NEW RESEARCH HORIZON

Potential involvement of iron in the pathogenesis of peritoneal endometriosis

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The aim of this study is to review the current literature associating endometriosis with iron and to discuss the potential causes and consequences of iron overload in the pelvic cavity. Indeed, iron is essential for all living organisms. However, excess iron can result in toxicity and is associated with pathological disorders. In endometriosis patients, iron overload has been demonstrated in the different components of the peritoneal cavity (peritoneal fluid, endometriotic lesions, peritoneum and macrophages). Animal models allow us to gather essential information on the origin, metabolism and effect of iron overload in endometriosis, which may originate from erythrocytes carried into the pelvic cavity mainly by retrograde menstruation. Peritoneal macrophages play an important role in the degradation of these erythrocytes and in subsequent peritoneal iron metabolism. Iron overload could affect a wide range of mechanisms involved in endometriosis development, such as oxidative stress or lesion proliferation. In conclusion, excess iron accumulation can result in toxicity and may be one of the factors contributing to the development of endometriosis. Treatment with an iron chelator could thus be beneficial in endometriosis patients to prevent iron overload in the pelvic cavity, thereby diminishing its deleterious effect.

Keywords: endometriosis; iron; macrophages; oxidative stress; retrograde menstruation

Endometriosis is defined as the presence of endometrial tissue, including both glandular epithelium and stroma, outside the uterine cavity. It is one of the most common benign gynecological disorders, affecting ~10–15% of all women of reproductive age and >30% of infertile women (Koninckx, 1999; Donnez et al., 2002). This pathology is associated with various distressing symptoms such as dysmenorrhea, dyspareunia, pelvic pain and subfertility. Despite an increasing number of studies on endometriosis, its etiology remains elusive due, in part, to its multifactorial characteristics. Indeed, a growing body of evidence suggests that a combination of genetic, hormonal, environmental, immunological and anatomical factors play a role in the pathogenesis of this disorder (Nisolle and Donnez, 1997; Van Langendonckt et al., 2002a; Giudice and Kao, 2004; Heilier et al., 2008).

Iron is an essential metal for almost all living organisms because of its involvement in a large number of iron-containing enzymes and proteins (Kaplan and O’Halloran, 1996; Aisen et al., 1999; Andrews, 1999). However, excess iron accumulation within tissues and cells can result in toxicity (Winterbourn, 1995; McCord, 1998) and is associated with the pathogenesis of a variety of diseases such as thalassemia, hemochromatosis, HIV or neurodegenerative diseases (Crichton et al., 2002). Moreover, in the case of hemorrhage, lysis of erythrocytes leads to iron overload, provoking iron-mediated damage, oxidative injury and inflammation (Sercome et al., 2002; Potts et al., 2006; Gorbunov et al., 2006; Xi et al., 2006; Levy et al., 2007). It was recently suggested that iron could be involved in endometriosis development (Arumugam and Yip, 1995; Defrère et al., 2006).

In this manuscript, iron metabolism and cellular uptake in humans are summarized, emphasizing the key role of macrophages in these processes. Current literature on iron involvement in endometriosis is reviewed and potential causes of iron overload in the pelvic cavity are reported. A model of metabolism and storage in the pelvic cavity of endometriosis patients is proposed on the basis of current knowledge of general iron metabolism, data collected from patient biopsies and in vivo and in vitro experimental models. Finally, the consequences of iron overload on the pathogenesis of endometriosis are discussed.

Iron metabolism in humans: an overview

Iron metabolism in humans is shown in Fig. 1. In humans, body iron content is ~45 mg Fe/kg body weight (Harrison-Findik, 2007), with typically higher values in men than in women. Circulating red blood cells contain most of this iron bound to the oxygen transport protein, hemoglobin (Hb; ~30 mg Fe/kg). A further 4 mg Fe/kg is found in muscle in the form of the oxygen storage protein, myoglobin, and ~2 mg Fe/kg in various tissues in the form of functional iron-containing proteins. Most of the remaining iron (10–12 mg Fe/kg in men and around 5 mg Fe/kg in women) is stored essentially in the liver, spleen, bone marrow and muscle in the form of ferritin and hemosiderin, whereas only a tiny fraction of total body iron, around 3 mg, circulates in the plasma and other extracellular fluids bound to the iron-transport protein, transferrin (Tf) (see Fig. 1) (Bothwell et al., 1979; Andrews, 1999). This transport compartment, despite
the non-pregnant uterus (Kunz and Leyendecker, 2001). Thanks to retrograde menstruation generated by uterine peristalsis of menstrual loss is limited, and body iron content in women is preserved because of gestation and lactation periods and blood loss during men- average daily iron absorption is about twice that in men, largely red cells, and one-third by exfoliation of cells from the skin and the gastrointestinal tract by exfoliation of mucosal cells and loss of absorption is the upper part of the gastrointestinal tract (the protein, promotes oxidation of Fe$^{2+}$ to Fe$^{3+}$). Under physiological conditions, there are many ferritin molecules available, and much of the iron will be trapped in ferritin and transformed into the form known as hemosiderin (Crichton, 2001) (Fig. 2).

**Iron absorption and transport by intestinal mucosa cells**

Normal subjects ingest $\sim$12–18 mg of dietary Fe/day, mainly as Fe$^{2+}$, of which 1–2 mg is absorbed (Crichton et al., 2002)

Iron is taken up by the intestinal mucosa cells (enterocytes). The two major uptake systems for dietary iron require reduction of Fe$^{3+}$ to Fe$^{2+}$ (Gräsbeck et al., 1982; Beale and Yeh, 1999; McKie et al., 2001). When Fe$^{2+}$ enters a mucosal cell, it has only two alternatives: either to encounter a ferritin molecule, which incorporates iron in the form of Fe$^{2+}$, oxidizes it to Fe$^{3+}$ and traps the Fe$^{3+}$ within the protein shell or to be transported to the basolateral membrane (Sharpe and Srai, 2007). Under physiological conditions, there are many ferritin molecules available, and much of the iron will be trapped in ferritin and retained in the mucosal cell, resulting in low mucosal transfer of iron (Crichton et al., 2002).

The diffusion of Fe$^{2+}$ across the basolateral membrane is facilitated by iron-regulated transporter 1 (IREG1), a transmembrane iron transporter protein (McKie et al., 2000). Hephaestin, a membrane-bound protein, promotes oxidation of Fe$^{2+}$ to Fe$^{3+}$ (Vulpe et al., 1999). Fe$^{3+}$ formed in this way is promptly bound to plasma ligands, essentially to the major iron transport protein, apotransferrin. Oxidation of Fe$^{2+}$ allows rapid binding of iron to Tf and its delivery to cells expressing Tf receptors, thus preventing endothelial damage and favoring iron uptake by other cells (Crichton et al., 2002).

**Intracellular iron uptake**

Iron in serum is present at concentrations between 3 and 5 µg/ml in normal subjects and is predominantly bound to Tf (Crichton, 2001). As illustrated in Fig. 2, the bilobal Tf molecule can bind two Fe$^{3+}$ ions tightly, but reversibly. It has become clear that iron uptake in almost all mammalian cells is mediated by Tf receptors (Lawrence et al., 1999). Diferric Tf binds to its receptor on the cell surface and the Tf–Tf receptor complex is internalized. Iron is released from the Tf–Tf receptor and transported out of the endosome. Apotransferrin, still bound to the Tf receptor, then returns to the cell surface, where the iron-free protein is released into the circulation for reutilization, completing a highly efficient cycle (Katz, 1961).

Once iron has entered a cell, it can become involved in a multitude of cellular processes, which include incorporation into a number of essential iron-containing proteins, as well as sequestration in ferritin. Ferritin can store up to 4500 iron atoms per molecule. In conditions of iron overload, the ferritin is transferred to the lysosomes, where it is transformed into the form known as hemosiderin (Crichton, 2001) (Fig. 2).

**Macrophages and iron metabolism**

Macrophages have two important functions: first, to orchestrate the inflammatory response; and second, to regulate iron homeostasis (Ward et al., 2002).

**Iron uptake by macrophages**

Macrophages acquire most of their iron by phagocytosing senescent red blood cells. After erythrophagocytosis, digestion of Hb liberates heme, which is catalyzed by heme oxygenase (HO) to produce biliverdin, carbon monoxide and Fe$^{2+}$ (Maines, 1997). The liberated iron is then either released from the macrophages or stored.

From kinetic studies of Hb turnover in humans, it has been calculated that 10–20% of normal erythrocyte destruction occurs intravascularly, resulting in the release of Hb (Garby and Noyes, 1959).

**Figure 1:** Schematic representation of iron metabolism in humans (adapted from Crichton, 2001). The plasma donates transferring-bound iron (Tf-Fe) to cells that need iron for cell division or production of iron proteins, mainly erythroblasts in the bone marrow. The majority of iron is used for hemoglobin synthesis. Iron enters the plasma from iron donor cells, mainly the macrophages and enterocytes. This iron is rapidly bound to Tf. Erythrocytes are destroyed in macrophages. Iron is lost from the body together with exfoliating cells or during blood loss.

**Figure 2:** Cellular iron uptake in mammals. Iron circulates in the plasma bound to Tf, which binds to its receptor (TIR) on the cell surface, and the Tf–TIR complex is internalized. Iron is released by endosomes. Apotransferrin, still bound to the TIR, then returns to the cell surface, where the iron-free protein is released into the circulation for reutilization, completing a highly efficient cycle. Released iron can become involved in a multitude of cellular processes, which include incorporation into a number of essential iron-containing proteins, as well as sequestration in ferritin. In conditions of iron overload, ferritin is transformed into hemosiderin.
Under normal circumstances, all of this Hb is rapidly bound by haptoglobin (Hp), which is then cleared from the circulation by parenchymal cells of the liver (Deiss, 1983). However, recent studies have identified a Hb scavenger receptor, CD163, expressed exclusively on monocytes and macrophages (Kristiansen et al., 2001). CD163 scavenges Hb by mediation of endocytosis and subsequent degradation of the Hb–Haptoglobin (Kristiansen et al., 2001). Uptake of Hb–Haptoglobin may represent a significant pathway of iron acquisition by macrophages. In conditions associated with increased intravascular hemolysis (e.g. hemolytic anemia, thalassemia, etc.), the Hb-binding capacity of Hp may be insufficient, so that free Hb appears in the plasma. Some of the circulating free Hb is degraded and releases heme, which then binds to the plasma glycoprotein hemopexin. Specific hemopexin receptors on hepatocytes clear the heme–hemopexin complex from the circulation (Alam and Smith, 1989). The detection of hemopexin receptors on human monocytic cell lines (Alam and Smith, 1989; Take-tani et al., 1990) also suggests that macrophages are able to acquire hemopexin-bound heme, but the amount taken up is likely to be low under normal circumstances.

Finally, like most other cells, macrophages express Tf receptors and are able to take up iron from Tf (Sizemore and Bassett, 1984).

**Iron storage by macrophages**

The main sites of body iron storage are the hepatic parenchyma and the reticuloendothelial system (RES). Iron acquired via erythropoiesis, which is not utilized or released, is first destined for storage in ferritin. As the amount of iron in the cell increases, a larger percentage is deposited in hemosiderin, an insoluble, aggregated form of partially digested ferritin. The highest concentrations of hemosiderin in the body are found in the RES (Bothwell et al., 1979).

**Iron release by macrophages**

Normal adult human plasma contains ~3–4 mg of iron, essentially all bound to Tf. Small amounts of plasma iron are acquired by the absorption of dietary iron from the duodenum, but most circulating iron issues from the RES through the release of iron by catabolized senescent red cells. Indeed, most of the iron released by macrophages into the plasma is bound by Tf (Kondo et al., 1988; Rama et al., 1988; Moura et al., 1998). A number of studies also indicate that RE cells release significant amounts of erythropoietic iron in the form of Hb (Custer et al., 1982; Kondo et al., 1988; Moura et al., 1998), heme (Kleber et al., 1981) or ferritin (Kleber et al., 1981; Custer et al., 1982; Kondo et al., 1988; Rama et al., 1988; Moura et al., 1998). It has been suggested that Hb release results from macrophage cell death after the ingestion of too many erythrocytes (Kondo et al., 1988), but other authors claim that Hb release represents a normal physiological process (Custer et al., 1982; Moura et al., 1998).

**Metabolism in the case of local iron overload**

In the case of internal hemorrhage, Hb released from erythrocytes constitutes a stimulus for oxidative stress and inflammation. An important defense mechanism to counteract the effects of hemorrhage is mediated by Hp, which binds extracellular Hb, thereby attenuating its oxidative and inflammatory potential. Hp also promotes the clearance of Hb via the CD163 scavenger receptor present on macrophages (Levy et al., 2007). This scavenging pathway is the only known mechanism for removing free Hb released from extravascular sites, i.e. sites of hemorrhage within atherosclerotic plaque.

**Iron overload in endometriosis: evidence of a relationship?**

As reported in Table I, 21 studies have demonstrated the presence of iron overload in the different components of the peritoneal cavity of endometriosis patients (peritoneal fluid, ectopic endometrial tissue, peritoneum adjacent to lesions and macrophages).

**Peritoneal fluid**

In the peritoneal fluid of patients with endometriosis, higher levels of iron (Arumugam, 1994; Arumugam and Yip, 1995; Van Langendonckt et al., 2002a; Lousse et al., 2008a), ferritin (Van Langendonckt et al., 2002a; Polak et al., 2006; Lousse et al., 2008a), Tf (Mathur et al., 1999) and Hb (Van Langendonckt et al., 2002b) were detected than in control patients. Saturation of Tf was also found to be higher in the peritoneal fluid of endometriosis patients (Lousse et al., 2008a).

**Endometriotic lesions and peritoneum**

In the stroma of endometriotic lesions and peritoneum, cytolological and histochemical data revealed the presence of iron conglomerates (Moen and Halvorsen, 1992; Petrozza et al., 1993; Van Langendonckt et al., 2004) and macrophages heavily laden with ferric pigment (Gaulier et al., 1983). In endometriotic cysts too, iron concentrations in cystic fluid were considered to be an indicator of endometriosis (Sugimura et al., 1992; Takahashi et al., 1996; Iizuka et al., 1998; Yamaguchi et al., 2008). Sharpe-Timms et al. showed that endometriotic lesions were able to synthesize and secrete Hp (Sharpe-Timms et al., 1998, 2000; Piva and Sharpe-Timms, 1999; Piva et al., 2001). Others have demonstrated strong expression of heme oxygenase-1 (HO-1), catalyzing heme degradation, in ectopic endometrium (Van Langendonckt et al., 2002b).

**Peritoneal macrophages**

Iron metabolism by macrophages appears to be enhanced in the 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 (Gaulier et al., 1983; Stowell et al., 1997). Lousse et al. (2008a) recently demonstrated increased iron storage (ferritin load) in the peritoneal macrophages of endometriosis patients compared with healthy subjects, correlating with iron load in peritoneal fluid. Bilirubin pigment, which is a normal metabolite of Hb (Hb→biliverdin→bilirubin), was also identified inside macrophages (Gaulier et al., 1983). Moreover, in the case of endometriosis, peritoneal macrophages were found to express more Tf receptors (Martinez-Román et al., 1997) and to be Hp-saturated (Sharpe-Timms et al., 2002).

Iron overload involves all the components of the peritoneal cavity in endometriosis patients. However, it is strongly localized and does not affect body iron content (Van Langendonckt et al., 2002a). On the contrary, endometriosis patients often experience longer and heavier menstrual periods (Sanfilippo et al., 1986; Darrow et al., 1993; Vercellini et al., 1997; Vinatier et al., 2001), resulting in anemia.

Most cells protect themselves from iron toxicity by expressing inducible HO-1 and scavenger proteins, such as Hp and hemopexin, binding Hb and heme, respectively. However, increased iron load, observed in all the components of the peritoneal cavity in endometriosis patients compared with controls, strongly suggests that iron homeostasis in the peritoneal cavity may be disrupted in these patients.
Table 1. Presence of iron overload in different components of the peritoneal cavity in case of endometriosis.

<table>
<thead>
<tr>
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<th>Peritoneal fluid (PF)</th>
<th>Macrophages in PF</th>
<th>Ectopic endometrium</th>
<th>Peritoneum</th>
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<td>Gaulier et al. (1983)</td>
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<td>Presence of iron-laden macrophages</td>
<td>Iron-laden macrophages in lesions</td>
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<td>Moen and Halvorsen (1992) and Petrozza et al. (1993)</td>
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<td>Iron deposits in lesions</td>
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<td>Sugimura et al. (1992), Takahashi et al. (1996), Iizuka et al. (1998) and Yamaguchi et al. (2008)</td>
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<td>High Fe levels in ovarian endometriotic cysts</td>
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<td>Arumugam (1994) and Arumugam and Yip (1995)</td>
<td>Higher Fe levels</td>
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<td>Stowell et al. (1997)</td>
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<td>Presence of iron-laden macrophages</td>
<td>Higher Tf expression in macrophages</td>
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<td>Van Langendonckt et al. (2002a)</td>
<td>Higher Hb levels</td>
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<td>Van Langendonckt et al. (2002b)</td>
<td>Higher ferritin and Fe levels</td>
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<td>Sharpe-Timms et al. (2002)</td>
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<td>Hp-saturated macrophages</td>
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<td>Polak et al. (2006)</td>
<td>Higher ferritin levels</td>
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<td>Lousse et al. (2008a)</td>
<td>Higher Tf saturation, ferritin and Fe levels</td>
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Tf, transferrin; Hb, haemoglobin; Hp, haptoglobin; HO, heme-oxygenase-1.

**Origin of iron in the pelvic cavity**

In the case of endometriosis, iron overload may originate from lysis of pelvic erythrocytes (Van Langendonckt et al., 2004).

Retrograde menstruation is considered an essential step in the pathogenesis of peritoneal endometriosis, according to Sampson’s theory (Sampson, 1927). This reflux, transporting menstrual endometrial tissue through the Fallopian tubes into the peritoneal cavity, is a common physiologic event in all menstruating women with patent tubes (Halme et al., 1984). Moreover, and red blood cells are present in the peritoneal fluid of most women. Why then would iron accumulate inside the pelvic cavity of some patients but not others? One hypothesis is that, in some patients, peritoneal protective mechanisms might be overwhelmed by menstrual reflux, either because of the abundance of reflux or because of defective scavenging systems (Sampfli et al., 1986). Menstrual periods are also frequently longer and heavier in endometriosis patients than in controls (Cramer et al., 1986; Darrow et al., 1993; Vercellini et al., 1997), although cycles tend to be shorter (Arumugam and Lim, 1997).

Indeed, in endometriosis patients, retrograde menstruation may be increased by certain anatomical dispositions often found in these patients, including hypertonia of the uterotubular junction, waves of retrograde contractions of the tubular and myometrial musculature (Salamanca and Beltran, 1995) and uterine malformations preventing or disturbing normal anterograde menstrual flux (Sanfilippo et al., 1986). Menstrual periods are also frequently longer and heavier in endometriosis patients than in controls (Cramer et al., 1986; Darrow et al., 1993; Vercellini et al., 1997), although cycles tend to be shorter (Arumugam and Lim, 1997).

Moreover, processes other than menstrual reflux, such as lesion bleeding, may contribute to the accumulation of erythrocytes in peritoneal fluid. Increased concentrations of erythrocytes have been reported in the peritoneal cavity of women with endometriosis (Halme et al., 1984; D’Hooghe and Debrock, 2002).

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. In a first study (Van Langendonckt et al., 2004), endometriosis was induced in nude mice by injection of unfractionated human menstrual effluent, endometrial fragments plus serum, endometrial fragments plus erythrocytes or endometrial cells alone. Iron deposits resembling those found in humans were observed in lesions induced by injection of menstrual effluent or endometrial cells with erythrocytes. In a second study (Defrére et al., 2006), human menstrual endometrium was injected into nude mice intraperitoneally, either alone (controls) or supplemented with erythrocytes or desferrioxamine (DFO), an iron chelator. Injection of erythrocytes caused iron overload in lesions, peritoneal macrophages and fluid, whereas DFO effectively reduced iron status in different components. Both studies clearly suggest that peritoneal iron overload encountered in lesions, peritoneal fluid and peritoneal macrophages of endometriosis patients may well originate from erythrocytes carried into the pelvic cavity by retrograde menstruation, or hemorrhaging foci of ectopic endometrium (Van Langendonckt et al., 2004; Defrére et al., 2006).

**Iron metabolism in the pelvic cavity**

Studies with experimental models and analysis of patient biopsies (see Table I) yielded further information on iron metabolism in the pelvic cavity in the case of endometriosis, which was interpreted in the light of data on erythrocyte metabolism available in the literature.

Postulated iron metabolism in the pelvic cavity in the context of endometriosis pathology is illustrated in Fig. 3. 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 peritoneal fluid of endometriosis patients (Gaulier et al., 1983; Stowell et al., 1997; Lousse et al., 2008a) and mice.
Iron and endometriosis

Effect of iron overload on endometriosis development

Endometriosis is a multifactorial disorder involving numerous mechanisms and a wide range of cell types, including endometrial cells (stromal and epithelial), mesothelial cells, endothelial cells and immune cells (macrophages, lymphocytes, ...). Iron overload could impair the functionality of these different cell types, thereby contributing to the development of the disease (see Fig. 4).

Iron, macrophages and oxidative stress

Peritoneal macrophages are known to play an important role in the initiation, maintenance and progression of endometriotic lesions (Dunsinlan, 1995; Lebovic et al., 2001). They may demonstrate differences in phenotype, as illustrated by higher expression of estrogen receptors-α and -β, differentiation markers (CD68, NCL-MACRO and HAM56) and inflammatory cytokines (interleukin-1β, tumor necrosis factor-α and IL-6) (Montagna et al., in press). As well as increasing in number, they have been found to be more activated in the case of endometriosis, releasing various products such as cytokines, growth and angiogenic factors (Oral et al., 1996; Gazvani and Templeton, 2002). In fact, activation of macrophages is an essential defense mechanism (acute inflammation), but in pathological conditions, such as endometriosis, their activation might become exacerbated and inflammation become chronic (Santanam et al., 2002).

Recently, Lousse et al. (2008a) showed iron storage levels (ferritin load) to be significantly higher in peritoneal macrophages of endometriosis patients than controls. Cellular iron storage within ferritin limits the capacity of iron to generate free radicals (Balla et al., 1992). However, continued delivery of iron to macrophages can overwhelm intraperitoneally injected with erythrocytes (Defrère et al., 2006). Macrophages usually phagocytose senescent erythrocytes or endocytose the Hb–Hp complex (Knutson and Wessling-Resnick, 2003). Metabolism of Hb and heme by HO releases iron, which is then incorporated into ferritin in macrophages or returned to the iron transporter Tf via the peritoneal fluid. Tf may then be incorporated by ectopic endometrial cells, resulting in the formation of iron deposits (ferritin or hemosiderin) inside lesions.

Moreover, erythrocyte lysis causes Hb release in the peritoneal cavity. Hb forms a complex with Hp which is, in part, secreted by endometriotic lesions (Sharpe-Timms et al., 1998, 2000; Piva and Sharpe-Timms, 1999) and then endocytosed by macrophages (Kristiansen et al., 2001; Knutson and Wessling-Resnick, 2003). This is a physiological event involved in iron recycling from senescent red blood cells. However, in the case of endometriosis, macrophages are Hp-saturated (Sharpe-Timms et al., 2002), suggesting that the scavenger mechanisms might be overwhelmed. Furthermore, macrophages are able to release ferritin (Knutson and Wessling-Resnick, 2003). This iron released by macrophages (in ferritin or Tf form) or by erythrocyte lysis (Hb) results in increased peritoneal fluid iron concentrations in endometriosis patients (Van Langendonckt et al., 2002a).

Dassen et al. (2008) recently showed that Hb is expressed by endometrial tissue, and metabolization of heme has been found to occur within endometrial implants. Indeed, active red endometrial lesions strongly express HO, the enzyme catalyzing degradation of the heme moiety of Hb into iron, carbon monoxide (CO) and biliverdin (Casanas-Roux et al., 2002; Van Langendonckt et al., 2002b). Iron is then incorporated into ferritin or hemosiderin in conditions of iron overload.

The presence of hemosiderin in ectopic endometrial tissue and macrophages, usually associated with toxic pathological states in humans, strongly suggests that peritoneal protective mechanisms might be overwhelmed in the case of endometriosis.

Figure 4: Cells and processes involved in endometriosis development. Iron overload may affect a wide range of cell types, modulating multiple mechanisms involved in endometriosis development.
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 \((\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^-)\) to potentiate oxygen and nitrogen toxicity by the generation of a wide range of free radical species, including hydroxyl radicals, \(\text{OH}\), or the peroxynitrite anion \((\text{ONOO}^-)\), produced by the reaction between \(\text{NO}\) and the superoxide anion \((\text{O}_2^-)\).

Hydroxyl radicals are the most reactive free radical species known and have the ability to react with a wide range of cellular constituents, including amino-acid residues and purine and pyrimidine bases of DNA, as well as attacking membrane lipids to initiate a free radical chain reaction known as lipid peroxidation. It is clear that reactive oxygen species (ROS) are generated within the cell in the course of normal cellular mechanisms and that the cell is adequately supplied with a range of cytoprotective enzymes and antioxidants to combat their toxicity. However, 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 harmful effects. Oxidative stress has been proposed as a potential factor involved in endometriosis pathophysiology (Van Langendonckt et al., 2002c; Szczepańska et al., 2003; Jackson et al., 2005; Gupta et al., 2006). Excessive release of ROS not only induces cellular damage, but may also alter cellular function by regulating protein activity and gene expression (Dalton et al., 1999). Indeed, ROS play an essential role in the regulation of the transcriptional factor NF-\(\kappa\)B (Dalton et al., 1999), which has been implicated in endometriosis (Guo, 2007; González-Ramos et al., 2007). This transcriptional factor induces expression of multiple genes encoding proinflammatory cytokines, growth and angiogenic factors, adhesion molecules and inducible enzymes, nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) (Viatour et al., 2005). All these products are expressed by activated peritoneal macrophages and are involved in the pathogenesis of endometriosis by inducing endometrial fragment adhesion, proliferation and neovascularization (Lebovic et al., 2001). Lousse et al. (2008b) recently showed NF-\(\kappa\)B activation to be significantly increased in the peritoneal macrophages of endometriosis patients, compared with controls.

**HO-1 detoxification system**

Hemolysis releases Hb and free heme into peritoneal fluid. Heme is essential to the activity of a wide range of enzymes, including COX-2 and iNOS, and has been shown to influence gene expression at the level of transcription, protein synthesis and post-translational modifications. However, in large amounts, it can become toxic by mediating oxidative stress and inflammation (Wagener et al., 2003). HO-1 is a heme-degrading enzyme strongly up-regulated by heme. HO protects cells from heme-induced oxidative stress by generating beneficial molecules like CO, bilirubin and ferritin. Indeed, HO-1 induction is accompanied by increased ferritin synthesis, scavenging of free iron and, subsequently, protection against the adverse effects of iron (Wagener et al., 2003). Bilirubin is an important antioxidant, providing potent protection against oxidative injury and inflammation (Stockner et al., 1987), whereas CO is a soluble gas acting as a signal molecule. Numerous functions have been ascribed to the HO–CO system, including regulation of neuroendocrine response, action as a vasodilator, and inhibition of muscle cell contractility (Maines, 1997; Elbirt and Bonkovsky, 1999).

In the case of endometriosis, Hb concentrations were increased in the peritoneal fluid, and higher HO expression was observed in eutopic endometrium, especially in red lesions, compared with eutopic endometrial and mesothelial cells (Van Langendonckt et al., 2002a). However, since inducible HO-1 was poorly expressed by macrophages and mesothelial cells, constituting the majority of cells in the peritoneal cavity, and because there was no concomitant increase in peritoneal fluid bilirubin, its final byproduct, it strongly suggests that detoxifying systems, although present, might be insufficient to metabolize hemoglobin in the case of endometriosis. Accumulation of heme in the peritoneal cavity might have a number of deleterious effects, including induction of oxidative stress, stimulation of cell adhesion and cytokine production by macrophages (Van Langendonckt et al., 2002a).

**Effect of iron overload on endometrial tissue adhesion**

The mesothelial lining, like other epithelium, might serve as a barrier to prevent adhesion of menstrual endometrial fragments to the peritoneal lining (Dunselman et al., 2001). However, some studies have shown that endometrial cells can adhere to mesothelium (Nisolle et al., 2000a, b). This may be because the mesothelium is a fragile membrane, which can be damaged by ectopic menstrual endometrium or inflammatory cells creating adhesion sites on its surface, facilitating the development of endometriosis (Kokorine, 1997; Demir et al., 2004). Oxidative stress was suggested to be responsible for local destruction of the peritoneal mesothelium, producing adhesion sites for ectopic endometrial cells (Arumugam and Yip, 1995; Van langendonckt et al., 2002b). This hypothesis is supported by the fact that the iron-binding protein Hb has been identified as one of the menstrual effluent factors harmful to mesothelium (Demir et al., 2004). Indeed, iron is known to induce oxidative stress, leading to macromolecular oxidative damage, tissue injury and chronic inflammation (Hippeli and Elstner, 1999).

**Effect of iron on endometriotic lesion proliferation**

Our murine endometriosis model has proven to be a useful tool to investigate the impact of pelvic iron overload on ectopic endometrium (Defrère et al., 2006). In this model, erythrocyte injection was shown to increase the proliferative activity of epithelial cells in endometriotic lesions, whereas DFO administration significantly decreased it, suggesting that iron overload may contribute to the further growth of endometriosis by promoting epithelial cell proliferation (Defrère et al., 2006).

Iron is an absolute requirement for proliferation, as iron-containing proteins catalyze key reactions involved in oxygen sensing, energy metabolism, respiration, folate metabolism and DNA synthesis (e.g. ribonucleotide reductase that catalyzes the conversion of ribonucleotides into deoxyribonucleotides for DNA synthesis). In fact, deprived of iron, cells are unable to proceed from the G1 to the S phase of the cell cycle (Le and Richardson, 2002). Iron chelators have proved to be efficient anti-proliferative agents for the treatment of cancer (Simonart et al., 2002; Pahl and Horwitz, 2005; Richardson, 2005; Brard et al., 2006).

After implantation onto the mesothelium, proliferation of lesions promotes the further development of endometriosis (Donmez et al., 1998; Nisolle et al., 2000a). Proliferation of epithelial cells and their differentiation into glandular structures are key events, likely to be under the control of factors in the local environment. Mitogens produced by stromal cells, like hepatocyte growth factor (Giudice and Kao, 2004) or growth factors and inflammatory cytokines present in peritoneal fluid, have indeed been shown to promote epithelial cell proliferation and ectopic endometrial cell growth. Iron could be one of the factors promoting further growth of implanted ectopic endometrial tissue (Defrère et al., 2006).

**Effect of iron on endothelial cells**

When shed menstrual endometrial tissue reaches the abdominal cavity and implants onto the peritoneum (Sampson, 1927), an adequate blood
supply is critical for the survival of the tissue (Donnez et al., 1998; Groothuis et al., 2005). Several studies have shown that endometrial tissue implantation (Nap et al., 2005) and subsequent growth (Dabrosin et al., 2002; Hull et al., 2003; Nap et al., 2004) require an adequate angiogenic response (Laschke and Menger, 2007).

Since vascularization is essential for lesion development, the impact of iron overload and iron chelation on endothelial cells in endometriotic lesions should be analyzed. Indeed, pro-oxidant iron has been shown to generate free radicals in endothelial cells (Zweier et al., 1994) and promote monocyte adhesion to these cells (Kartikasari et al., 2004) by inducing adhesion molecules such as intracellular adhesion molecule and vascular adhesion molecule (Wagener et al., 1997). Binding and transmigration of leukocytes through endothelium to gain access to inflamed sites is an essential inflammatory process implicated in the development of many diseases, such as atherosclerosis and neurodegenerative diseases.

Involvement of iron in endometriosis-associated subfertility

Endometriosis and infertility are commonly associated. In 1994, Arumugam investigated the role of accelerated lipid peroxidation of spermatozoa by peritoneal fluid in patients with endometriosis as a factor for this association. This study suggested that increased iron concentrations found in the fluid of these patients acted as a catalyst for the process. Indeed, a decrease in acrosome reaction rates was associated with increased iron concentrations in peritoneal fluid (Arumugam, 1994).

Excessive activation of macrophages is considered to be an etiological factor of marital infertility. Furthermore, iron ingested by peritoneal macrophages could be responsible for their increased phagocytosis and contribute to the subfertility observed in endometriosis patients (Skowron, 2000).

Iron chelators as endometriosis treatment

Treatment with DFO, a common iron chelator, has proved beneficial and is currently used for pathologies characterized by iron overload, such as β-thalassemia and hereditary hemochromatosis (Tam et al., 2003).

In a murine endometriosis model, DFO was found to decrease the number of lesions with iron deposits, iron concentrations in peritoneal fluid and the percentage of iron-loaded pelvic macrophages (Défremé et al., 2006). Moreover, DFO treatment was effective at reducing cellular proliferation of lesions. Treatment with an iron chelator like DFO could thus be beneficial in the case of endometriosis to prevent iron overload in the pelvic cavity, thereby diminishing its possible deleterious effects. However, in women suffering from endometriosis, menstrual periods are often longer and heavier (Sanfilippo et al., 1986; Darrow et al., 1993; Vercellini et al., 1997; Vinatier et al., 2001), and cycles tend to be shorter (Arumugam and Lim, 1997). Therefore, iron overload observed in these patients is generally localized in the pelvic cavity, whereas body iron content may actually be decreased due to abundant menstruation. For this reason, iron chelator treatment should be applied locally, only inside the peritoneal cavity, by means of intrapelvic implants that release DFO over several months or years.

Conclusions

Retrograde menstruation transports menstrual endometrial tissue and red blood cells through the Fallopian tubes into the peritoneal cavity. This phenomenon, which preserves the body iron content of women, is a physiologic event affecting all menstruating women with patent tubes. Indeed, red blood cells are present in the peritoneal fluid of most women. However, in endometriosis patients, retrograde menstruation is often increased and may overwhelm peritoneal protective mechanisms, resulting in iron overload in all the components of the peritoneal cavity (peritoneal fluid, endometriotic lesions, peritoneum and macrophages) (Fig. 3).

Iron overload may affect a wide range of cell types, including endometrial cells (stromal and epithelial), mesothelial cells, endothelial cells and immune cells (macrophages, lymphocytes, ...), impairing their functionality and thereby contributing to the development of the disease (Fig. 4).

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References


Iron and endometriosis


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