Human Reproduction Update, Vol.7, No.6 pp. 556–566, 2001

Peritoneal healing and adhesion formation/reformation

Y.C.Cheong, S.M.Laird, T.C.Li[1], J.B.Shelton, W.L.Ledger and I.D.Cooke

The Jessop Hospital for Women, Leavygreave Road, Sheffield S3 7RE, UK

[1]To whom correspondence should be addressed at: The Jessop Hospital for Women, Level 4, Room 126, The Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK. E-mail: yingcheong@hotmail.com

Intra-abdominal adhesion formation and reformation after surgery is a cause of significant morbidity, resulting in infertility and pain. The understanding of the pathogenesis of adhesion formation and reformation especially at the cellular and molecular level can help to further develop more effective treatments for the prevention of adhesion formation and reformation. Following an injury to the peritoneum, fibrinolytic activity over the peritoneal surface decreases, leading to changes in the expression and synthesis of various cellular mediators and in the remodelling of the connective tissue. The cellular response to peritoneal injury and adhesion formation and reformation are reviewed. Analysis of the available literature data on the cellular mediators in the peritoneal fluid showed variation in results from different investigators. The potential sources of variability and error are examined. It is still unclear if there is significant individual variation in the peritoneal response to injury.

Key words: adhesion formation/cytokines/fibrinolysis/metalloproteinases/peritoneal fluid

TABLE OF CONTENTS

Introduction

Intra-abdominal adhesion formation and reformation after surgery is a significant cause of morbidity (Ellis *et al*., 1999). In a recent study, this phenomenon was found to result in 33% of patients who had previous surgery being readmitted an average of twice to a hospital for related complications. Congenital or inflammatory adhesions rarely give rise to intestinal obstruction. Post-operative adhesions, however, account for 40% of all cases of intestinal obstruction and 60–70% of those involve the small bowel (Tulandi, 1986; Menzies, 1993). Moreover, the presence of adhesions during surgery may result in longer operating times and increase intraoperative complications, including damage to the bowel, bladder, ureters, and bleeding. Other recognized causes of adhesions include bacterial peritonitis, radiotherapy, chemical peritonitis, foreign body reaction, long-term continuous ambulatory peritoneal dialysis, endometriosis and pelvic inflammatory disease.

Pelvic adhesions can cause infertility and pain (Rosenthal *et al*., 1984; Stout *et al*., 1991). Adhesions can result in infertility by causing mechanical blockage to the Fallopian tubes thus preventing oocyte retrieval. Several studies have reported on the beneficial effect of salpingo-ovariolysis on the conception rate (Tulandi *et al*., 1990; Saravelos *et al*., 1995a). The distortion of the pelvic anatomy due to adhesions can also make ultrasonographic interpretation of the pelvis difficult. For invasive procedures such as oocyte retrieval for IVF, access to the ovaries can be difficult owing to alteration in the pelvic anatomy caused by pelvic adhesive disease. Unilateral pelvic pain is usually associated with adhesions on the same side as the symptom (Stout *et al*., 1991). A number of studies have shown that division of adhesions at surgery is useful in the treatment of chronic pelvic pain (Sutton and MacDonald, 1990; Steege and Stout, 1991; Mueller *et al*., 1995; Saravelos *et al*., 1995b). A randomized controlled trial of adhesiolysis by laparotomy versus no surgery, however, showed that there was no difference in pain level at 9–12 months post-operatively (Peters *et al*., 1992). This result is not surprising because laparotomy is known to cause more adhesions post-operatively compared with laparoscopy (Luciano *et al*., 1989; Lundorff *et al*., 1991). To date, no randomized study has been published that examines the benefit of laparoscopic adhesiolysis in pelvic pain.

Currently, there is no ideal method of preventing adhesion formation/reformation. In terms of surgical technique, gentle tissue handling, the no-touch technique, meticulous haemostasis, copious irrigation, prevention of infection, avoidance of powdered gloves which can evoke a foreign body response in the peritoneum and prevention of extensive thermal injury have all been described as means of adhesion prevention. Multiple adjuvants to post-surgical adhesion prevention have also been evaluated. They include agents that prevent inflammation such as both steroidal and non-steroidal anti-inflammatory medications, agents that degrade fibrin such as recombinant tissue plasminogen activator and barrier methods involving the application of an absorbable material/solution/gel intraperitoneally to prevent the peritoneal surfaces from adhering together (diZerega, 1999). The two commercially available synthetic barriers for adhesion prevention, namely, oxidized regenerated cellulose (Interceed) and polytetrafluoroethylene (PTFC, Gore-Tex), have both been shown to be safe and efficacious in reducing the incidence of post-operative adhesions (Farquhar *et al*., 2000). Interceed (TC 7) Absorbable Adhesion Barrier (Ethicon, Inc., Somerville, NJ, USA) is absorbable and can be cut to the size required to cover a surgical field without needing suturing. It should be placed on the raw tissue area after adequate haemostasis has been achieved. The other commercially available barrier is Gore-Tex (WL Gore & Associates, Inc., Flagstaff, AZ, USA) which is non-absorbable and needs to be sutured in place, and has the disadvantage that it needs to be removed during a subsequent surgical procedure. Though meta-analyses showed that these adhesion prevention barriers are capable of reducing adhesions after surgery, they do not completely eliminate the formation and reformation of adhesions from all patients. Other newer products such as Seprafilm (Hal-F, Bioresorbable Membrane; Genzyme Cooperation, Cambridge, MA, USA), Sepracoat (HAL-C; Genzyme Corporation), Intergel (Ethicon, Inc.) and Icodextrin solution (M.L. Laboratories, Plc, Leicester, UK) are still being evaluated at the moment.

A better understanding of the pathogenesis of adhesion formation/reformation at the cellular and molecular level would undoubtedly help to develop more effective treatment strategies. In the next section, we review the cellular events involved in the peritoneal healing and its relevance to the pathogenesis of adhesion formation/reformation.

The peritoneum

The serous membranes of the peritoneal, pleural and pericardial cavities are of similar embryological derivation and are lined by a layer of mesothelial cells. These mesothelial cells are anchored to the basement membrane. The submesothelial layer consists of the extracellular matrix (ECM) made up of different types of collagen, glycoproteins (i.e. laminin and fibronectin), glycosaminoglycans and proteoglycans. Vascular structures and lymphatics are found in the subserous space. Diffusion and resorption of fluid occur freely through the mesothelium and submesothelial stroma.

Mesothelial cells are loosely attached to the basement membrane and can be readily detached by the slightest trauma (Raftery, 1973). They have microvilli. The nuclei of mesothelial cells are large and prominent, occupying most of the cytoplasm. They have an even distribution of chromatin, except at the periphery where the chromatin forms narrow, dense bands on the inner aspect of the nuclear membrane. The mesothelial cells secrete interleukin (IL)-1, –6 and –8 (Douvdevani *et al*., 1994; Bachus *et al*., 1995; Offner *et al*., 1995; Arici *et al*., 1996), tumour necrosis factor (TNF)-α (Bachus *et al*., 1995) and transforming growth factor (TGF)-β (Offner *et al*., 1996) when stimulated *in vitro*. The mesothelial cells can contribute to the fibrinolytic process by secreting tissue plasminogen activator (Berborowicz *et al*., 1997) and plasminogen activator inhibitors (PAI) (van Hinsburgh *et al*., 1990; Sitter *et al*., 1995; Berborowicz *et al*., 1997). The expression of intracellular adhesion molecule-1 (ICAM-1) by the mesothelial cells when stimulated *in vitro* has been described (Liberek *et al*., 1996). Hyaluronic acid (Yung *et al*., 1996) and prostaglandins (Topley *et al*., 1994) can also be synthesized by the mesothelial cells *in vitro*.

The peritoneal fluid

The peritoneum is in constant contact with the peritoneal fluid. This fluid facilitates the normal function of the gastrointestinal tract, bladder and, in the female genital tract, plays an important role in the motility of the Fallopian tubes and oocyte retrieval. Peritoneal fluid circulates within the abdominal cavity and is in continuity, via the lymphatic system, with the pleural fluid within the thoracic cavity and the vascular system. Molecules can enter or exit the peritoneal cavity by transudation, exudation and facilitated transport or via the lymphatic system.

It is likely that the activity of cellular mediators in the peritoneal fluid plays an active role in peritoneal healing. These cellular mediators are produced by the cellular components of the peritoneal fluid such as the macrophages as well as the mesothelial cells. Because of its mobile nature, the peritoneal fluid can potentially modulate the inflammatory response over a large surface area.

Oestrogen and progesterone concentrations in the peritoneal fluid are higher in the luteal phase compared to the follicular phase (Padilla *et al*., 1986). Women on the oral contraceptive pill and post-menopausal women have lower volumes of peritoneal fluid (4.2 ± 2.3 ml) compared with those women with regular menstrual cycles. Peritoneal fluid volume increases progressively during the follicular phase, being highest during the luteal phase (20 ± 6.3 ml), and declines thereafter (Koninckx *et al*., 1980). In the presence of pelvic pathology such as endometriosis, the volume of peritoneal fluid is significantly increased throughout the menstrual cycle (Drake *et al*., 1980). More recently, it has been shown that inhibin A, a dimeric protein that modulates ovarian steriodogenesis and pituitary FSH production, is significantly elevated in peritoneal fluid in the luteal phase compared with the follicular phase (Florio *et al*., 1998)

The fibrinolytic property of the peritoneal fluid also varies with the menstrual cycle. During the luteal phase, peritoneal fluid plasminogen and PAI are significantly increased above that of the follicular phase (Boukaert *et al*., 1984; Padilla *et al*., 1986; Dorr *et al*., 1993).

The peritoneal fluid contains a vast number of leukocytes and a small number of macrophages, eosinophils and basophils (Haney, 1999). However, the cellular population in the peritoneal fluid can vary depending on the state of the peritoneal environment. In the early follicular phase, the peritoneal leukocyte numbers are

elevated. In pathological conditions such as endometriosis, the number of macrophages is increased in the peritoneal fluid (Syrop and Halme, 1987). During infection, the number of macrophages increases greatly (Olive *et al*., 1987).

Thus it appears that in the normal female pelvis, the volume and composition of the peritoneal fluid is highly dependent on the stage of menstrual cycle and hence the process of ovulation. Moreover, peritoneal fluid content and volume can be altered in the presence of pelvic pathology. Consequently, to further our understanding of the role of cellular components and cytokines in adhesion formation/reformation, it will be necessary to establish how these mediators fluctuate normally during the menstrual cycle, before comparison can be made with studies of pathological states and assessment in individuals who are at risk of adhesion formation/reformation.

Cellular components of peritoneal healing

A summary of the cellular changes in the peritoneal healing process is summarized in Figure 1. The peritoneal leukocytes, mesothelial cells and macrophages are important cellular components of peritoneal healing. Peritoneal healing is characterized by cellular infiltration and a growth response by the mesothelial cells in the damaged area. In response to the initial injury, resident cells in the peritoneum such as macrophages and mesothelial cells produce cellular mediators, which serve to modulate and orchestrate the subsequent response of the other cells involved in the inflammatory response. The kinetics of the cellular infiltration in response to inflammation is as follows (diZerega, 1990): the earliest cells to appear in the damaged peritoneum are mainly the polymorphonuclear neutrophils (PMN), which persist for 1–2 days. This is followed by the entry of monocytes which later differentiate into macrophages and become adherent to the wound surface. By day 3, mesothelial cells begin to cover the peritoneal macrophages at the wound surface and these macrophages become embedded more deeply in the wound (Raftery, 1973; Haney, 2000). On days 4–7, the predominant cells on the peritoneal surface are mesothelial cells. After post-operative day 5, the main cell type in the peritoneal fluid is the macrophage. These mesothelial cells then proliferate throughout the wound base and form multiple islands of cells. Confluence of these islands of cells allows larger wounds to heal in the same amount of time as smaller wounds. This form of healing contrasts with that of the skin in which healing takes place from the skin edge; thus larger injuries take longer to heal than smaller ones.

Tissue injury and formation of fibrin bands

It has been postulated that peritoneal injury leads to ischaemia, either from the inadequate ingrowth of vessels in the base of the wound, or, if adequate ingrowth does occur, from an inadequate blood flow in the vessels (Ellis, 1962; Raftery, 1973). Adhesion bands are more likely to occur after surgery when both contacting surfaces of the peritoneum are injured (Haney and Doty, 1994). After trauma/injury to the peritoneum, there is increased vascular permeability in vessels supplying the damaged area, followed by an exudation of inflammatory cells, ultimately leading to the formation of a fibrin matrix. The fibrin matrix is gradually organized and replaced by tissue containing fibroblasts, macrophages and giant cells. This fibrin matrix connects two injured peritoneal surfaces forming fibrin bands. These fibrin bands can be broken down by fibrinolysis into smaller molecules as fibrin degradation products (FDP). Under conditions of aberrant peritoneal healing, ischaemia results in a reduction in fibrinolytic activity and thus the persistence of the fibrin bands. The organisation of the fibrin bands over time results in the adhesions persisting. Adhesion tissue is a mixture of macrophages, eosinophils, red blood cells, tissue debris, mast cells and fibroblasts. The cell population changes with the maturity of the adhesion tissue, with the initial cell type at days 1–3 being mainly polymorphonuclear leukocytes (PMN) and on days 5–7 mainly fibroblasts (Milligan and Raftery, 1974). Adhesion tissue also contains nerve fibres (Kligman *et al*., 1993; Tulandi *et al*., 1998) and small vascular channels of endothelial cells (Milligan and Raftery, 1974).

The fibrinolytic system

The role of the fibrinolysis in adhesion formation/reformation is to breakdown fibrin clots that are formed during the healing

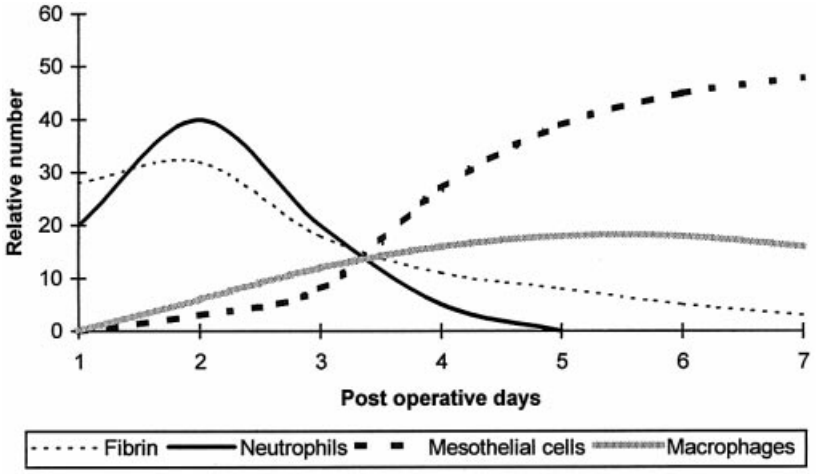

Figure 1. Changes in the relative number of cellular elements and fibrin during normal rat peritoneal repair. [Modified from diZerega, G.S. (1999) Peritoneum, peritoneal healing and adhesion formation. In diZerega G.S. (ed.), *Peritoneal Surgery*, p. 15. With the permission of Springer-Verlag, New York.]

process. The zymogen precursor, plasminogen, is converted to plasmin by the action of plasminogen activators (PA), namely tissue plasminogen activator (tPA) and urokinase-like plasminogen activator (uPA). PA are serine proteinases, which belong to the family of endoproteinases. Other endoproteinases include the cysteine proteinases, aspartyl proteinases and metalloproteinases. Plasmin can be produced by macrophages or by mesothelial cells lining the peritoneal cavity (Jones and Werb, 1980; van de Poll *et al*., 1991). The main role of plasmin is to degrade fibrin. To counteract this process, plasminogen activator inhibitor-1 (PAI-1) and PAI-2, both glycoproteins, inhibit both tPA and uPA to different degrees, thus contributing to the active interplay between the activators and inhibitors of this system. In the pathological process of adhesion formation, the fibrinolytic process is altered, allowing the formation and persistence of adhesions. Figure 2 shows the current thinking about the role of the fibrinolytic system in the formation of adhesions.

Porter *et al*. first described the presence of plasminogen activator activity in the human peritoneum (Porter *et al*., 1969) and later work localized plasminogen-activating activity to the mesothelium (Raftery, 1979). Fibrinolytic activity has also been detected in the peritoneal fluid (Pattinson *et al*., 1981; Batzofin *et al*., 1985) in both humans and animal models. Studies have suggested that there is marked interspecies variation in fibrinolytic activity and this may in part be due to differences in the concentrations of plasminogen inhibitors (Pugatch and Poole, 1969). PAI activity has been identified in normal human peritoneal cells (Holmdahl *et al*., 1997).

In the inflamed peritoneal tissue, the activity of peritoneal plasminogen activators is significantly reduced (Porter *et al*., 1969; Hau *et al*., 1979; Raftery, 1981b; Thompson *et al*., 1989; Holmdahl *et al*., 1997), partly because the PAI concentration is increased (Vipond *et al*., 1994b; Holmdahl *et al*., 1997). Holmdahl *et al*. (1997) obtained peritoneal tissue samples during laparotomy from patients with and without peritonitis. They employed immunocytochemistry to determine the differential expression of PA and PAI proteins in two different layers (mesothelium and submesothelium) of the peritoneum. In the normal mesothelial layer, tPA, uPA and PAI-1 protein were present, compared with the submesothelial layer, where only PAI-1 and uPA but not tPA were present. In inflamed peritoneum, tPA expression in the mesothelium was substantially reduced, whereas PAI-1 expression in the submesothelial layer was intensified. Expression of tPA in the mesothelium but not the submesothelium suggested that tPA was responsible for the clearance of fibrin in the peritoneal cavity proper. The authors further postulated that injury to the mesothelium during surgery/trauma may have deleted the tPA source and exposed PAI-1, thus accounting for the elevated PAI-1 detected in the peritoneal tissue samples obtained.

Biopsy of adhesion tissue and the peritoneal tissue of patients undergoing laparotomy has been performed (Ivarsson *et al*., 1998). These patients had varying degrees of adhesions and the severity of the adhesions was scored during surgery. Compared to individuals who formed mild or moderate adhesions, individuals who formed severe adhesions were found to overexpress PAI-1 and had decreased tPA activity, not only in adhesion tissue, but more importantly, also in the peritoneal tissue adjacent to the sites of the adhesions. Their findings suggested that post-operative adhesion tissue has a reduced ability to degrade fibrin. They also postulated that individuals who overexpress PAI-1 in their peritoneum are at increased risk of adhesion formation/reformation. Others studies have also positively correlated the severity of adhesion formation to decreased fibrinolytic activity in the injured peritoneum (Buckman *et al*., 1976; Raftery, 1981a; Vipond *et al*., 1994a).

In the presence of adhesions, the fibrinolytic activity in the mesothelium is reduced, but data on the fibrinolytic activity in the peritoneal fluid are conflicting; some reported a reduction in PA activity with adhesive disease in endometriosis (Ohtsuka, 1980; Malick, 1982), some reported no difference in activity of PA or its inhibitor, PAI (Pattinson *et al*., 1981; Batzofin *et al*., 1985), and others an increase in PA activity (Edelstam *et al*., 1998). The factors resulting in these inconsistencies will be discussed later.

The fibrinolytic system clearly plays a significant role in adhesion formation/ reformation. There are also interactions between the fibrinolytic system and other proteinases, particularly the metalloproteinases (MMP) and their inhibitors: tissue inhibitors of metalloproteinases (TIMP). MMP and TIMP are both important players in the remodelling of the ECM (Figure 2).

Proteases and protease inhibitors

The MMP are a family of enzymes that can degrade various components of the ECM; they require zinc for their catalytic action and their actions are opposed by tissue-derived inhibitors called TIMP. More than 17 different MMP have been described to date. Five groups of MMP have a recognized role in wound healing: the collagenases (MMP-1, –8 and –13), gelatinases (MMP-2 and –9), stromelysins (MMP-3, –7, –10 and –11), membrane-type metalloproteinases (MMP-14–17) and other matrix metalloproteinases (MMP-12). Collectively, MMP are capable of degrading all of the components of the ECM. To date, four TIMP have been characterized; TIMP-1–4. The relative concentrations of MMP and TIMP and their resulting proteolytic activity are important in many normal and pathological conditions. MMP play a role in reproductive processes including menstruation, ovulation, implantation and uterine, breast and prostate involution (Hulboy *et al*., 1997). The importance of these proteinases and their inhibitors has also been well described in the process of wound healing (Saarialho-Kere *et al*., 1993; Cooper *et al*., 1994; Bullen *et al*., 1995; Wlaschek *et al*., 1997; Yager *et al*., 1997). Aberrant MMP and TIMP expression has been associated with many gynaecological conditions including endometriosis (Sharpe-Timms *et al*., 1998a; Carolien *et al*., 2000), and adhesion formation/reformation (Chegini *et al*., 1998; Sharpe-Timms *et al*., 1998b).

MMP and TIMP: their significance in fibrinolysis and adhesion formation/reformation

Figure 2 illustrates the possible role of MMP and TIMP in the adhesion formation process. Plasmin can activate latent MMP. It has been shown that human parietal peritoneum and serosal tissue of several intraperitoneal organs express MMP and TIMP (Chegini *et al*., 1998). Mesothelial cells express significantly less MMP-1, higher MMP-3 and similar levels of TIMP mRNA compared to macrophages (Chunfeng *et al*., 1999). Surgical manipulation resulting in adhesion formation has been shown to alter both the PA/PAI and MMP/TIMP equilibrium in the

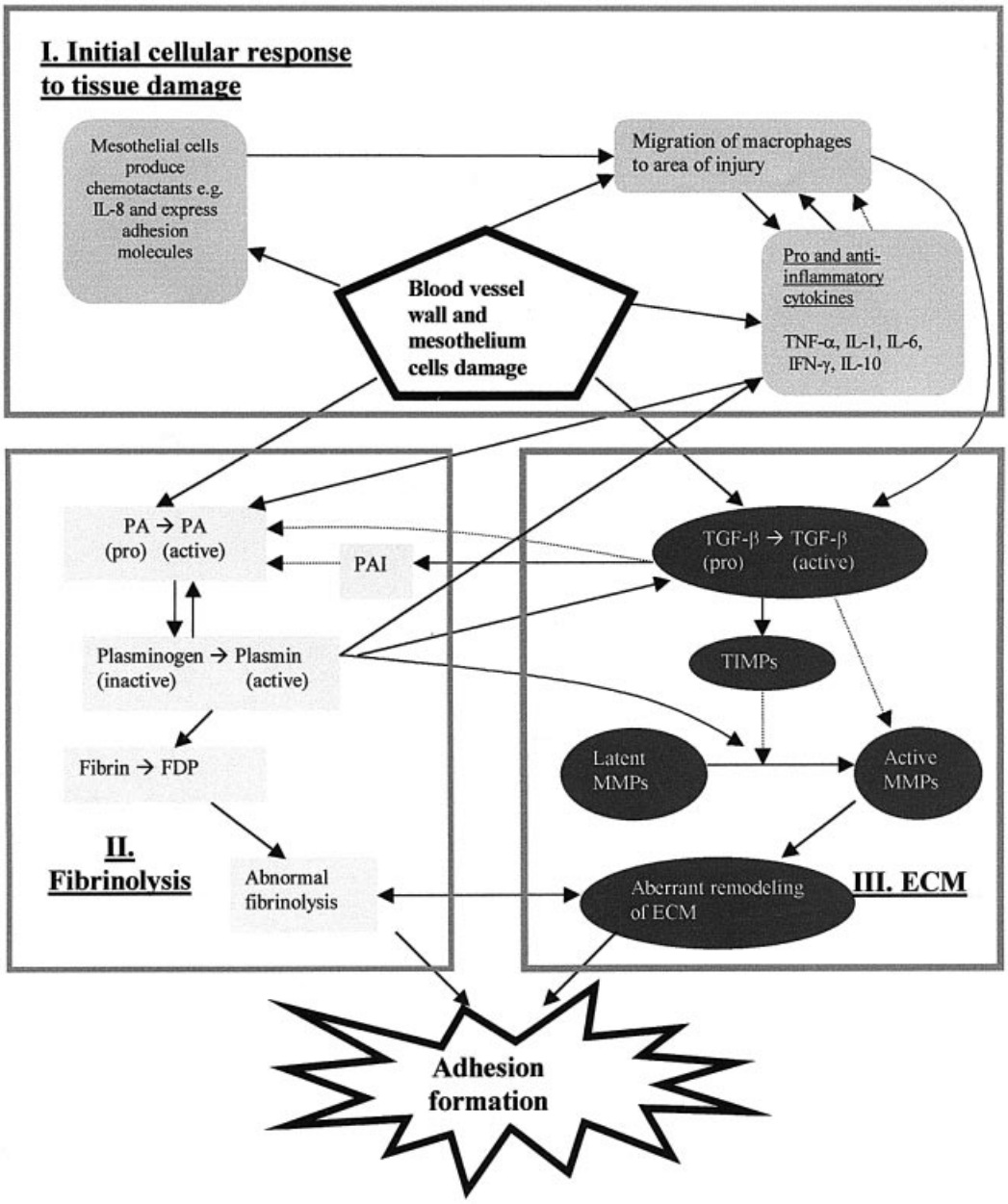

Figure 2. A summary of three important pathways leading to adhesion formation: (I) initial cellular response to tissue damage; (II) fibrinolysis ; and (III) components of ECM . Bold arrows: stimulatory effect; dotted arrows: inhibitory effect. For clarity, functions of the components considered important in adhesion formation are illustrated. ECM = extracellular matrix; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of metalloproteinase; PA = plasminogen activator; PAI = plasminogen activator inhibitor; FDP = fibrin degradation products; TNF-α = tumour necrosis factor-α; INF-γ = interferon-γ; IL = interleukin.

peritoneal fluid (Sharpe-Timms *et al*., 1998b). Pre-surgical gonadotrophin-releasing hormone (GnRH) agonist therapy was shown to reduce adhesion formation after surgery (Wright and Sharpe-Timms, 1995) and this was associated with decreased PA and MMP activity and increased PAI and TIMP activity in the peritoneal fluid. This alteration in the balance of the PA/PAI and MMP/TIMP systems is postulated to induce a shift to a less invasive phenotype that alters fibrinolysis and ECM remodelling, thus accounting for a possible mechanism for the reduction in adhesion formation after surgery following GnRH agonist treatment.

The role of transforming growth factor (TGF)-β

In peritoneal healing and adhesion formation, latent TGF-β is activated by plasmin (Sato and Rifkin, 1989). In its active form, TGF-β not only interacts with the fibrinolytic system and ECM but also with many other cellular mediators involved in the process of adhesion formation (Figure 2). It is normally found in platelets, macrophages and wound fluid (Assoian *et al*., 1983, 1987; Cromack *et al*., 1987). It is a key factor in normal wound healing and is also a potent inducer of tissue fibrosis in peritoneal wound healing (Border and Noble, 1994). During the acute phase

of the inflammatory response, peritoneal macrophages and/or mesothelial cells produce TGF-β (Offner *et al*., 1996). It can contribute to the synthesis of the ECM by stimulating fibroblastic cell production of collagen and fibronectin (Ignotz and Massaque, 1986).

TGF-β overexpression by the parietal peritoneum and the serosal surfaces of the pelvic organs as well as increased concentrations of TGF-β in the peritoneal fluid have been associated with an increased incidence of adhesion formation in both humans and animals (Williams *et al*., 1992; Chegini *et al*., 1994, 1999; Chegini, 1997). Using immunocytochemical staining, Chegini *et al*. (1994) showed the presence of various TGF-β isoforms in surgically induced adhesion tissue in rats. Moreover, rats given intraperitoneal TGF-β daily for 5 days develop significantly more adhesions than the controls (Chegini, 1997).

In an in-vitro model, Tietze *et al*., using a cell/fibrin clot assay, showed that TGF-β increased *PAI-1* mRNA and decreased *tPA* mRNA expression in mesothelial cells (Tietze *et al*.,1998), resulting in decreased fibrinolytic activity. TGF-β administration to human mesothelial cells decreased *MMP-1* but increased *TIMP-1* mRNA expression *in vitro* (Chunfeng *et al*., 1999). However, the underproduction of TGF-β *in vivo* was associated with increased adhesion formation in transgenic mice carrying the heterozygous *TGF-β* allele compared to the homozygous controls (Krause *et al*., 1999). The literature shows that TGF-β can differentially regulate *MMP* and *TIMP*, *PAI* and *PA* at the transcription level and that it can increase ECM production. Thus it exerts substantial influence over the outcome of peritoneal healing and adhesion formation.

Adhesion molecules and chemotactic cellular mediators

Mesothelial cells lining the peritoneal cavity play an important part in the adhesion formation process via expression of cell adhesion molecules (CAM) and production of chemotactic cytokines. CAM play a key role in the inflammatory response. Selectins, integrins and immunoglobulin (Ig) gene family adhesion receptors are selectively expressed by tissue cells and can help to mediate the different steps of leukocyte attachment and migration to the inflammatory foci.

Cell adhesion molecules

Mesothelial cells express adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) (Liberek *et al*., 1996). The integrin family of adhesion receptors is also important in the process of adhesion formation. In adults, various types of cells expressed the integrin adhesion receptors (Damjanovich *et al*., 1992). Integrins interact with proteins primarily via the tripeptide sequence Arg-Gly-Asp (RGD). This tripeptide sequence functions as the core cell-binding sequence in many proteins and cells including fibronectin, platelet glycoprotein IIb/IIIa receptor, laminin and in leukocytes (leukocyte antigen receptor family). The activation of the integrin adhesion molecule receptor also has profound effects on the genetic expression of MMP and various cytokines (Werb *et al*., 1989; Miyakes *et al*., 1993). Integrins can thus enhance the adhesion formation process by: (i) activating platelet aggregation, the coagulation process and fibrin deposition; (ii) accelerating the inflammatory process; and (iii) enhancing the attachment of the mesothelial cells to fibrin and ECM.

Chemotactic cytokines

Mesothelial cells also secrete chemotactic cytokines such as IL-8, monocyte chemotactic protein-1 (MCP-1) and Regulated on Activation and Normally T cell Expressed and presumably Secreted (RANTES) to create a chemical gradient to recruit more inflammatory cells (Zeillemakerm *et al*., 1995; Fear *et al*., 1997). IL-8, a potent chemoattractant is elevated in the peritoneal fluid in cases of endometriosis (Arici *et al*., 1996). IL-8 concentrations are higher in early (stage 1) disease compared with later stages of endometriosis (Gazvani *et al*., 1998), although adhesions generally are more severe at later stages of the disease. This could be explained by the fact that the role of IL-8 is recruitment of cells and thus it could be expected to have a more important role in the initial rather than the later phase of the inflammatory response. Mesothelial cells, by up-regulating the secretion of chemotactic cytokines and expression of adhesion molecules on their surfaces, are able to control the phenotype of leukocyte recruitment (Robson *et al*., 1997).

The pro- and anti-inflammatory cytokines (TNF-α, IL-1, –6, –8 and 10 and IFN-γ)

Evidence to date suggests that there is alteration in peritoneal fluid cytokine profile in the presence of adhesions. Table I shows a summary of the changes in peritoneal fluid factors associated with established peritoneal adhesions. In order to more precisely delineate the role of cytokines in the adhesion formation process, it will be important to consider the alteration of cytokine profile in the following situations: (i) in acute inflammation, a condition that can predispose to adhesion formation; (ii) during the adhesion formation process; and (iii) in adhesions which have been well-established some time ago.

Acute inflammation

In acute inflammation, the peritoneal fluid shows increased concentrations of the pro-inflammatory cytokines IL-1, IL-6, IL-8 and TNF-α (Brauner *et al*., 1993, 1996; Zemel *et al*., 1994). Acute inflammation in the peritoneum is followed by a large influx of cells, predominately macrophages, by chemotactic mechanisms. IL-1 and TNF-α are both pro-inflammatory cytokines important in the early phase of wound healing (Lowry, 1993), and are produced by activated macrophages in the peritoneal fluid (Halme, 1986; Mori *et al*., 1991). IL-6 is expressed by macrophages and its production is up-regulated by IL-1 during the inflammatory process (Hirano, 1998). Both IL-1 and TNF-α are potent inducers of IL-6 (Bauer *et al*., 1988).

It is known that TNF-α, IL-1 and IL-6 interact with the fibrinolytic system; the latter is an important component of the adhesion formation process (Figure 2). Plasmin has been shown to mobilize and release TNF-α, IL-1 and IL-6 (Whawell *et al*., 1994; Ivarsson *et al*., 1998). TNF-α and IL-1β can in turn down-regulate tPA expression both at the protein and the mRNA level (Sitter *et al*., 1996; Tietze *et al*., 1998), thus helping to regulate the inflammatory process and, possibly, the extent and severity of adhesion formation and reformation subsequently. However, none of the studies attempted to correlate these alterations in cytokine

Table I. A summary of changes in peritoneal fluid factors associated with established peritoneal adhesions

Cytokines in peritoneal fluid	Human, animal or in-vitro studies
Growth factors	
TGF-β	↑ (Chegini *et al*., 1999)
VEGF	↑ (Mahneke *et al*., 2000; Mclauren *et al*., 1996)
Metalloproteinases and their tissue inhibitors	
MMP	↑ (Chegini *et al*., 1998; Sharpe-Timms *et al*., 1998b)
TIMP	↑ (Chegini *et al*., 1998; Sharpe-Timms *et al*., 1998b)
Pro- and anti-inflammatory cytokines	
TNF-α	↔ (Mori *et al*., 1991; Chegini *et al*., 1999) ↑ (Kaidi *et al*., 1995b; Krahenbuhl *et al*., 1998; Guerra-Infante *et al*., 1999)
INF-γ	↑ (Chegini *et al*., 1999)
IL-1	↔ (Mori *et al*., 1991; Chegini *et al*., 1999) ↑ (Hershlag *et al*., 1991)
IL-6	↑ (Buyalos *et al*., 1992; Saba *et al*., 1996)
IL-8	↑ (Arici *et al*., 1996; Gazvani *et al*., 1998)
L-10	↔ (Chegini *et al*., 1999)
Chemoattractants	
GM-CSF	↔ (Chegini *et al*., 1999)
MCP-1	↔ (Zeyneloglu *et al*., 1998b)
RANTES	↑ (Khorram *et al*., 1993)
Fibrinolytic factors	
PA	↑ (Bakkum *et al*., 1996; Edelstam *et al*., 1998) ↔ (Pattinson *et al*., 1981; Batzofin *et al*., 1985; Sharpe-Timms *et al*., 1998b) ↓ (Ohtsuka, 1980; Malick, 1982)
PAI	↓ (Edelstam *et al*., 1998) ↑ (Sharpe-Timms *et al*., 1998b)

↑ = increased concentrations; ↓ = decreased concentrations; ↔ = no change. TGF-β = transforming growth factor-β; VEGF = vascular endothelial growth factor; MMP = matrix metalloproteinase; TIMP = tissue inhibitors of metalloproteinases; TNF-α = tumour necrosis factor-α; INF-γ = interferon-γ; IL = interleukin; GM-CSF = granulocyte–macrophage colony-stimulating factor; MCP-1 = monocyte chemotactic protein-1; RANTES = Regulated on Activation and Normally T cell Expressed and presumably Secreted; PA = plasminogen activator; PAI = plasminogen activator inhibitor.

profile to the extent and severity of adhesion formation/reformation.

During the adhesion formation process

From the literature, we can find no human studies to date that examined the alteration of cytokine profile during the adhesion formation process. Clearly, in establishing a casual or causal role of cytokines in adhesion formation, this will be an interesting and important aspect for future research.

Chronic preformed adhesions

Compared with patients with no adhesions, the altered cytokine profile found in patients with existing, well-established adhesions during surgery could either be a consequence of the presence of adhesions, or it could represent an altered environment that predisposes to the formation of adhesions in the first instance. Notwithstanding the difficulty in the interpretation of any altered cytokine profile, significant controversies exist concerning the concentrations of a number of cytokines in the peritoneal fluid of subjects with well-established adhesions. Although some studies showed elevated TNF-α concentration in the serum (Saba *et al*., 1998) and peritoneal fluid (Guerra-Infante *et al*., 1999) of patients with adhesions during surgery, others did not (Mori *et al*., 1991; Chegini *et al*., 1999). Similarly, even though administration of IL-1 into the peritoneal cavity in experimental rat models has been shown to contribute to adhesion formation (Hershlag *et al*., 1991), IL-1 concentrations in the peritoneal fluid of patients with adhesive disease were not elevated (Mori *et al*., 1991; Chegini *et al*., 1999). More of the possible reasons for the discrepancies will be discussed later.

IL-6 has consistently been reported to be adhesiogenic. Administration of IL-6 into the peritoneal cavity of rats was also found to increase adhesion formation significantly (Saba *et al*., 1996). Similarly, elevated concentrations of IL-6 have been found to correlate with non-endometriotic pelvic adhesions (Buyalos *et al*., 1992).

As for the anti-inflammatory cytokines, low concentrations of IL-10 and INF-γ are reported in the peritoneal fluid of subjects with adhesions or endometriosis (Chegini *et al*., 1999), and in rats, the peritoneal fluid after surgery contains low concentrations of IL-10 (Holschneider *et al*., 1997). Intraperitoneal administration of IL-10 has also been shown to reduce adhesions in rats (Holschneider *et al*., 1997).

None of the above studies demonstrated the actual source of the cytokines, which could be derived from inflammatory cells such as the macrophages, the mesothelial cells, and/or other pelvic organs such as the uterus and ovaries.

A critical analysis of published data on peritoneal fluid biochemistry

A summary of the published literature on the changes in peritoneal fluid factors associated with established adhesions is shown in Table I. There appear to be significant discrepancies in results between the various studies reported, which may be due to a number of reasons. The concentration of any substance-including cytokines, in the peritoneal fluid is influenced by the rate of production, and degradation, as well as dilution. Thus the concentration of cellular mediators in the peritoneal fluid will be influenced by the amount of fluid present. To analyse the cytokine data in a meaningful way, the volume of the fluid present in the peritoneal cavity ought to be measured so that the impact of dilution can be evaluated. Unfortunately, none of the published studies on peritoneal biochemistry and cytokines takes into consideration the importance of fluid volume in their results. There are of course many factors that could potentially influence the peritoneal fluid volume, for example, ovulation can release ~5 ml of follicular fluid into the peritoneal cavity. The peritoneal fluid volume is generally small, and thus the impact on the result is likely to be significant.

In addition to the fluid released during ovulation, there are several other potential sources of peritoneal fluid including transudation or exudation directly via the peritoneal membrane and secretions from the endometrial cavity and Fallopian tube. It is not known whether the secretion from the endometrial cavity and Fallopian tubes into the cavity is influenced by steroid hormones and hence follows a cyclical pattern. The variation in

the fluid volume during the cycle means that timing of the collection of the fluid in relation to the physiological events is of critical importance.

In the case of adhesion formation/reformation, the most important time to collect the fluid is within the 7 days of tissue injury, when the process of fibrin deposition and resorption (fibrinolysis) is actively taking place. The collection of a single sample of peritoneal fluid from individuals with established intra-abdominal adhesions provides little information about the adhesion formation process that occurred some time earlier. Even if the peritoneal fluid cytokine concentration in patients with adhesions is significantly different from controls, we do not know whether it is a cause or an effect of the disease process. Whilst there are several animal studies that have examined peritoneal fluid cytokine concentration within a week of surgical insult, there are no prospective human studies on the relationship between peritoneal fluid cytokine concentration and subsequent adhesion formation/reformation. Moreover, as the process of adhesion formation and reformation is a dynamic one, serial measurement of concentrations of cytokines in the peritoneal fluid during the adhesion formation process is more likely to provide important physiological information than single measurements.

Studies on adhesion formation and reformation in humans need to consider the heterogeneous nature of adhesions. For example, it is still unclear if the adhesions from previous surgery differ from those associated with pelvic infection or endometriosis. In contrast to post-surgical adhesions which are often considered to be non-progressive, endometriosis has been thought to be a progressive disease (Leyendecker *et al*., 1998). GnRH analogue, by suppressing endometriosis deposits, is expected to reduce adhesion formation associated with endometriosis. Interestingly, using rat models, Sharpe-Timms *et. al*. showed that GnRH analogue treatment resulted in a similar decrease in adhesion formation in rats with post-operative adhesions as well as rats with endometriosis by altering the balance of both PA/PAI and MMP/TIMP systems (Sharpe-Timms *et al*., 1998). Until we understand the pathogenesis of adhesions in both diseases further, it will be important to examine the groups separately.

Moreover, the actual collection process and the assays used for analysis of the peritoneal fluid differ among different researchers. Finally, the majority of the studies reported in humans were of small sample size, generally fewer than 50 subjects. Therefore, it is not surprising that there is much controversy within the literature concerning the concentration of cytokines in peritoneal fluid and their relationship to adhesion formation/reformation. Future studies on peritoneal fluid biochemistry should take into consideration these potential sources of variability/error mentioned above.

Prevention of adhesion formation/reformation: the cellular approach

An increased understanding of the cellular mechanism of adhesion formation and reformation should lead to improved means of prevention. In animal models, adhesion formation has been reduced by three different strategies: (i) enhancing the fibrinolytic process using tPA; (ii) immunomodulation; and (iii) disrupting the cell interaction with the ECM.

Altering the fibrinolytic pathway to decrease adhesion formation

A number of animal studies have demonstrated that tPA is effective in reducing adhesion formation. Recombinant tPA can be delivered as a gel to delay its release and absorption as tPA is rapidly absorbed into the peritoneal cavity (Doody *et al*., 1989; Dorr *et al*., 1990; Vipond *et al*., 1994a). Possible side-effects of tPA include risks of post-operative haemorrhage and delay in wound healing. Nevertheless, experimental studies had not reported complications with wound healing. So far, only one study reported associated haemorrhagic consequences (Gehlbach *et al*., 1994). The only pilot clinical study performed to date consisted of 15 patients receiving recombinant tPA after surgery. The investigators found a significant decrease in adhesion formation with no laboratory changes post-operatively and reported no complications such as bleeding or altered healing (Dunn and Mohler, 1994).

The fibrinolytic pathway can also be altered by the administration of GnRH analogue (Sharpe-Timms *et al*., 1998b). These authors showed that GnRH analogue adhministration altered the tPA/PAI ratio, resulting in the reduction of adhesion formation in women. The exact mechanism by which GnRH analogue exerts its anti-adhesion effect is yet unknown. In this study, the authors again found no significant post-operative complications such as bleeding or abnormal healing. Thus the results so far has been encouraging and therefore further clinical trials consisting of a larger group of patients should be justifiable in the near future.

Immunomodulation

Selective immunosuppression has been used for reduction of adhesion formation/ reformation. Rats injected with TGF-β_1 antibodies into their abdominal cavity developed comparatively fewer adhesions compared with the controls (Lucas *et al*., 1996). Another group showed that adhesions were significantly reduced in rats administered IL-1 or TNF-α antibodies and the fewest adhesions were formed in the group where antibodies to IL-1 and TNF-α were administered together (Kaidi *et al*., 1995a). They later showed that IL-6 antibodies also reduced adhesion formation in rats when administered pre-operatively. Furthermore, they did not find any alteration in the collagen content in the wound, nor any adverse effect of treatment on wound healing. Administration of anti-MCP-1 antibodies to inhibit chemotaxis has also been successfully used in mice to prevent adhesion formation (Zeyneloglu *et al*., 1998a).

Montz *et al*. showed that IL-10 administration was effective at limiting post-operative intraperitoneal adhesion formation without significant systemic effects in mice (Montz *et al*., 1994). It was later observed that treatment with IL-10 and/or ketorolac (a non-steroidal anti-inflammatory drug) caused a reduction in adhesion formation and also thinner and filmier adhesions (Holschneider *et al*., 1999). Surprisingly, immunosuppression systemically did not seem to reduce adhesion formation/reformation, as demonstrated by the administration of cyclosporin in rats (Leondires *et al*., 1995).

Disruption of cell interaction with the ECM

As discussed earlier, adhesion molecules play a part in peritoneal healing. The integrin family is known to interact with the ECM also primarily via the Arg-Gly-Asp (RGD) tripeptide. Thus the

inhibitory effects of RGD-containing peptides have been explored for their ability to reduce adhesion formation in rats. RGD peptides were administered via a pump over a 7 day period or in the form of a viscous gel at the end of the surgery. In both experimental models, adhesion scores were significantly less compared with controls (Rodgers, 1999).

Conclusion

Adhesion formation and reformation are important and recognized causes of mortality and morbidity. The understanding of the complex relationship between peritoneal healing and adhesion formation is still in its infancy. Studies on peritoneal fluid should allow for large variation/error and should be performed in a more systematic and standardized manner. Further studies into the differences between what is normal and aberrant peritoneal healing may help in the design of an effective treatment for adhesion prevention in the future.

References

Arici, A., Tazuke, S., Attar, E. *et al*. (1996) Interleukin-8 concentration in peritoneal fluid of patients with endometriosis and modulation of interleukin-8 expression in human mesothelial cells. *Mol. Hum. Reprod*., **2**, 40–45.

Assoian, R., Fleurdelys, B. and Stevenson, H. (1987) Expression and secretion of type 2 beta transforming growth factor by activated human macrophages. *Proc. Natl Acad. Sci. USA*, **84**, 6020–6024.

Assoian, R., Kornoriya, A. and Meyers, C. (1983) TGF-beta in human platelets. *J. Biol. Chem*., **258**, 7155–7160.

Bachus, K., Doty, E. and Haney, A. (1995) Differential effects of interleukin-1 alpha, tumour necrosis factor-alpha, indomethacin, hydrocortisone, and macrophage co-culture on the proliferation of human fibroblasts and peritoneal mesothelial cells. *J. Soc. Gynecol. Invest*., **2**, 636–642.

Bakkum, E., van Blitterswiijk, C., Emeis, J. *et al*. (1996) Long term analysis of plasminogen activator activity and adhesion formation after surgical trauma in the rat model. *Fertil. Steril*., **66**, 1018–1022.

Batzofin, J., Holmes, S., Gibbons, W. *et al*. (1985) Peritoneal fluid plasminogen activator activity in endometriosis and pelvic adhesive disease. *Fertil. Steril*., **44**, 277–279.

Bauer, J., Ganter, U., Geiger, T. *et al*. (1988) Regulation of IL-6 expression in cultured human blood monocytes and monocyte-derived macrophages. *Blood*, **72**, 1134–1140.

Berborowicz, A., Rodela, H., Karon, J. *et al*. (1997) *In vitro* stimulation of the effect of peritoneal dialysis solution on mesothelial cells. *Am. J. Kidney Dis*., **29**, 404–449.

Border, W. and Noble, N. (1994) Transforming growth factor beta in tissue fibrosis. *N. Engl. J. Med*., **331**, 1286–1289.

Boukaert, P., Van Wersch, J. and Schellekens, L. (1984) Haemostatic and fibrinolytic properties of peritoneal fluid in the menstrual cycle. *Br. J. Obstet. Gynaecol*., **91**, 256–259.

Brauner, A., Hylander, B. and Wretlind, B. (1993) IL-6 and IL-8 in dialysate and serum from patients on CAPD. *Am. J. Kidney Dis*., **22**, 430–435.

Brauner, A., Hylander, B. and Wretlind, B. (1996) TNF-alpha, IL-1beta, and IL-1 receptor antagonist in dialysate and serum from patients on CAPD. *Am. J. Kidney Dis*., **27**, 402–408.

Buckman, R., Buckman, P., Hufnagel, H. *et al*. (1976) A physiological basis for the adhesion-free healing of deperitonealised surfaces. *J. Surg. Res*., **21**, 67–76.

Bullen, E., Longaker, M., Updike, D. *et al*. (1995) Tissue inhibitor of metalloproteinase-1 is decreased and activated gelatinases are increased in chronic wounds. *J. Invest. Dermatol*., **104**, 236–240.

Buyalos, R.P., Funari, V.A., Azzizz, R. *et al*. (1992) Elevated interleukin-6 levels in peritoneal fluid of patients with pelvic pathology. *Fertil. Steril*., **58**, 302–306.

Carolien, A., Koks, M., Groothuis, P. *et al*. (2000) Matrix metalloproteinases and their tissue inhibitors in antegradely shed menstruum and peritoneal fluid. *Fertil. Steril*., **73**, 604–612.

Chegini, N. (1997) The role of growth factors in peritoneal healing: transforming growth factor beta (TGF-beta) *Eur. J. Surg*., **577**, 17–23.

Chegini, N., Gold, L., Williams, S. *et al*. (1994) Localisation of transforming growth factor beta isoforms TGF-beta1, TGF-beta2, TGF-beta3 in surgically induced pelvic adhesions in rat. *Obstet. Gynecol*., **83**, 449–454.

Chegini, N., Zhao, Y. and Kotseos, K. (1998) Comparative analysis of matrix metalloproteinase (MMP-1), tissue inhibitor of MMP (TIMP-1) and MMP/TIMP-1 complex expression in intraperitoneal environment and their relation to adhesion development (abstract). *Fertil. Steril*., **70** (Suppl.), 26.

Chegini, N., Rong, H., Bennett, B. *et al*. (1999) Peritoneal fluid cytokine and eicosanoid levels and their relation to the incidence of peritoneal adhesion. *J. Soc. Gynecol. Invest*., **6**, 153–157.

Chunfeng, M., Tarnuzzer, R. and Chegini, N. (1999) Expression of matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases in mesothelial cells and their regulation by transforming growth factor-beta. *Wound Repair Regen*., **7**, 477–485.

Cooper, D., Yu, E., Hennessey, P. *et al*. (1994) Determination of endogenous cytokines in chronic wounds. *Ann. Surg*., **219**, 688–692.

Cromack, D., Spom, M., Roberts, A. *et al*. (1987) TGF-beta levels in rat wound chambers. *J. Surg. Res*., **42**, 622–628.

Damjanovich, L., Albelda, S., Mette, S. *et al*. (1992) Distribution of integrin cell adhesion receptors in normal and malignant lung tissue. *Am. J. Respir. Cell Mol. Biol*., **6**, 197–206.

diZerega, G.S. (1990) The peritoneum and its response to surgical injury. In diZerega, G.S., Malinak, L., Diamond, M. and Linsky, C. (eds), *Treatment of Post Surgical Adhesions*. Wiley–Liss, New York, pp. 166–171.

diZerega, G.S. (1999) Use of adhesion prevention barriers in pelvic reconstructive and gynaecological surgery. In DiZerega, G.S. (ed.), *Peritoneal Surgery*. Springer-Verlag, New York, pp. 379–399.

Doody, K., Dunn, R. and Buttram, V. (1989) Recombinant tissue plasminogen activator reduces adhesion formation in rabbit uterine horn model. *Fertil. Steril*., **51**, 509–512.

Dorr, P., Vemer, H., Brommer, E. *et al*. (1990) Prevention of postoperative adhesions by tissue-type plasminogen activator (t-PA) in the rabbit. *Eur. J. Obstet. Gynecol. Reprod. Biol*., **37**, 287–291.

Dorr, P., Dooijewaard, G. and Vemer, H. (1993) Parameters of fibrinolysis in peritoneal fluid and plasma in different stages of the menstrual cycle. *Thromb. Haem*., **70**, 873–875.

Douvdevani, A., Raport, J., Konforty, A. *et al*. (1994) Human peritoneal mesothelial synthesize IL-1 alpha and beta. *Kidney Int*., **46**, 993–1001.

Drake, T., Metz, S., Grunert, G. *et al*. (1980) Peritoneal fluid volume in endometriosis. *Fertil. Steril*., **34**, 280–281.

Dunn, R.C. and Mohler, M. (1994) Tissue plasminogen activator. *Infertil. Reprod. Med. Clin. North Amer*., **5**, 242 – 238.

Edelstam, G., Lecander, I., Larsson, B. *et al*. (1998) Fibrinolysis in the peritoneal fluid during adhesions, endometriosis and inflammatory disease. *Inflammation*, **22**, 341–351.

Ellis, H. (1962) The aetiology of post-operative abdominal adhesions. An experimental study. *Br. J. Surg*., **50**, 10–16.

Ellis, H., Moran, B., Thompson, J. *et al*. (1999) Adhesion-related hospital readmissions after abdominal and pelvic surgery: a retrospective cohort study. *Lancet*, **353**, 1476–1480.

Farquhar, C., Vandekerckhove, P., Watson, A. *et al*. (2000) Barrier agents for preventing adhesions after surgery for subfertility. The Cochrane Library (The Cochrane Collaboration). *Cochrane Review Syst. Rev*. 2000 (2) CD 000475.

Fear, H., Williams, J. and Topley, N. (1997) Staphylococci direct leukocyte recruitment across mesothelium via IL-8 dependent and independent mechanisms. *Kidney Int*., **52**, 1117–1118.

Florio, P., Luis, S., Vigano, P. *et al*. (1998) Healthy women and patients with endometriosis show high concentrations of inhibin A, inhibin B, and activin A in peritoneal fluid throughout the menstrual cycle. *Hum. Reprod*., **13**, 2606–2611.

Gazvani, M., Christmas, S., Quenby, S. *et al*. (1998) Peritoneal fluid concentrations of interleukin-8 in women with endometriosis: relationship to the stage of the disease. *Hum. Reprod*., **13**, 1957–1961.

Gehlbach, D., O'Hair, K., Parks, A. *et al*. (1994) Combined effects of tissue plasminogen activator and carbosymethycellulose on adhesion reformation in rabbits. *Int. J. Fertil*., **39**, 172–176.

Guerra-Infante, F.M., Flores-Medina, S., Lopex-Hurtado, M. *et al*. (1999) Tumour necrosis factor in peritoneal fluid from asymptomatic infertile women. *Arch. Med. Res*., **30**, 138–143.

Halme, J. (1986) Release of tumour necrosis factor-alpha by human peritoneal macrophages *in vivo* and *in vitro*. *Am. J. Obstet. Gynecol*., **61**, 1718–1725.

Haney, A. (1999) Peritoneal fluid. In DiZerega, G. (ed.), *Peritoneal Surgery*. Springer-Verlag, New York, pp. 39–49.

Haney, A. (2000) Identification of macrophages at the site of peritoneal injury: evidence supporting a direct role for peritoneal macrophages in healing injured peritoneum. *Fertil. Steril*., **73**, 988–995.

Haney, A. and Doty, S. (1994) The formation of coalescing peritoneal adhesions requires injury to both contacting peritoneal surfaces. *Fertil. Steril*., **61**, 767–775.

Hau, T., Payne, W. and Simmons, R. (1979) Fibrinolytic activity of the peritoneum during experimental peritonitis. *Surg. Gynecol. Obstet*., **148**, 415–418.

Hershlag, A., Otterness, I., Bliven, M. *et al*. (1991) The effect of IL-1 on adhesion formation in the rat. *Am. J. Obstet. Gynecol*., **163**, 771–774.

Hirano, T. (1998) IL-6 and its receptor: ten years later. *Int. Rev. Immunol*., **16**, 249–284.

Holmdahl, L., Falkenberg, M., Ivarsson, M. *et al*. (1997) Plasminogen activators and inhibitors in peritoneal tissue. *A.P.M.I.S*., **105**, 25–30.

Holschneider, C., Cristoforoni, P., Ghost, K. *et al*. (1997) Endogenous versus exogenous IL-10 in post-operative intraperitoneal adhesion formation in a murine model. *J. Surg. Res*., **70**, 138–143.

Holschneider, C., Nejad, F. and Montz, F. (1999) Immunomodulation with interleukin-10 and interleukin-4 compared with ketorolac trimethamine for prevention of postoperative adhesions in a murine model. *Fertil. Steril*., **71**, 67–73.

Hulboy, D., Rudolph, L. and Matrisian, L. (1997) Matrix metalloproteinases as mediators of reproductive function. *Mol. Hum. Reprod*., **3**, 27–45.

Ignotz, R. and Massaque, J. (1986) TGF-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J. Biol. Chem*., **261**, 4337–4345.

Ivarsson, M., Holmdahl, L., Falk, P. *et al*. (1998) Characterisation and fibrinolytic properties of mesothelial cells isolated from peritoneal lavage. *Scand. J. Clin. Lab. Invest*., **58**, 195–203.

Jones, P. and Werb, Z. (1980) Degradation of connective tissue matrices by macrophages. III. Influence of matrix composition on proteolysis of glycoprotein, elastin and collagen by macrophages in culture. *J. Exp. Med*., **152**, 1527–1536.

Kaidi, A., Nazzal, M., Gurchumelidze, T. *et al*. (1995a) Preoperative administration of antibodies against tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) and their impact on peritoneal adhesion formation. *Am. Surg*., **61**, 569–572.

Kaidi, A.A., Gurchumelidze, T., Nazzal, M. *et al*. (1995b) Tumour necrosis factor alpha: a marker for peritoneal adhesion formation. *J. Surg. Res*., **58**, 516–518.

Khorram, O., Taylor, R.N., Ryan, I.P. *et al*. (1993) Peritoneal fluid concentrations of cytokine RANTES correlate with severity of endometriosis. *Am. J. Obstet. Gynecol*., **169**, 1545–1549.

Kligman, I., Drachenberg, C., Papadimitriou, J. *et al*. (1993) Immunohistochemical demonstration of nerve fibres in pelvic adhesions. *Obstet. Gynecol*., **82**, 566–568.

Koninckx, P., Renaer, M. and Brosens, I. (1980) Origin of peritoneal fluid in women: an ovarian exudation product. *Br. J. Obstet. Gynaecol*., **87**, 177–183.

Krahenbuhl, L., Schafer, M., Kuzinkovacs, P. *et al*. (1998) Experimental study of adhesion formation in open and laparoscopic fundoplication. *Br. J. Surg*., **85**, 826–830.

Krause, T., Katz, D., Wheeler, C. *et al*. (1999) Increased levels of surgical adhesions in TGF-beta1 heterozygous mice. *J. Invest. Surg*., **12**, 31–38.

Leondires, M., Stubblefield, P., Tarraza, H. *et al*. (1995) A pilot study of cyclosporine for the prevention of postsurgical adhesion formation in rats. *Am. J. Obstet. Gynecol*., **172**, 1537–1539.

Leyendecker, G., Kunz, G., Noe, M. *et al*. (1998) Endometriosis: a dysfunction and disease of archimetra. *Hum. Reprod. Update*, **4**, 752–762.

Liberek, T., Topley, N., Luttmann, W. *et al*. (1996) Adherence of neutrophils to human peritoneal mesothelial cells: role of intercellular adhesion molecule-1. *J. Am. Soc. Nephrol*., **7**, 208–217.

Lowry, S. (1993) Cytokines mediators of immunity and inflammation. *Arch. Surg*., **128**, 1235–1241.

Lucas, P., Warejcka, D., Young, H. *et al*. (1996) Formation of abdominal adhesions is inhibited by antibodies to transforming growth factor-beta1. *J. Surg. Res*., **65**, 135–138.

Luciano, A., Maier, D., Koch, E. *et al*. (1989) A comparative study of post-operative adhesions following laser surgery by laparoscopy versus laparotomy. *Obstet. Gynecol*., **74**, 220–224.

Lundorff, P., Hahlin, M. and Kallfely, B. (1991) Adhesion formation after laparoscopic surgery in tubal pregnancy: a randomised trial versus laparotomy. *Fertil. Steril*., **55**, 911–915.

Mahneke, J.L., Dawood, Y. and Huang, J.C. (2000) Vascular endothelial growth factor and interleukin-6 in peritoneal fluid of women with endometriosis. *Fertil. Steril*., **73**, 166–170.

Malick, J. (1982) The etiology of endometriosis. *J. Am. Osteopath. Assoc*., **81**, 407–412.

Mclauren, J., Prentice, A., Charnock-Jones, D. *et al*. (1996) Vascular endothelial growth factor (VEGF) concentrations are elevated in peritoneal fluid of women with endometriosis. *Hum. Reprod*., **11**, 220–223.

Menzies, D. (1993) Postoperative adhesions: their treatment and relevance in clinical practice. *Ann. R. Coll. Surg. Engl*., **75**, 147–153.

Milligan, D. and Raftery, A. (1974) Observations on the pathogenesis of peritoneal adhesion: a light and electron microscopical study. *Br. J. Surg*., **61**, 270–280.

Miyakes, S., Yagita, H., Maruyama, T. *et al*. (1993) Beta-1 integrin-mediated interaction with extracellular matrix proteins regulates cytokine gene expression in synovial fluid cells of rheumatoid arthritis patients. *J. Exp. Med*., **177**, 863–868.

Montz, F., Holschneider, C., Bozuk, M. *et al*. (1994) Interleukin 10: ability to minimize postoperative intraperitoneal adhesion formation in a murine model. *Fertil. Steril*., **61**, 1136–1140.

Mori, H., Sawairi, M., Nakagawa, M. *et al*. (1991) Peritoneal fluid IL-1 beta and TNF in patients with benign gynaecological disease. *Am. J. Reprod. Immun*., **26**, 62–67.

Mueller, M., Tshudi, J., Herrmann, U. *et al*. (1995) An evaluation of laparosocpic adhesiolysis in patients with chronic abdominal pain. *Surg. Endosc*., **9**, 802–804.

Offner, F., Feichtinger, H., Stadlmann, S. *et al*. (1996) TGF-beta synthesis by human peritoneal mesothelial cells. Induction by interleukin-1. *Am. J. Pathol*., **148**, 1679–1688.

Offner, F., Obrist, P., Stadlmann, S. *et al*. (1995) IL-6 secretion by human peritoneal mesothelial and ovarian cancer cells. *Cytokine*, **7**, 542–547.

Ohtsuka, N. (1980) Study of pathogenesis of adhesions in endometriosis. *Acta Obstet. Gynecol. Jpn*., **32**, 1758–1766.

Olive, D., Haney, A. and Weinberg, J. (1987) The nature of exudate associated with infertility: peritoneal fluid and serum lysozome. *Fertil. Steril*., **48**, 802–806.

Padilla, S., Galle, P., Ellegood, J. *et al*. (1986) Plasminogen in peritoneal fluid: possible nonsteroidal indicator of ovulation. *Fertil. Steril*., **46**, 1071–1076.

Pattinson, H., Koninckx, P., Brosens, I. *et al*. (1981) Clotting and fibrinolytic activities in peritoneal fluid. *Br. J. Obstet. Gynaecol*., **88**, 160–166.

Peters, A., Trimbos-Kemper, G., Admiraal, C. *et al*. (1992) A randomised clinical trial on the benefit of adhesiolysis in patients with intraperitoneal adhesions and chronic pelvic pain. *Br. J. Obstet. Gynaecol*., **99**, 59–62.

Porter, J., McGregor, G., Mullen, D. *et al*. (1969) Fibrinolytic activity of the mesothelial surfaces. *Surg. Forum*, **20**, 80–82.

Pugatch, E. and Poole, J. (1969) Inhibitor of fibrinolysis from mesothelium. *Nature*, **221**, 269–270.

Raftery, A. (1973) Regeneration of parietal and visceral peritoneum in the immature animal: a light and electronmicroscopical study. *Br. J. Surg*., **60**, 969–975.

Raftery, A. (1979) Regeneration of the peritoneum: a fibrinolytic study. *J. Anat*., **129**, 659–664.

Raftery, A. (1981a) The effect of peritoneal trauma on peritoneal fibrinolytic activity and intraperitoneal adhesion formation. *Eur. Surg. Res*., **13**, 379–401.

Raftery, A. (1981b) Method of measuring fibrinolytic activity in a single layer of cells. *J. Clin. Pathol*., **34**, 625–629.

Robson, R., Witowski, J., Loetscher, P. *et al*. (1997) Differential regulation of C-C and C-x-C chemokine synthesis in cytokine-activated human peritoneal mesothelial cells by IFN-gamma. *Kidney Int*., **52**, 1123.

Rodgers, K.E. (1999) The role of intergins in peritoneal healing. In DiZerega, G. (ed.), *Peritoneal Surgery*. Springer-Verlag, New York, pp. 85–99.

Rosenthal, R., Ling, F., Rosenthal, T. *et al*. (1984) Chronic pelvic pain: psychological features and laparoscopic findings. *Psychosomatics*, **25**, 833–841.

Saarialho-Kere, U., Kovacs, S., Petland, A. *et al*. (1993) Cell–matrix interactions modulate interstitial collagenase expression by keratinocytes actively involved in wound healing. *J. Clin. Invest*., **92**, 2858–2866.

Saba, A., Kaida, A., Godziachvili, V. *et al*. (1996) Effects of IL-6 and its neutralising antibodies on peritoneal adhesions formation and wound healing. *Am. Surg*., **62**, 569–772.

Saba, A.A., Godziachvili, V., Mavani, A.K. *et al*. (1998) Serum levels of interleukin 1 and tumour necrosis factor alpha correlate with peritoneal adhesion grades in human after major abdominal surgery. *Am. Surg*., **8**, 734–737.

Saravelos, H., Li, T. and Cooke, I. (1995a) An analysis of the outcome of microsurgery and laparoscopic adhesiolysis for infertility. *Hum. Reprod*., **109**, 2887–2894.

Saravelos, H., Li, T. and Cooke, I. (1995b) An analysis of the outcome of microsurgical and laparoscopic adhesiolysis for chronic pelvic pain. *Hum. Reprod*., **10**, 2895–2901.

Sato, Y. and Rifkin, D. (1989) Inhibition of endothelial cell movememt by pericytes and smooth muscle cells: activation of latent transforming growth factor-beta-1 like molecule by plasmin during co-culture. *J. Cell Biol*., **109**, 309–315.

Sharpe-Timms, K., Keisier, L.W., McIntush, E.W. *et al*. (1998a) Tissue inhibitor of metalloproteinase-1 concentrations are attenuated in peritoneal fluid and sera of women with endometriosis and restored in sera by gonadotropin-releasing hormone agonist therapy. *Fertil. Steril*., **69**, 1128–1134.

Sharpe-Timms, K., Zimmer, R., Jolliff, W. *et al*. (1998b) Gonadotropin-releasing hormone agonist (GnRH-a) therapy alters activity of plasminogen activators, matrix metalloproteinases, and their inhibitors in rat models for adhesion formation and endometriosis: potential GnRH-a regulated mechanisms reducing adhesion formation. *Fertil. Steril*., **69**, 916–923.

Sitter, T., Spannagl, M., Schiffl, H. *et al*. (1995) Imbalance between intraperitoneal coagulation and fibrinolysis during peritonitis of CAPD patients: the role of mesothelial cells. *Nephrol. Dial. Transplant*., **10**, 677–683.

Sitter, T., Godde, M., Spannagl, M. *et al*. (1996) Intraperitoneal coagulation and fibrinolysis during inflammation: *in vivo* and *in vitro* observations. *Fibrinolysis*, **10**, 99–104.

Steege, J. and Stout, A. (1991) Resolution of chronic pelvic pain after laparoscopic lysis of adhesions. *Am. J. Obstet. Gynecol*., **165**, 278–281.

Stout, A., Steege, J., Dodson, W. *et al*. (1991) Relationship of laparoscopic findings to self-report of pelvic pain. *Am. J. Obstet. Gynecol*., **46**, 39–73.

Sutton, C. and MacDonald, R. (1990) Laser laparosocpic adhesiolysis. *J. Gynecol. Surg*., **6**, 155–159.

Syrop, C. and Halme, J. (1987) Cyclic changes of peritoneal fluid parameters in normal and infertile patients. *Obstet. Gynecol*., **69**, 416–418.

Thompson, J., Paterson-Brown, S., Harbourne, T. *et al*. (1989) Reduced human peritoneal plasminogen activating activity: a possible mechanism of adhesion formation. *Br. J. Surg*., **76**, 382–384.

Tietze, L., Elbrecht, A., Schauerte, C. *et al*. (1998) Modulation of pro and antifibrinolytic properties of human peritoneal mesothelial cells by transforming growth factor beta 1, tumour necrosis factor alpha and interleukin 1beta. *Thromb. Haemost*., **79**, 362–370.

Topley, N., Peterson, M., Mackenzie, R. *et al*. (1994) Human peritoneal mesothelial cell prostaglandin synthesis: induction of cyclooxygenase mRNA by peritoneal macrophage derived cytokines. *Kidney Int*., **46**, 900–909.

Tulandi, T. (1986) Salpingo-ovariolysis: a comparison between laser surgery and electrosurgery. *Fertil. Steril*., **45**, 489–491.

Tulandi, T., Collins, J., Burrows, E. *et al.* (1990) Treatment-dependent and treatment independent pregnancy among women with periadnexal adhesions. *Am. J. Obstet. Gynecol*., **162**, 354–357.

Tulandi, T., Chen, M., Al-Took, S. *et al*. (1998) A study of nerve fibers and histopathology of postsurgical, postinfectious, and endometriosis-related adhesions. *Obstet. Gynecol*., **92**, 766–768.

van de Poll, T., Levi, M., Buller, H. *et al*. (1991) Fibrinolytic response to tumour necrosis factor in healthy subjects. *J. Exp. Med*., **175**, 729–732.

van Hinsburgh, V., Kooistra, T., Scheffer, M. *et al*. (1990) Characterisation and fibrinolytic properties of human omental tissue mesothelial cells. Comparison with endothelial cells. *Blood*, **75**, 1490–1497.

Vipond, M., Whawell, S., Scott-Coombes, D. *et al*. (1994a) Experimental adhesion prophylaxis with recombinant tissue plasminogen activator. *Ann. R. Coll. Surg. Engl*., **76**, 412–415.

Vipond, M., Whawell, S., Thompson, J. *et al*. (1994b) Effect of experimental peritonitis and ischaemia on peritoneal fibrinolytic activity. *Eur. J. Surg*., **160**, 471–477.

Werb, Z., Tremble, P., Behrendtsen, O. *et al*. (1989) Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. *J. Cell. Biol*., **109**, 877–889.

Whawell, S., Scott-Coombes, D., Vipond, M. *et al*. (1994) Tumour necrosis factor mediated release of plasminogen activator inhibitor-1 by human peritoneal mesothelial cells. *Br. J. Surg*., **81**, 214–216.

Williams, R.S., Rossi, A.M., Chegini, N. *et al*. (1992) Effect of transforming growth factor beta on postoperative adhesion formation and intact peritoneum. *J. Surg. Res*., **52**, 65–70.

Wlaschek, M., Pees, D., Achterberg, V. *et al*. (1997) Protease inhibitors protect growth factor activity in chronic wounds. *Br. J. Dermatol*., **137**, 646–647.

Wright, J. and Sharpe-Timms, K. (1995) Gonadotropin-releasing hormone agonist therapy reduces postoperative adhesion formation and reformation after adhesiolysis in rat models for adhesion formation and endometriosis. *Fertil. Steril*., **63**, 1094–1100.

Yager, D., Chen, S., Ward, S. *et al*. (1997) Ability of chronic wound fluids to degrade peptide growth factors is associated with increased levels of elastase activity and diminished levels of proteinase inhibitors. *Wound Repair Regen*., **5**, 23–32.

Yung, S., Coles, G. and Davies, M. (1996) IL-1 beta, a major stimulator of hyaluronan synthesis *in vitro* of human peritoneal mesothelial cells: relevance to peritonitis in CAPD. *Kidney Int*., **50**, 1337–1343.

Zeillemakerm, A., Mul, F., Hoynck van Papendrecht, A. *et al*. (1995) Polarised secretion of interleukin-8 by human mesothelial cells: a role in neutrophil migration. *Immunology*, **84**, 227–232.

Zemel, D., Koomen, G., Hart, A. *et al*. (1994) Relationship of TNF-alpha, IL-6 and prostaglandins to peritoneal permeability for macromolecules during longitudinal follow-up of peritonitis in CAPD. *J. Lab. Clin. Med*., **122**, 686–696.

Zeyneloglu, H., Seli, E., Senturk, L. *et al*. (1998a) The effect of monocyte chemotactic protein 1 in intraperitoneal adhesion formation in mouse model. *Am. J. Obstet. Gynecol*., **179**, 438–443.

Zeyneloglu, H.B., Senturk, L.M., Seli, E. *et al*. (1998b) The role of monocyte chemotactic protein-1 in intraperitoneal adhesion formation. *Hum. Reprod*., **13**, 1194–1199.

Received on January 2001; accepted on July 30, 2001