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Endometriotic disease: the role of peritoneal fluid

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Peritoneal fluid and the intraovarian milieu are a specific microenvironment. Peritoneal fluid originates mainly as an ovarian exudation product caused by increased vascular permeability, with cyclic variation in volume and steroid hormones which are always higher than in plasma. It contains large amounts of macrophages and their secretion products, and has a large exchange area with plasma through the peritoneum, which is highly permeable for small molecules. Diffusion becomes virtually zero for molecules with a molecular weight of >100 000 Da. In women with the luteinized unruptured follicle (LUF) syndrome, concentrations of oestrogens and progesterone are much lower in the luteal phase. Endometriosis is associated with sterile low-grade inflammation, increased concentrations of activated macrophages and many of their secretions, such as cytokines, growth factors and angiogenic factors. Concentrations of CA-125 and of glycodelins are also increased, secreted locally by the endometrial cells. Natural killer (NK) cell function declines, possibly mediated by glycodelins or local intercellular adhesion molecule (ICAM) -1 shedding. The ovary is also a specific microenvironment, with steroid hormone concentrations 1000-fold higher in follicles than in plasma. Endometrial and superficially implanted cells are influenced by peritoneal fluid concentrations so that local environment, rather than inherent cellular differences could explain differences between superficial endometriosis and eutopic endometrium. Differences between superficial implants and endometriotic disease, deep infiltrating or cystic ovarian endometriosis, may thus arise via different endocrine environments. Superficial endometrial implants are regulated by peritoneal fluid factors, whereas deep endometriosis and cystic ovarian endometriosis are influenced by blood or ovarian factors. The endometriotic disease theory considers superficial endometriotic implants and their remodelling as a physiological process in most women, and concentrates on the causes of severe endometriosis such as differences in the eutopic endometrium from women with and without endometriosis (which may indicate hereditary differences), the invasiveness of some endometriotic cells *in vitro*, focal 'shielding' of endometriotic foci by adhesions, and inhibition of NK activity by ICAM-1 and glycodelins. Endometriotic disease is thus seen as a benign tumour. The type of cellular lesion, hereditary and immunological environments and local hormone concentrations in the ovary and in peritoneal fluid, will decide expression as cystic ovarian endometriosis, deep endometriosis or adenomyosis externa, and whether the latter is associated with adhesions.

Key words: endometriosis/human/peritoneal fluid/steroid hormones

Introduction

Endometriosis, defined as the presence of endometrial glands and stroma outside the uterine cavity, is a common disease which affects between 5% and 40% of 'normal' women and up to 60–80% of women with pelvic pain and/or infertility (Koninckx *et al*., 1991). The most severe forms of the disease are deeply infiltrating endometriosis, with pelvic pain as the predominant symptom (Cornillie *et al*., 1990; Koninckx *et al*., 1991) and cystic ovarian

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endometriosis, which is associated with extensive intrapelvic adhesions, pain and infertility.

The pathophysiology and the natural history of the disease remain enigmatic. The Sampson hypothesis of retrograde menstruation of viable endometrial cells which may attach, implant and grow, is intellectually attractive since each aspect of this process is supported by data (for review see van der Linden, 1996). Retrograde menstruation is indeed a frequent phenomenon in most women (Koninckx *et al*., 1980c; Badawy *et al*., 1984; Halme *et al*., 1984; Bartosik *et al*., 1986; van der Linden *et al*., 1995). Peritoneal fluid contains viable endometrial cells (Willemsen *et al*., 1985; Kruitwagen *et al*., 1991) which can attach themselves to the peritoneum (van der Linden *et al*., 1994; D'Hooghe *et al*., 1995a). The metaplasia hypothesis is also attractive since it can explain some occurrences of endometriosis such as in the absence of menstruation (Doty *et al*., 1980; El Mahgoub and Yaseen, 1980), and because it is necessary to attribute a role to the secondary Müllerian system (Fujii, 1991). The implantation and the metaplasia theory can be viewed to a large extent as complementary, as metaplasia can be induced experimentally with menstrual debris.

The weakness of both the retrograde menstruation and metaplasia theories is that growth and proliferation of these endometriotic cells is accepted on indirect evidence such as the disruption of the basal membrane only (Evers and Willebrand, 1987). Moreover, these theories fail to explain why implantation and endometriosis does not occur in all women, considering the prevalence of retrograde menstruation, and why endometriotic disease does not develop in all women, considering the prevalence of subtle/minimal endometriosis. The theories also do not explain why some women preferentially develop cystic ovarian and others deep endometriosis, nor why infiltration occurs in some deep lesions whereas in others retraction or adenomyosis externa are the most prominent features (Koninckx and Martin, 1992).

To explain the specific behaviour of endometrial cells in endometriosis, much effort has been devoted to identify cellular differences between endometriosis and endometrium, and to delineate immunological deficiencies in women with endometriosis. Many observed differences between endometrium and endometriosis can, however, also be explained as the direct consequence of the different endocrine environment, of peritoneal fluid and of the intraovarian milieu as opposed to plasma, for steroid hormones, cytokines, angiogenic and growth factors. Since the late 1970s, over 600 articles have investigated in peritoneal fluid those factors which might explain the growth of endometrial cells and their development into endometriotic disease (for review see Oral *et al*., 1997). We now review the data of the specific intraperitoneal and intraovarian environment which may prove beneficial in improving our understanding of the aetiology and pathophysiology of endometriosis.

Peritoneal fluid: a specific microenvironment

Origin and volume

The origin and content of peritoneal fluid was investigated in the late 1970s. Peritoneal fluid is mainly formed as an ovarian exudate (Koninckx *et al*., 1980a–d), more specifically from the developing follicle/corpus luteum, and results from the increased vascular permeability, probably due to the high local oestrogen concentrations. This explains why the volume of peritoneal fluid increases progressively during the follicular phase of the menstrual cycle and decreases thereafter, that the volume is increased following ovarian hyperstimulation, and that the volume is low in women without ovarian activity such as post-menopausal women, in women taking oral contraceptives, and in men (Koninckx *et al*., 1980a). It is still unclear (for review see Hurst and Rock, 1991) whether in women with endometriosis the volume is slightly increased (Mahmood and Templeton, 1991) or is comparable with that in women without endometriosis (Koninckx *et al*., 1980e). The overwhelming picture is, however, the inter-individual variability in volume, which around ovulation ranges from 5 ml to >200 ml.

The slightly higher peritoneal fluid protein concentration in the late follicular and early luteal phases in comparison with the follicular phase (Koninckx *et al*., 1980c) has also been considered as an indirect argument for an oestrogen-driven vascular permeability as the mechanism of peritoneal fluid formation.

Content: exudation of plasma and local secretion

The total surface area of the peritoneal cavity is estimated at >2 m^2 (for review see Dizerega and Rodgers, 1992). This large area permits a quantitatively important passive dialysis of substances between peritoneal fluid and the blood stream, a phenomenon that is clinically used in peritoneal dialysis. Small molecules, such as urea and electrolytes, diffuse rapidly. Diffusion rates decrease with the molecular weight to become extremely slow for molecules with a molecular weight >100 000 Da (Dunselman *et al*., 1988a). Thus, it is logical that the concentrations of small molecules in peritoneal fluid and plasma are similar, whereas the concentration of larger molecules is lower. In peritoneal fluid, prolactin (mol. wt

20 000 Da) and albumin (mol. wt 60 000 Da) concentrations are 67% of those in plasma, whereas the concentration of clotting factors (mol. wt >100 000 Da), is less than 30% of plasma concentrations (Koninckx *et al*., 1980c; Pattinson *et al*., 1981).

Since the ovarian exudate occurs mainly around the developing follicle and the corpus luteum, it is logical that the ovarian steroid hormone concentrations in peritoneal fluid are always higher in peritoneal fluid than in plasma. From the early follicular phase onwards, free 17β-oestradiol concentrations in peritoneal fluid are slightly higher than in plasma, since the total concentrations are comparable with those in plasma whereas the sex hormone binding globulin concentration is only 67% of the plasma concentration. The concentration differences with plasma increase progressively and, when following ovulation the content of the follicle is released into the peritoneal fluid, the 17β-oestradiol concentration increases abruptly, reaching up to 40 000 pg/ml, i.e. 100-fold higher than in plasma (Koninckx *et al*., 1980c; Donnez *et al*., 1983; Dhont *et al*., 1984; Scheenjes *et al*., 1990). During the luteal phase, 17β-oestradiol concentrations decrease progressively.

Progesterone concentrations in peritoneal fluid also are much higher in peritoneal fluid than in plasma. During the follicular phase, concentrations of 5–10 ng/ml are found which are comparable with luteal phase concentrations in plasma. The peritoneal fluid concentration of progesterone rises abruptly following ovulation, reaching 2000 ng/ml in some women, but decreases slowly thereafter. It has been calculated that during the early luteal phase, little progesterone is secreted directly into the blood stream. Until the corpus luteum is vascularized—a process that takes days to be completed—an exudate containing progesterone leaks from the corpus luteum and is subsequently reabsorbed from the peritoneal cavity.

Peritoneal fluid also contains cellular elements such as monocytes (mainly macrophages), red blood cells and endometrial cells with their large granular lymphocytes. Endometrial cells secrete a range of products as glycodelins, formerly called placental protein 14 (PP14; Koninckx *et al*., 1992) and a 60 kDa heat shock protein (Kligman *et al*., 1996). The peritoneum secretes substances such as CA125, while macrophages secrete a range of products including cytokines, growth factors and angiogenic factors. As these substances are secreted locally, they are all present in supraphysiological concentrations.

A specific microenvironment

Peritoneal fluid is a specific microenvironment which contains steroid hormones, cytokines, growth factors and angiogenic factors, etc. in concentrations and with time courses during the cycle which are different from those in plasma.

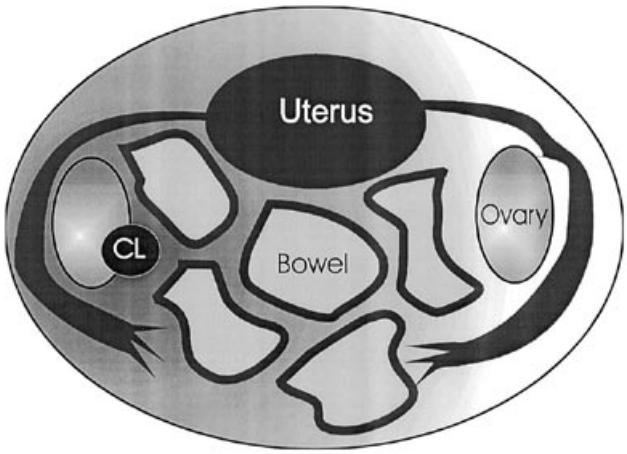

Figure 1. Steroid hormone concentration gradient in peritoneal fluid, which is formed mainly as an ovarian exudate from the growing follicle and/or corpus luteum (CL).

The role of peritoneal fluid compartimentalization is well recognized in processes such as postoperative adhesion formation and the prevention of peritonitis. In accepting the concept of compartimentalization, it is conceivable that concentrations around endometriotic implants—especially those accompanied with adhesions—might differ from those in peritoneal fluid. Similarly, it can be postulated that ovarian secretion products directly influence the ipsilateral oviduct since they are drained locally into the peritoneal fluid (Figure 1). This mechanism could be functionally similar to the arteriovenous counter-current systems in some species.

Peritoneal fluid circulation around the abdominal cavity (reviewed by Dizerega and Rodgers, 1992) may be important for the spread of pelvic infections or for postoperative adhesion formation, but this has not been investigated in relation to reproductive biology and endometriosis. Although fertilizations have been observed in women with an oviduct on one side and an ovary on the other side, we still do not know whether aspiration of the oocyte from the peritoneal fluid is the rule rather than an occasional mechanism. The pregnancy rates following intraperitoneal insemination suggest, however, that this mechanism and peritoneal fluid circulation might be important (Campos Liete *et al*., 1992). This could explain the effect of intraperitoneal adhesions upon fertility.

Cells suspended in peritoneal fluid and the superficial cells of the peritoneum are clearly influenced by peritoneal fluid factors rather than by plasma factors. Thus, the

concentrations of peritoneal fluid factors rather than of plasma factors should be considered in order to understand the physiology of intraperitoneal endometrial cells, both those arriving by retrograde menstruation and those attached to the peritoneum, i.e. minimal endometriosis.

The intraovarian milieu

The concentrations of steroid hormones and other substances (Lopez Bernal *et al*., 1992) in follicular fluid are well documented (for reviews see Baird and Fraser,1975; Channing *et al*., 1980; Edwards *et al*., 1980; McNatty *et al*., 1980; Hsueh, 1986; Hillier, 1987). Whereas peritoneal fluid concentrations are some 10- to 100-fold higher than plasma concentrations, intraovarian concentrations are even higher. These very high concentrations of oestrogens lead to specific, direct membrane effects rather than being mediated via the receptor and immunosuppressive effects of progesterone. These high oestrogen concentrations and their effects may be important in understanding why cystic ovarian endometriosis develops almost exclusively in the ovary.

Pathophysiology of endometriosis

Peritoneal fluid in endometriosis

For reviews of this subject, the reader is referred to Ramey and Archer (1993) and Oral *et al*. (1997).

Endometriosis is associated with the luteinized unruptured follicle (LUF) syndrome and with a sterile low-grade inflammatory reaction in the peritoneal cavity, as judged by an increased amount of activated macrophages and their secretion products.

The association between the LUF syndrome and endometriosis was described in women (Brosens *et al*., 1978; Donnez *et al*., 1983; Dhont *et al*., 1984; Holtz *et al*., 1985; Koninckx *et al*., 1994) and primates (D'Hooghe *et al*., 1996). In women with a LUF syndrome, steroid hormone concentrations in peritoneal fluid are much lower following ovulation (Koninckx *et al*., 1980b; Donnez *et al*., 1983; Dhont *et al*., 1984), It was suggested that this may facilitate the development of endometriosis. Therefore, the LUF syndrome may not be a consequence, but rather a cause or cofactor in the development of endometriosis (Koninckx *et al*., 1980d).

Peritoneal fluid of women with endometriosis contains an increased concentration of macrophages and activated macrophages. This has been considered as the consequence of low-grade inflammation, although it can also be caused directly by endometriosis as women with endometriosis have higher chemotactic activity for macrophages in their peritoneal fluid, (Weil *et al*., 1997) and medical treatment of endometriosis can reduce this chemotactic activity (Leiva *et al*., 1993). Other conditions which play a role in the recruitment of macrophages into the peritoneal cavity are monocyte chemotactic protein-1, secreted by cytokine-stimulated endometriotic cells *in vitro* (Akoum *et al*., 1995), and RANTES, which is increased in peritoneal fluid of women with endometriosis (Khorram *et al*., 1993; Hornung *et al*., 1997) and which has potent chemotactic activity for human monocytes and T lymphocytes.

The proportion of Bax-positive peritoneal fluid macrophages is elevated in women without endometriosis, which could have important consequences for the survival and proliferation of the ectopic endometrial tissue by resisting apoptosis (McLaren *et al*., 1997b). Peritoneal fluid of women with endometriosis contains higher concentrations of macrophage secretion products. Particularly important for endometriosis is the increased angiogenic activity *in vivo* (Oosterlynck, 1993a), the increased concentrations of transforming growth factor-β (TGF-β; Oosterlynck *et al*., 1994b) and vascular endothelial growth factor (VEGF; McLaren *et al*., 1996b), the secretion of which by activated macrophages is regulated directly by ovarian steroids (McLaren *et al*., 1996a). In peritoneal fluid of women with endometriosis, increased concentrations of cytokines were found for tumour necrosis factor-α (TNF-α; Halme, 1989; Taketani *et al*., 1992; Rana *et al*., 1996), interleukin (IL)-8 (Ryan *et al*., 1995; Rana *et al*., 1996), IL-10 (Rana *et al*., 1996), IL-6 (Boutten *et al*., 1992; Keenan *et al*., 1994; Rier *et al*., 1995), the IL-6 soluble receptor (Schroder *et al*., 1996), IL-13 (McLaren *et al*., 1997a), macrophage-derived growth factor (Halme *et al*., 1988) and macrophage colony stimulating factor (M-CSF; Fukaya *et al*., 1994). Interferon-γ (Khorram *et al*., 1993; Keenan *et al*., 1994) was reported not to be elevated, whereas some reports did not confirm the increased concentration of TNF-α (Vercellini, 1993; Keenan *et al*., 1995) and IL-13 (McLaren *et al*., 1997a). Macrophages of women with endometriosis were reported to secrete higher amounts of fibronectin (Kauma *et al*., 1988), and mild endometriosis has been shown to be associated with decreased platelet-activating factor acetylhydrolase activity in peritoneal fluid (Hemmings *et al*., 1993). Peritoneal fluid also contains the complete insulin-like growth factor (IGF) system, i.e. IGFs, their binding proteins and an IGFBP protease (Giudice *et al*., 1994). Peritoneal fluid of women with endometriosis contains potent mitogens for fibroblasts and endometrium-derived epithelial cells (Koutsilieris *et al*., 1993), such as the N-terminal truncated form of IGFBP-3 with a preferential mitogenic action on epithelial-derived

endometrial cells (Koutsilieris *et al*., 1995). Women with endometriosis have higher concentrations of inflammatory prostaglandins in their peritoneal fluid (Drake *et al*., 1983; Dawood, 1984; Koskimies *et al*., 1984; Mudge *et al*., 1985; Alber *et al*., 1986; Morita *et al*., 1990), and a higher phospholipase-α2 activity (De Leon *et al*., 1986; Sano *et al*., 1994; Sharma *et al*., 1994).

Women with endometriosis have a decreased cellular immunity and more specifically a decreased natural killer (NK) cell activity in peripheral blood (for review see Hill, 1997). In peritoneal fluid, this decrease in NK cell activity (Oosterlynck *et al*., 1992) is not a consequence of a decreased concentration of NK cells, but due to an inhibition of their function (Oosterlynck, 1993b; Ho *et al*., 1995). The factor(s) responsible for this has not been identified, although they were shown to differ from those in follicular fluid (Oosterlynck, 1993b). A role was suggested for increased shedding of intercellular adhesion molecule (ICAM) -1 by endometrial cells (Somigliana *et al*., 1996) and for the high concentrations of glycodelins (Koninckx *et al*., 1992), known to inhibit NK activity (Okamoto *et al*., 1991).

Women with endometriosis have higher concentrations of a protease inhibitor (Fazleabas *et al*., 1987), possibly related to an altered fibrinolytic system (Pattinson *et al*., 1981; Dunselman *et al*., 1988b). Other proteins in peritoneal fluid with an uncertain role comprise a 32 kDa protein (Nothnick *et al*., 1994), glycodelins and CA-125 (Koninckx *et al*., 1992). Finally, not only macrophages, but also the concentrations of other monocytes (Oosterlynck *et al*., 1994a) were reported to differ in the peritoneal fluid of women with endometriosis.

The hypothesis in these studies which investigated cytokines, growth factors and angiogenetic factors in peritoneal fluid was that these substances might stimulate implantation of endometrial cells and growth and development into endometriotic disease. Although stimulatory effects of individual substances are obvious, the concept that peritoneal fluid could explain the development of endometriotic disease has never been proven. Indeed, the overall effect *in vitro* of peritoneal fluid upon the proliferation of purified endometrial stromal and epithelial cells was stimulatory, but no differences were found between women with and without endometriosis (Overton *et al*., 1997). Possibly more important is the observation that the area of pelvic endometriosis is inversely, and not directly, proportional to the pelvic inflammatory macrophage response. This suggests that the overall effect of peritoneal fluid on growth and development of endometriosis may be inhibitory rather than stimulatory (Haney *et al*., 1991). In considering the variety of secretory products, the two concepts that peritoneal fluid may stimulate and inhibit growth of endometriosis are not necessarily contradictory, as peritoneal fluid could be inhibitory overall for endometrial cells in most women, whereas in some women stimulatory effects could prevail.

The intraovarian milieu and cystic ovarian endometriosis

Isolated reports showed differences in follicular fluid of women with and without endometriosis, such as higher levels of NK cells, B lymphocytes and monocytes (Lachapelle *et al*., 1996). To the best of our knowledge, there have been no data linking the development of ovarian cystic endometriosis to the intraovarian milieu, although the extremely high (steroid) hormone concentrations may be a significant factor explaining why large ‘chocolate’ cysts occur only in the ovary.

Implantation versus infiltration

For reviews of this subject, the reader is referred to Koninckx and Martin (1994) and Oral and Arici (1997.

Although the precise role of factors such as the LUF syndrome and macrophage secretory products remains unclear, it is obvious that the peritoneal fluid micro-environment regulates endometrial cells floating in peritoneal fluid and superficial endometriosis, whereas the endometrium is regulated by factors in the blood stream. Similarly, deep endometriotic lesions are also regulated by blood stream factors rather than peritoneal fluid factors. This concept, that superficial endometriotic lesions are regulated by peritoneal factors whereas from a certain depth onwards the lesions are regulated by blood stream factors, is self-evident. It is moreover supported by the observation that superficial lesions secrete CA-125 and PP14, mainly towards the peritoneal cavity, whereas deep lesions secrete mainly towards the blood stream (Koninckx *et al*., 1992) by the morphological differences in deep lesions (Cornillie *et al*., 1990), and by the biphasic frequency distribution of depth of infiltration (Koninckx *et al*., 1991).

This concept of different environment and regulation of endometrium and of superficial and deep endometriosis is rarely considered when eutopic endometrium and endometriosis are compared (Figure 2). Indeed, the observed differences have generally been interpreted as differences in tissue characteristics, rather than arising as a result of the different environment of hormones. A typical example is the following. It is well known that oestrogens induce progesterone receptors, whereas progesterone decreases the concentration of both receptors in the

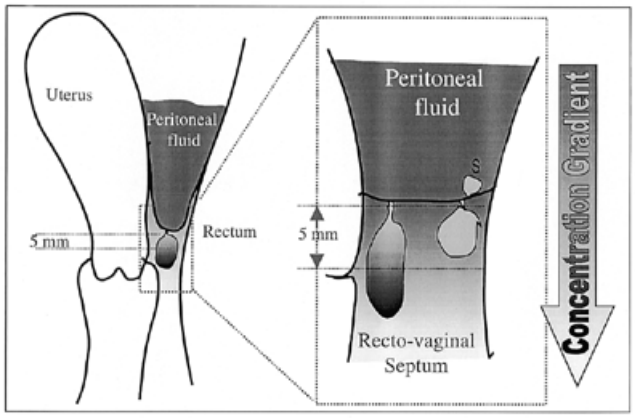

Figure 2. Endocrine gradients from peritoneal fluid and their effect on superficial and deep endometriosis suggest that deep endometriosis has 'escaped' from peritoneal fluid

endometrium. Taking into account the high concentrations of progesterone in peritoneal fluid throughout the menstrual cycle, it is not surprising that the receptor concentrations in superficial endometriosis differ from those in the endometrium. Similarly, the conclusion that deep endometriosis differs from superficial endometriosis because its mitotic activity and vascularization are different, and because the effects of medical therapy are different (Donnez *et al*., 1996) may be wrong, since the observed differences could result simply from the different local environment

The fact that superficial endometriosis is regulated by peritoneal fluid whereas deep endometriosis is regulated by substances in the blood stream, has led to the concept that endometriosis and endometriotic disease are two different conditions. In this respect, endometrial cells in peritoneal fluid are a natural phenomenon, and superficial endometriosis is considered a natural condition which occurs intermittently in all women as implantation of endometrial cells will occur regularly in all women (Koninckx, 1994). The removal of these implants by the body's defence mechanisms is seen clinically as active remodelling of minimal endometriosis. This endometriosis should perhaps not be considered pathological since it generally disappears spontaneously, and associated pain or infertility occurs only rarely. The deeper lesions that penetrate 5–6 mm beneath the peritoneum invariably display more aggressive behaviour, causing pelvic pain and probably infertility. In this respect, the depth of invasion or the distance from the peritoneal fluid milieu is considered to be the major factor influencing tissue behaviour. It is less important whether these deep lesions result from infiltration (type I), retraction (type II) or local metaplasia, suggested by the morphological appearance of adenomyosis externa (type III) (Koninckx and Martin, 1992).

We believe that this concept of the local endocrine environment is essential for our understanding of the pathophysiology of endometriosis. The Sampson theory emphasizes retrograde menstruation and implantation as driving forces, which is not necessarily very different from the metaplasia theory, as degenerating endometrium may release a biochemical factor(s) into the peritoneal environment which induces ectopic endometrium formation (Ramey and Archer, 1993). What these theories have in common is that they explain why endometrial cells appear on the peritoneum, assuming that their subsequent growth, albeit at a different speed through modulation by peritoneal fluid, is unavoidable. The newer concept proposed by us, which also emphasizes the local microenvironment of peritoneal fluid, considers endometrial cells that are implanted superficially on the peritoneum as a normal condition and relatively unimportant. The key question to ask is why these cells infiltrate and behave aggressively in some women, but not in others. Without an answer to this question, endometriosis—or rather endometriotic disease—cannot be understood. A first element of this answer could be that endometriotic disease has 'escaped' from the influence of peritoneal fluid. In addition, other factors such as genetic and immunological factors, either acquired or hereditary, should be considered.

Are endometriotic cells different from endometrial cells?

Much effort has been devoted to finding cellular differences between eutopic and ectopic endometrium, which could explain the aggressive behaviour of endometriosis. Steroid hormone receptors are different in eutopic and ectopic endometrium, and from the expression of oestrogen and progesterone receptors observed in columnar cells of the pelvic peritoneum and in typical endometriosis it was suggested that endometriosis may originate from such columnar cells (Fujishita *et al*., 1997). The level of the proteolytic enzyme cathepsin D is significantly higher in endometriotic tissue than in endometrium (Bergqvist *et al*., 1996). The expression of the adhesion glycoproteins laminin and fibronectin, their receptors $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$ and $\alpha_6\beta_1$ and E-cadherin was found to be similar in endometriotic and endometrial samples (Sillem *et al*., 1997), whereas fibronectin receptor expression was different (Beliard *et al*., 1997). In comparison with endometrium, the integrin α_3 subunit was found to be upregulated, whereas the integrin α_6 subunit was downregulated. Also, the cycle stage-dependent expression of α_v and β_3 was absent in endometriosis (Rai *et al*., 1996).

From differences in the expression pattern of IGF-II and ICE-I GF between endometrium and endometriosis it was suggested that the mechanisms regulating cell proliferation and differentiation are altered in endometriosis (Sbracia *et al*., 1997). Since in the endometrium the expression of plasminogen and plasmin was cycle-dependent, whereas in ectopic endometrium the expression was maintained continuously at a high level (Fernandez Shaw *et al*., 1995), it was suggested that this could reflect the more invasive nature of the endometriotic implants.

As discussed previously, these observations should be interpreted with great caution, as it is unclear whether they reflect real differences between eutopic and ectopic endometrium, or whether the differences found are a mere consequence of differences in the endocrine environment between peritoneal fluid and plasma.

Are endometrial cells different in women with and without endometriosis?

Recently, evidence of differences in endometrium between women with and without endometriosis has emerged. The observation of a loss in E-cadherin receptors in some foci of endometriosis (Gaetje *et al*., 1997), and the fact that suppressing metalloproteinase secretion *in vitro* with progesterone or with a natural metalloproteinase inhibitor, inhibits endometriosis formation in an animal model (Bruner *et al*., 1997) makes it attractive to consider endometriosis as a benign (infiltrating) tumour. Studies on NK cell activity and endometriosis showed that the endometrium of women with severe endometriosis was more resistant to lysis by NK cells than the endometrial cells of normal women. As the NK cells were derived from a male control person, the defect must be localized in the endometrial cell (Oosterlynck *et al*., 1991), a conclusion supported by the recent observation that endometrium from women with endometriosis releases more ICAM-1, known to inhibit NK activity (Somigliana *et al*., 1996). P450arom transcripts, indicating aromatase activity, and IL-6 and IL-11 transcripts, were detected in endometriotic implants and in eutopic endometrium from patients with endometriosis, but not in endometrium from women without endometriosis (Noble *et al*., 1996). Increased expression of heat shock protein 27, regardless of the menstrual phase, was found in eutopic endometrium from patients with endometriosis or adenomyosis (Ota *et al*., 1997). The dominant-positive behaviour of the oestrogen receptor exon 5 slicing variant might be masked by the functional cascade of oestrogen receptor wild-type in normal endometria, but not in endometriotic tissue. This might result in an incomplete response to endogenous steroids, and contribute to the growth potential of endometriosis (Fujimoto *et al*., 1997).

Conclusions

The role of the peritoneal fluid compartment in the pathophysiology of endometriosis is undoubtedly important. Peritoneal fluid is a self-contained microenvironment with specific products and hormones in concentrations which are very different from those in plasma. This milieu is likely to play a crucial role, but the role attributed to peritoneal fluid varies whether the implantation theory as opposed to the endometriotic disease theory is considered. In the former theory, peritoneal fluid will stimulate growth and development. If, however, superficial endometriosis is considered a physiological condition, and if superficial endometriosis is normally removed by the body's defence mechanisms, our concepts of the role of peritoneal fluid need to change. Indeed, rather than considering peritoneal fluid as a stimulus towards endometrial implantation and growth, it may be more appropriate to see it as having a protective role in preventing the development of endometriosis through various mechanisms such as macrophage digestion and the release of inhibitory factors. This is consistent with the observation that peritoneal inflammation is inversely and not directly proportional to the extent of pelvic endometriosis (Haney *et al*., 1991). When the normal peritoneal defence mechanisms fail even temporarily, for example because of low progesterone concentrations associated with LUF syndrome, massive retrograde menstruation, or of defective NK cell function (for review see Vinatier *et al*., 1996), endometriotic cells could infiltrate and escape from the direct influence of peritoneal fluid. These cells may change their behaviour and give rise to deeply infiltrating and cystic ovarian endometriosis, the two forms of severe endometriosis that we suggest represent the expression of endometriotic disease.

In addition to the concept that endometriotic disease has escaped from peritoneal fluid, compartmentalization of peritoneal fluid should be considered. This concept is well recognized in processes such as local ischaemia and adhesion formation. Similarly, in endometriosis associated with adhesions, peritoneal fluid should not be considered a homogeneous fluid, and factors secreted locally by endometrial cells, such as glycodelins and ICAM-1, which can inhibit NK function (Okamoto *et al*., 1991) could 'shield' the endometriotic implant from immunological attack.

Endometrial cells will behave differently in different hormonal environments. We therefore question whether the observed differences between ectopic and eutopic endometrium reflect true differences in tissue characteristics, or whether they are merely the result of the different hormonal environments (Figure 2). Importantly, these observations have been made in superficial endometriotic implants clearly influenced by peritoneal

fluid in contrast to the endometrium, which is influenced by the circulation. More important for the endometriotic disease theory are the recent data describing differences between the endometrial cells of women with and without endometriosis, possibly pointing to genetic/hereditary differences between those women. Also, the observation that cystic ovarian endometriosis might be monoclonal in origin (Tamura *et al*., 1998), the increase in severe endometriosis in rhesus monkeys exposed to dioxin (Rier *et al*., 1993) or total body irradiation (Wood *et al*., 1983) with a delay of many years, and the metalloproteinase activity, support the concept that endometriotic disease might be considered as a benign tumour.

Besides peritoneal fluid and cellular differences in local hormonal concentrations, the pathophysiology of endometriosis undoubtedly involves genetic and immunological factors (Dmowski *et al*., 1981; Steele *et al*., 1984) Affected women have a decreased cellular immunity and decreased NK cell activity (Vinatier *et al*., 1996), both in plasma and in peritoneal fluid. It is still unclear, however, whether this decrease in NK activity is the cause of endometriosis or the mere consequence of inhibition by factors associated with endometriosis. In this context, it is possibly important that decreases in NK activity were not found in baboons with endometriosis (D'Hooghe *et al*., 1995b). The argument for a genetic factor in the aetiology of endometriosis is strong in both humans and non-human primates (Kennedy *et al*., 1995; Hadfield *et al*., 1997). The age of onset of pain symptoms is identical in non-twin sisters concordant for endometriosis (Kennedy *et al*., 1996) and there is an increased prevalence among sisters of affected women compared with the general population. (Simpson *et al*., 1980; Coxhead and Thomas, 1993; Moen and Magnus, 1993). We therefore believe that, ultimately, the identification of susceptibility genes could prove to be the best way of identifying those molecular and cellular mechanisms that are aberrant in endometriosis.

In conclusion, we suggest that the endometriotic disease theory could become more important than the implantation/metaplasia theory. Varying with the type of genetic differences, either spontaneous or induced by environmental agents as dioxin or total body irradiation, and acknowledging that the susceptibility may depend on hereditary factors, and taking into account tumour mechanisms such as critical mass, extracellular matrix breakdown and local shielding from immunological attack, the local hormone concentrations in the ovary and peritoneal fluid will determine whether endometriotic disease is expressed as cystic ovarian endometriosis, as invasive deep endometriosis or as adenomyosis externa, and whether this disease is associated with adhesions.

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