil Steril* 82, 1023-1028 (2004)

137. E.L. Sheldrick, K. Derecka, E. Marshall, E.C. Chin, L. Hodges, D.C. Wathes, D.R. Abayasekara and A.P. Flint: Peroxisome-proliferator-activated receptors and the control of levels of prostaglandin-endoperoxide synthase 2 by arachidonic acid in the bovine uterus. Biochem J 406,175-183 (2007)

138. K. Derecka, E.L. Sheldrick, D.C. Wathes, D.R. Abayasekara and A.P. Flint: A PPAR-independent pathway

to PUFA-induced COX-2 expression. Mol Cell Endocrinol 287, 65-71 (2008)

139. W.G. Cao, M. Morin, C. Metz, R. Maheux and A. Akoum: Stimulation of macrophage migration inhibitory factor expression in endometrial stromal cells by interleukin 1, beta involving the nuclear transcription factor NF-kappa B. Biol Reprod 73, 565-570 (2005)

140. V. Veillat, C.H. Lavoie, C.N. Metz, T. Roger, Y. Labelle and A. Akoum: Involvement of nuclear factor-kappa B in macrophage migration inhibitory factor gene transcription up-regulation induced by interleukin- 1 beta in ectopic endometrial cells. Fertil Steril 91, 2148-2156 (2009)

141. F. Wieser, J.L. Vigne, I. Ryan, D. Hornung, S. Djlali and R.N. Taylor: Sulindac suppresses nuclear factor-kappa B activation and RANTES gene and protein expression in endometrial stromal cells from women with endometriosis. J Clin Endocrinol Metab 90, 6441-6447 (2005)

142. T. Yagyu, H. Kobayashi, H. Matsuzaki, K. Wakahara, T. Kondo, N. Kurita, H. Sekino, K. Inagaki, M. Suzuki, N. Kanayama and T. Terao: Thalidomide inhibits tumor necrosis factor-alpha-induced interleukin-8 (IL-8) expression in endometriotic stromal cells possibly through suppression of nuclear factor-kappa B activation. J Clin Endocrinol Metab 90, 3017-3021 (2005)

143. S. Horie, T. Harada, M. Mitsunari, F. Taniguchi, T. Iwabe and N. Terakawa: Progesterone and progestational compounds attenuate tumor necrosis factor alpha-induced interleukin-8 production via nuclear factor kappa B inactivation in endometriotic stromal cells. Fertil Steril 83, 1530-1535 (2005)

144. Y. Tagashira, F. Taniguchi, T. Harada, A. Ikeda, A. Watanabe and N. Terakawa: Interleukin-10 attenuates TNF-alpha-induced interleukin-6 production in endometriotic stromal cells. Fertil Steril 91, 2185-2192 (2009)

145. W. Xiu-li, C. Wen-jun, D. Hui-hua, H. Su-ping and F. Shi-long: ERB-041, a selective ER beta agonist, inhibits iNOS production in LPS-activated peritoneal macrophages of endometriosis via suppression of NF-kappa B activation. Mol Immunol 46, 2413-2418 (2009)

146. K. Nasu, M. Nishida, T. Ueda, A. Yuge, N. Takai and H. Narahara: Application of the nuclear factor-kappa B inhibitor BAY 11-7085 for the treatment of endometriosis: an *in vitro* study. Am J Physiol Endocrinol Metab 293, 16-23 (2007)

147. M. Güney, S. Nasir, B. Oral, N. Karahan and T. Mungan: Effect of caffeic acid phenethyl ester on the regression of endometrial explants in an experimental rat model. Reprod Sci 14, 270-279 (2007)

148. S. Netsu, R. Konno, K. Odagiri, M. Soma, H. Fujiwara and M. Suzuki: Oral eicosapentaenoic acid supplementation as possible therapy for endometriosis. Fertil Steril 90, 1496-1502 (2008)

149. M.W. Laschke, C. Schwender, C. Scheuer, B. Vollmar and M.D. Menger: Dietary glycine does not affect physiological angiogenesis and reproductive function, but inhibits apoptosis in endometrial and ovarian tissue by down-regulation of nuclear factor-kappa B. Fertil Steril 90, 1460-1469 (2008)

150. A.V. Huber, J.C. Huber, A. Kolbus, M. Imhof, F. Nagele, D. Loizou, U. Kaufmann and C.F. Singer: Systemic HCG treatment in patients with endometriosis: a new perspective for a painful disease. Wien Klin Wochenschr 116, 839-843 (2004)

151. A.V. Huber, L. Saleh, J. Prast, P. Haslinger and M. Knöfler: Human chorionic gonadotrophin attenuates NF-kappa B activation and cytokine expression of endometriotic stromal cells. Mol Hum Reprod 13, 595-604 (2007)

Abbreviations: 5-LO: 5-lipoxygenase; 15-PGDH: 15-hydroxy prostaglandin dehydrogenase; AP-1: activator protein-1; COX: cyclooxygenase; DNA: deoxyribonucleic acid; HIF-1: hypoxia-inducible factor-1; ICAM-1: intercellular adhesion molecule-1; IL: interleukin; MCP-1: monocyte chemotactic protein-1; MIF: macrophage migration inhibitory factor; MMP: matrix metalloproteinase; mPGES-1: microsomal prostaglandin E synthase-1; mRNA: messenger ribonucleic acid; NF-kappa B: nuclear factor-kappa B; PF: peritoneal fluid; PG: prostaglandin; PGE2: prostaglandin E2; RANTES: regulated on activation and normally T-cell expressed and secreted; ROS: reactive oxygen species; sPLA2-IIA: type IIA secretory phospholipase A2; TNF: tumor necrosis factor; uPA: urokinase-type plasminogen activator; VEGF: vascular endothelial growth factor

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