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PERITONEAL MOLECULAR ENVIRONMENT, ADHESION FORMATION AND CLINICAL IMPLICATION

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TABLE OF CONTENTS

1. ABSTRACT

Whether induced by infection, inflammation, ischemia, and/or surgical injury, peritoneal adhesions are the leading cause of pelvic pain, bowel obstruction and infertility. It is clear that while postsurgical peritoneal wounds heal without adhesions in some patients, others develop severe scarring from seemingly equal procedures; in addition, in the same patient, adhesions can develop at one surgical site and not in another. The mechanisms underlying the predisposition to form adhesions as well as their site specificity are completely unknown. However, a large number of intraperitoneal surgical procedures are performed each day in the USA, and thus many patients are at risk of developing postoperative adhesions. Therefore, understanding of adhesion formation at the molecular level is essential and in the absence of such information, attempts to prevent patients from developing adhesions will remain an empirical process. The unprecedented advancement in molecular biology during the past decade has led to the identification of many biologically active molecules with the potential of regulating inflammatory and immune responses, angiogenesis and tissue remodeling, events that are central to normal peritoneal wound healing and adhesion formation. Although, the insight into their importance in the development of tissue fibrosis has substantially increased, their major roles in peritoneal biological functions and adhesion formation remain at best speculative. This article reviews the clinical implications of adhesions and attempts to highlight some of the key molecules i.e. growth factors, cytokines, chemokines, proteases and extracellular matrix, that are recognized to regulate inflammation, fibrinolysis, angiogenesis, and tissue remodeling, events that are central to peritoneal wound repair and adhesion formation. Finally, the article discusses the potential application and site specific delivery of several active compounds that are developed to alter the local inflammatory and immune response i.e., cytokine/chemokine network, targeted gene delivery and development of a new generation of biomaterials to prevent adhesion formation. Such understanding of peritoneal biology not only assist us to better manage patients with adhesion, but also those with endometriosis and malignant diseases that affect the peritoneal cavity.

2. CLINICAL IMPLICATIONS

In humans, postoperative adhesion formation has been a problem since the advent of intra-abdominal manipulation. Even today, after immense improvement in surgical instrumentation and techniques as well as the development of various postsurgical devices to prevent

adhesion formation, intraperitoneal adhesions remain a major clinical concern. During the past two decades, clinical observations have consistently confirmed and estimated that between 60 to 100% of patients undergoing abdominal/pelvic surgery develop peritoneal adhesions. These observations have also revealed a direct proportional link between the frequency of surgery and the incidence of adhesion formation. Although postoperative adhesions are recognized as a consequence of surgery, patients with pelvic inflammation or endometriosis also are at risk of developing adhesions. For this reason, common use of laparoscopic intervention in various abdominal and pelvic surgical procedures has led to the belief that such an approach is less likely to cause adhesions with lower postoperative morbidity compared with laparatomy. Laparoscopic surgery often is more precise than open surgery, causing less tissue drying and more localized tissue trauma. However, the difference between the incidence of postoperative adhesion formation caused by laparatomy versus laparoscopic procedures is, at best, controversial.

While most of the earlier studies have been conducted in female infertility patients, recent studies showed that 94% of women without infertility and men who had undergone colectomy developed adhesions immediately beneath the midline abdominal incision at second-look (1-8). The most recent epidemiological studies, which examined a ten year follow-up of over 54,000 patients' medical records from the Scottish National Health Service Registrar Database, have clearly pointed out both the immediate and long term scope of peritoneal adhesion associated morbidity following gynecological and lower abdominal surgeries (2,3). These studies have concluded that 30 to 35% of all hospital readmissions were associated with potential adhesion associated complications, of which 4.5 to 5.1% were directly related to adhesions. Patients who underwent gynecological surgery involving ovaries and fallopian tubes were even at the highest relative risk of hospital readmission for complication directly related to adhesions, with an overall rate of readmission of 80 to 100 per 100 initial procedures, with 15% occurring during the first year and continued over the next 10 years. A similar trend was also found in hospital readmission of patients who had undergone gastrointestinal surgery, with an overall rate 2.5 times higher than other lower abdominal surgical procedures. Another 22.1% of patients required additional surgery for reasons that were possibly related to adhesions (3). Peritoneal adhesions affecting the uterus, fallopian tubes, or ovaries have been shown to account for approximately 20% of all infertility, a problem that is experienced by 10% of reproductive age women (1). Collectively, adhesions are a key initiating cause of infertility, chronic abdominal/pelvic pain and bowel obstruction, and they impair the organs' unique physiological functions. Although controversial, the presence of abdominal-pelvic pain has often been attributed to adhesions, which are estimated to be a contributing factor in up to 30% of women with chronic pain (1). Extensive adhesions can also lead to serious complications for peritoneal dialysis patients or those who require intraperitoneal drugs or nutrition. Even for patients who do not suffer from adhesion-associated complications, the presence of adhesions in re-operative procedures can prolong operative time and increase the risk of intraoperative/postoperative complications due to injuries to the bowel, bladder, blood vessels or other sites (1-8). Failure to identify and acknowledge the scope of adhesion-associated problems may be a contributing factor that has hampered our goal of developing measures to prevent adhesions, and is due in large part to ethical and logistical restraints to the performance of second-look operations to assess the extent of postoperative adhesion development.

The recognition that adhesions contribute to the above clinical problems has generated a great deal of interest in development of creative techniques or products to prevent postoperative adhesion development (5-8). Despite continued efforts and advancements, these measures have been found to be ineffective; rather, at best, they only reduce the incidence of adhesion formation. Even currently available devices, i.e. bio-absorbable membranes, gels, and coating solutions, as well as materials presently undergoing clinical trials, are formulated such that they have a casual relationship with the ultimate goal of serving as an ideal tool to prevent postoperative adhesion development. In addition to a significant clinical burden associated with surgical and medical work loads, the economic impact of adhesion related to medical expenses, currently estimated to be around 2-4 billon dollars annually in the United States alone (1-8), as well as the indirect costs associated with reduced business efficiency or time lost from work, make finding a successful adhesion prevention strategy an urgent necessity. However, to reach this goal we must first increase our understanding of the peritoneal wound environment at the molecular level, which will allow the development of devices that far exceed the need of satisfying the basic biocompatibility with the surrounding environment. Most of our current knowledge of peritoneal wound healing and adhesion formation derives from analogous studies of dermal wound healing and scar tissue formation. Such comparisons have been instrumental in increasing our overall knowledge of the peritoneal wound molecular environment, since peritoneal adhesion formation is a defective wound healing process that is similar to dermal scar formation. However, there are reasons to address the issue of peritoneal wound healing and adhesion formation in a different context in order to identify those differences that may aid us to more effectively reach our ultimate goal. We still poorly understand why adhesions form more frequently in an individual tissue and/or patient than in others. Although improved surgical techniques and limited manipulation of organs during surgical procedures are probably important in preventing adhesions, the post-genomic era with its wealth of information may be useful in answering this question at the molecular level. In addition, the development of superior postsurgical devices that have biocompatibility with their surrounding environment and carrying wound modifying agents could improve the management of adhesion development. In this review, we attempt to highlight some of the key molecules recognized to modulate the outcome of wound healing and scar tissue formation and discuss potential avenues that could be utilized to locally alter their expression to reach an adhesion-free peritoneal environment.

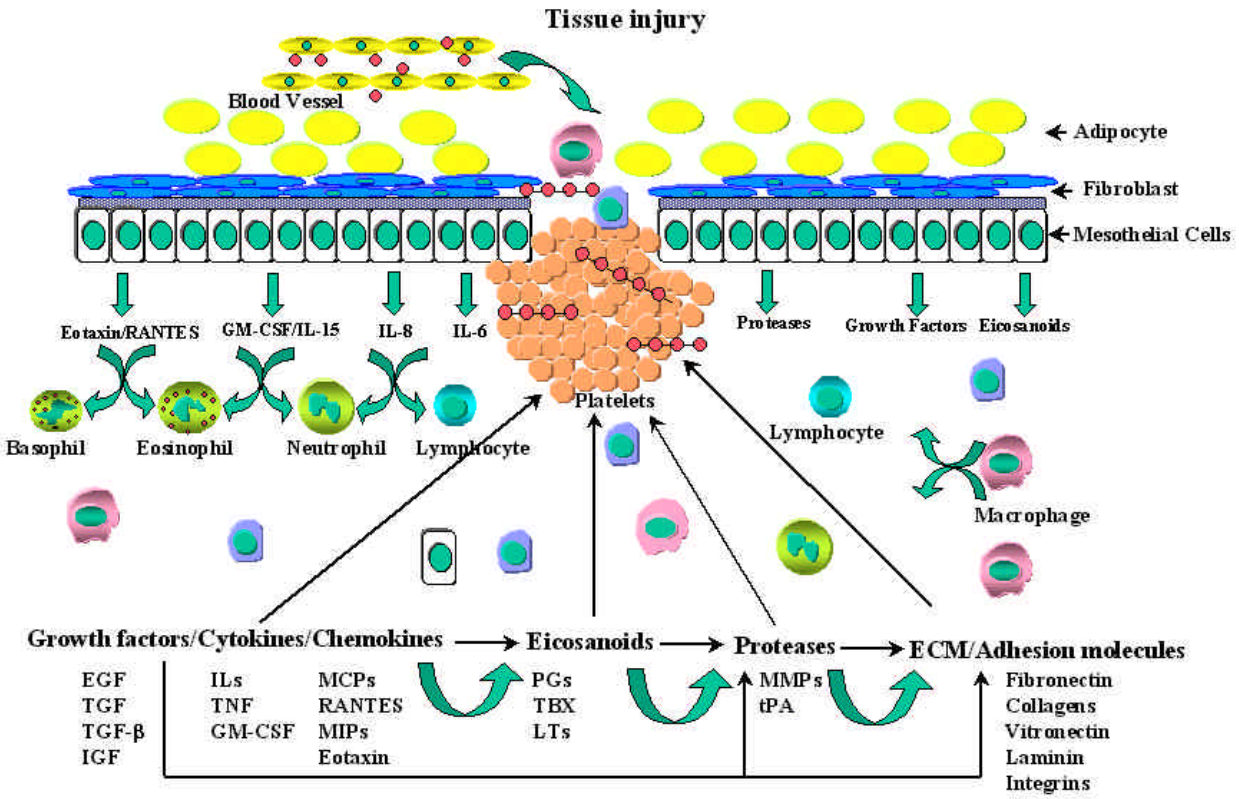

Figure 1. Schematic representation of early events following peritoneal injury and involvement of a number of cytokines, chemokines, growth factors, eicosanoids, proteases and adhesion molecules that regulate blood clot formation, inflammation, cell migration involving leukocytes, macrophages, and fibroblasts to initiate the wound repair.

3. MOLECULAR EVENTS IN PERITONEAL ADHESION FORMATION

The serosal surface of intraperitoneal organs and the parietal peritoneum is composed of mesothelial cells. Taken together, these surfaces comprise the largest surface area in the body that almost equals that of the skin. Peritoneal inflammation, ischemia, infection, and surgically-induced tissue trauma can easily damage or permanently remove these cells, exposing the submesothelial connective tissue (Figures 1 and 2). The onset of cellular and tissue injury is associated with remarkable morphological and biochemical alterations which promote repair of the defective region. However, when this process is unregulated, the outcome of wound healing is associated with excessive tissue or scar formation (9). In contrast, fetal wounds heal with near-perfect regeneration or scarless healing (9). The regulating mechanism underlying fetal tissue regeneration is unknown, although the extent of trauma and degree of inflammation are considered important elements. Interestingly, this phenomenon appears to apply only to dermal wound healing, because injury to other fetal tissues, including intraperitoneal organs, results in scarring similar to that which occurs in adult tissues. In addition, aging slows the rate of dermal wound healing, while peritoneal wounds do not appear to follow this trend.

The overlapping and dynamic processes that lead to peritoneal wound healing include inflammatory response, cell growth and differentiation, angiogenesis, extracellular matrix turnover, tissue remodeling, and apoptosis. While many phases of peritoneal wound repair and specific mechanisms regulating these activities resemble dermal wound healing, two fundamental differences must be taken into consideration. First, dermal injuries heal inward from the edges such that the rate of healing is dependent on the size of the lesion. On the other hand, peritoneal wounds are thought to heal by the differentiation of underlying progenitor cells, the movement of mesothelial cells from the edge of the wound towards the center, and by seeding of the wound with mesothelial cells detached from other regions, such that healing occurs simultaneously throughout the lesion and independent of the surface area of the injury (10). Second, peritoneal wounds are continuously exposed to many substances that are synthesized and released by mesothelial, inflammatory and immune cells in peritoneal fluid and various other cell types within the wound. Therefore, direct and indirect autocrine/paracrine feedback regulation initiated by local expression of these molecules is an important component and determinant of the outcome of peritoneal healing and adhesion formation.

The identity and precise nature of the molecules that are involved in peritoneal repair processes are not yet known. However, emerging evidence from studies examining peritoneal wound and peritoneal fluid during healing and comparative analogy with dermal wound

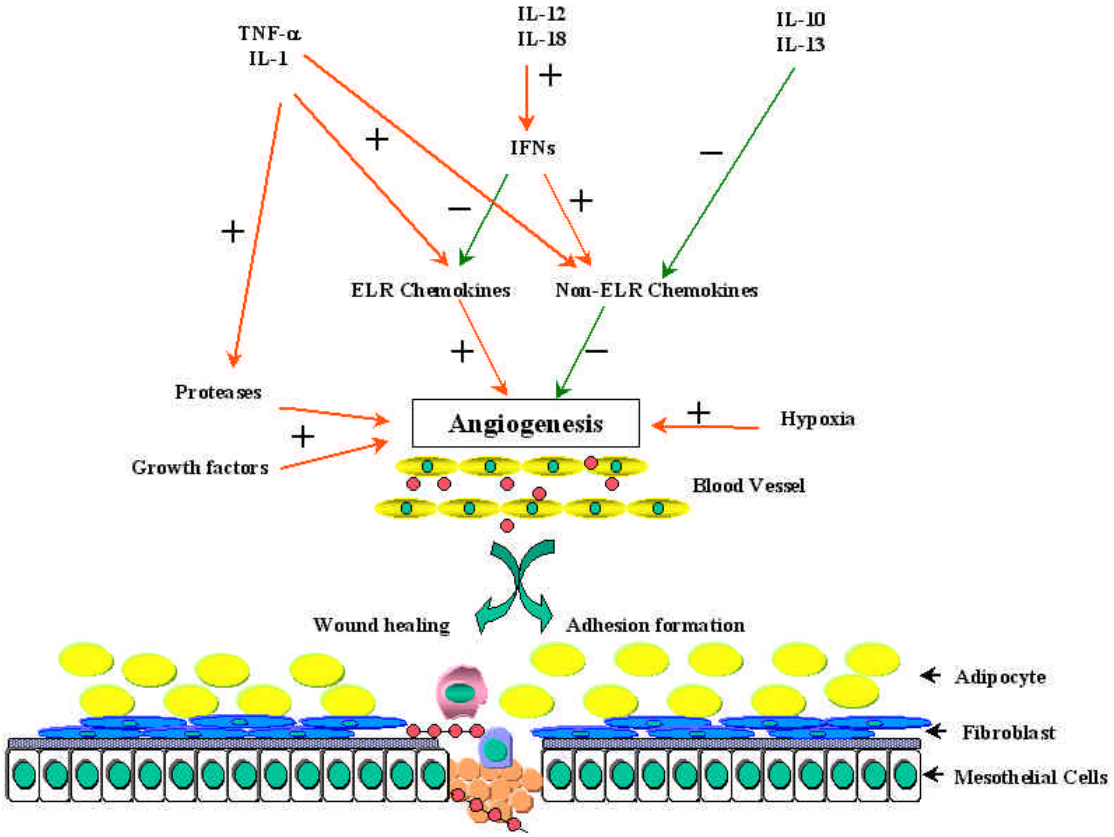

Figure 2. Schematic representation and involvement of a number of cytokines, chemokines and growth factors that regulate angiogenesis that ultimately leads to either peritoneal wound healing or adhesion formation.

healing has led to the hypothesis that local expression of autocrine/paracrine growth factors, cytokines, chemokines, proteases, adhesion molecules and the extracellular matrix, etc. plays a critical role in these events. As evidenced by different degrees of peritoneal tissue repair, the expression of these molecules and their biological signals must be optimal, precise, and synchronized. The correct and timely progression of expression and interaction among these signals appear to provide an optimal environment for near perfect tissue repair. In contrast, the aberrant function of some of these factors seems to be responsible for inappropriate wound healing processes affecting the inflammatory response, cellular migration, proliferation, angiogenesis, and tissue remodeling that result in peritoneal adhesions.

3.1. Inflammatory Mediators and Adhesion Formation

Most of our understanding of the process of peritoneal inflammation and its key regulators has been accumulated from clinical and basic science research on pelvic inflammatory disease and peritoneal infection in peritoneal dialysis patients (11). Peritoneal dialysis is performed in a substantial number of patients annually and is also the leading cause of adhesion formation (11). These studies have led to recognition of an array of molecules that are established as key regulators of peritoneal host defense mechanisms and functional activity generated to heal the defected area in response to infection, inflammation or cellular/tissue injury (10-12). The inflammatory and immune cells that reside or migrate into the peritoneal cavity, mesothelial cells lining the visceral and parietal peritoneum, fibroblasts which reside within the submesothelial tissue, and their secretory products are the key regulators of the peritoneal response to these events (10-12). The individual and combined actions of these molecules are to initiate, amplify, and control many molecular events that ultimately lead either to resolution of the inflammatory response, peritoneal wound repair, or adhesion formation (Figures 1 & 2).

In the case of peritoneal injury, coagulation and platelet aggregation are initiated to prevent excessive blood loss. Cell surface activation of Hagemann factor, a tissue procoagulant factor released from damaged cells, as well as the activation of surface membrane coagulation factors and phospholipids, expressed by activated platelets and vascular endothelial cells, initiate these events and promote the healing of peritoneal defects (13-15). At the same time, as a requirement for initiation of tissue repair, the clots must begin to lyse. Both clot formation and degradation are regulated by many intrinsic substrates including eicosanoids such as prostacyclin, thromboxane, leukotrienes, antithrombin III, protein C, plasminogen activators, and plasminogen activator inhibitors (13-21).

The activities of these substances are regulated by the local release of various soluble factors produced by platelets and, later in the process, by infiltrating and activated inflammatory and immune cells. Platelets release a network of highly active substances into the wound environment, some with adhesive properties that act as ligands for platelet aggregation, e.g. fibrinogen, fibronectin, thrombospondin, and von Willebrand factor VIII. Von Willebrand factor VIII also mediates platelet adhesion to fibrillar collagens through interaction with integrin and subsequent platelet activation (14-18). Activated platelets also release leukocyte chemotactic factors, platelet derived growth factor (PDGF), transforming growth factor alpha (TGF-α), heparin binding epidermal growth factor (HB-EGF), transforming growth factor beta (TGF-β), etc. (22-27). The fibrin clot, which provides an early form of extracellular matrix (ECM), retains many of these factors that potentiate the migration of inflammatory cells into the wound (Figures 1 & 2).

The induction of a local inflammatory reaction due to infiltration of peripheral blood neutrophils and monocytes is an important feature of soft tissue repair, and animals depleted of macrophages have defective tissue debridement, fibroblast proliferation, and wound repair (9). Macrophage recruitment into the tissues is further sustained in part by platelet-derived cytokines as well as other chemoattractant factors. These factors include fibrinopeptides cleaved from fibrinogen by thrombin, degradation products of fibrin produced by plasmin, platelet factor 4 (PF4), eicosanoids (LTB_4, LTC_4, and PGE_2), and platelet-activating factor (PAF) released from endothelial cells or activated neutrophils (28). The recruitment of inflammatory cells into the wound is also facilitated by adhesive molecules such as fibrin, fibronectin, and vitronectin that are present in the fibrin clot (provisional matrix), as well as integrins that recognize these molecules (9,19-21). The infiltrating monocytes that become activated and differentiate into macrophages are a major source of growth factors, cytokines, chemokines, eicosanoids, and proteases (22,25-27). In addition, peritoneal macrophages, neutrophils, T cells, mast cells, and mesothelial cells are the sources of many of these molecules. Collectively, these molecules, either individually or through their interactions, regulate the peritoneal wound as it proceeds through a degradative phase into a reparative stage that ultimately results in peritoneal tissue repair.

Many of the cytokines and chemokines released into the wound regulate the production of eicosanoids and proteases that are essential factors in the coagulation cascade and inflammatory response (19-29). The production of cytokine-induced eicosanoids through the cyclooxygenase pathway appears to antagonize, while products of lipoxygenase pathway mediate or amplify the effect of cytokine action (30,31). The stimulatory actions of TGF-β, EGF, and TNF-α on fibroblasts, a key cell type in adhesion formation, are augmented by cyclooxygenase inhibitors and reduced by the presence of PGE_2, PGE_1, and PGI_2 (9,22,24,32). In addition, eicosanoids act as intracellular mediators of cytokine and growth factor actions in various cell types. Elevated production of eicosanoids and their receptors has been associated with an increased incidence of adhesion formation, and their inhibition through the use of non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, indomethacin and meclofenamate, has proven effective in reducing adhesion formation in animal models. Although limited studies performed to establish efficacy in humans have had mixed results (33-35), evidence generated from patients with peritoneal inflammation due to peritoneal dialysis, animal models of peritoneal inflammation, and in vitro studies, support the use of agents to suppress peritoneal eicosanoid production in the peritoneal cavity following infection or surgical trauma. Intraperitoneal (IP) administration of indomethacin in continuous ambulatory peritoneal dialysis patients during peritonitis has been reported to decrease the intrinsic permeability of the peritoneum to macromolecules without effectively altering other peritoneal functional parameters (36). In addition, IP administration of indomethacin following peritoneal bacterial challenge enhances leukocyte migration and peritoneal permeability to protein and dialysate concentrations of PGE2, PGF1α and IL-8. Such enhancement occurs without affecting the transperitoneal migration of leukocytes or the production of IL-8, a potent inflammatory cytokine (37). Leukotrienes, which are potent chemoattractant factors for neutrophils, macrophages, and eosinophils have been implicated in the pathogenesis of a variety of inflammatory processes. Surgically-induced peritoneal injury results in upregulation of prostaglandin, thromboxine, 5-lipoxygenase, and receptors for LTB-4, LTC-4 and LTD-4 throughout healing and peritoneal adhesion formation (33,34). LTB-4 mediates its action through a G protein-coupled receptor, the LTB-4 receptor (BLTR), and targeted gene disruption of BLTR has further implicated LTB-4 as a key factor in leukocyte activation. Despite a functional redundancy with other chemoattractant-receptors, it is apparent that LTB-4 and BLTR are central to the recruitment and/or retention of leukocytes, particularly eosinophils, to the inflamed peritoneum (38). BLTR deficiency did not result in any apparent abnormalities and peritoneal neutrophils displayed normal responses to the inflammatory mediators such as PAF; however, neutrophil influx in response to arachidonic acid was significantly reduced following the induction of peritonitis in BLTR deficient mice. Surprisingly, female mice deficient in BLTR displayed selective survival relative to males in response to PAF-induced anaphylaxis (39). This finding may be due, in part, to the protective action of ovarian steroids.

3.2. The Role of Chemokines and Cytokines

Chemokines are small polypeptides divided into several subgroups according to the spatial arrangement of the first two cysteine residues that include CXC or α, CC or β, C or γ and CX3C or δ chemokine **(**40). Chemokines have a potent chemoattractant activity for leukocytes and regulate inflammation, angiogenesis, hematopoesis, and host response to infection. They mediate their biological activities through a distinct family of G-protein-coupled receptors that have been identified on various cells types (41). The two most prominent chemokines are the CC

which include monocyte chemoattractant protein-1 (MCP-1), MCP-5, macrophage inflammatory protein-1α (MIP-1α), MIP-1β, eotaxin, regulated upon activation, normal T-cell-expressed and secreted (RANTES), C10, stromal cell-derived factor 1 (SDF-1) and CXC such as IFN-inducible protein-10 (IP-10), MIP-2, KC and growth-related oncogene, Gro-*a* (40,41).

Recent in vitro and in vivo studies also point to the critical role for chemokines in peritoneal biology, specifically, the inflammatory response that often leads to adhesion formation (11). Plasma-derived fibrinogen released in response to endothelial cell retraction at sites of inflammation has been shown to induce peritoneal macrophage expression and release of several chemokines, including MIP-1α, MIP-1β, MIP-2, and MCP-1 (42). MCP-1 in turn stimulates peritoneal macrophage LTB-4 production. The LTB-4 receptor antagonist, CP-105-696, inhibits peritonitis-induced recruitment of neutrophils and macrophages, accompanied by a reduction in peritoneal MCP-1 expression and survival of mice (43). These results suggest that induction of chemokines following the initiation of peritoneal injury occurs directly or indirectly through the production of LTB-4, and that chemokines mediate the recruitment of neutrophils to the site of injury (43). Induced peritoneal macrophages are also associated with the induction of chemokines such as RANTES, MCP-1 and MIP-1α (44). IP administration of MCP-1 promotes chemotaxis of specific leukocyte populations predominately consisting of granulocytes and macrophages, while eotaxin selectively enhances eosinophils, and MIG induces T cell migration. Administration of methotrexate, piroxicam or dexamethasone inhibited cellular migration, and MCP-1-mediated trafficking was impaired by treatment with anti-MCP-1 antibody or IL-10, a cytokine with anti-inflammatory properties (44). Interestingly, MIP-2 expression was found to be limited to localized inflamed regions, independent of the expression of proinflammatory cytokines such as TNFα or IL-1β production (45). IFN-γ treatment selectively inhibited LPS-induced MCP-1, MIP-1α, MIP-1β and MIP-2, but induced IP-10 and MCP-5 expression (46). Peritoneal B lymphocytes which express the SDF-1 receptor accumulate and proliferate in response to SDF-1 chemotactic and growth-promoting action, and IP administration of neutralizing SDF-1 antibody significantly decreased their numbers, suggesting that peritoneal derived SDF-1 may play a role in the peritoneal migration of B cells (47). In addition, SDF-1 reversed the antigen-induced T-cell recruitment into the peritoneal cavity, a phenomenon described as a movement away from a chemokine, which represents a previously unknown mechanism regulating the localization of mature T cells (48). IL-3, IL-4, GM-CSF, IL-10 and IL-13, cytokines that are expressed by mesothelial cells and are present in peritoneal fluid, induce macrophage expression of C10, a CC chemokine that regulates the chronic stage of host defense reaction. In addition, MCP-1 and MIP-1α expression is induced by IL-3 and GM-CSF, and inhibited by IL-4 or IFN-γ. In contrast MCP-1 and MIP-1α inhibit IL-3- and GM-CSF-induced C-10 expression. The peritoneal level of C10 is increased following the induction of peritonitis, and IP administration of C-10 induced a rapid production of TNF-α and MCP-1, and a later increase in IL-13 levels, which were negatively impacted following IP anti-C10 antiserum therapy (49,50).

IL-13 is a Th2 cytokine with a potent anti-inflammatory activity. Induction of peritonitis in mice increased tissue expression of IL-13 in liver, lung, and kidney, but not in peritoneal fluid or in serum, and the inhibition of IL-13 with anti-IL-13 antibodies resulted in a decreased rate of survival (51). Interestingly, treatment with anti-IL-13 antibodies did not alter peritoneal leukocyte infiltration and bacterial load, but it did increase the influx of neutrophils into these tissues, as well as the expression of MIP-2, KC, MIP-1α and TNF-α (51). These finding suggest that endogenous IL-13 mediates its protective action through suppression of tissue expression of inflammatory cytokines/chemokines. Another cytokine with anti-inflammatory properties is IL-10, which effectively inhibits the expression of MCP-1, MCP-5, MIP-1α, MIP-1β, MIP-2, IP-10, KC and RANTES. In addition, IFN-γ selectively induces IP-10 and MCP-5 expression, but inhibits LPS-induced MCP-1, MIP-1α, MIP-1β, MIP-2 and KC (46). Human peritoneal fibroblasts constitutively express MCP-1 and IL-8 and their expression is augmented by the pro-inflammatory cytokines, IL-1β and TNF-α, and by peritoneal macrophage-conditioned medium (52). These effects were partly due to the presence of IL-1β, since co-treatment with the IL-1 receptor antagonist reduced their production (52). IL-1β, TNF-α, IFN-γ or their combinations also modulate the expression of MCP-1, RANTES, IP-10 and GRO*a* by mesothelial cells, by inducing GRO*a* and IP-10 expression, and they augment constitutive production of MCP-1, with limited effect on RANTES expression (53). Furthermore, GRO*a* (8-73), a CXC chemokine receptor antagonist, prevented MIP-2 and KC-induced neutrophil migration (54). Prior treatment with GRO*a* (8-73) or an analogue of PF4- (9-70) resulted in inhibition of leukocyte infiltration into the peritoneal cavity in response to MIP-2. Moreover, GRO*a* (8-73) treatment effectively inhibited TNF-α-, IL-1β- or LPS-induced leukocyte recruitment. These observations point out the importance of complex interactions among cytokines and chemokines in peritoneal inflammatory and immune responses (50), and suggest that their modulation in the peritoneal environment might influence peritoneal inflammation, healing, and adhesion formation.

Hyaluronan (HA) and its derivatives are extensively used in fabrication of biodegradable materials such as membranes, gels, and solutions to prevent adhesion formation (6-8). Degradation products of HA often accumulate at sites of inflammation, and experimentally generated HA fragments have been shown to enhance the expression of MCP-1 and IL-8 in human mesothelial cells (55). Therefore, increased HA levels in the peritoneal cavity of peritoneal dialysis patients as well as the byproducts of biodegradable HA-based devices may alter the local production of these and other chemokines, resulting in prolonged inflammation (55). In addition, neutrophil elastase produced during peritoneal inflammation and at the site of tissue injury enhance the production of MCP-1 by peritoneal macrophages, which was inhibited by serine protease inhibitor,

phenylmethylsulfonyl fluoride (56). This suggests that, in addition to degradation of ECM, proteases may stimulate the release of chemokine production by macrophages, leading to impairment of peritoneal healing and adhesion formation.

The resolution of the inflammatory reaction, which begins with reduced neutrophil infiltration, is also essential for the repair process to proceed**.** Tissue neutrophils are entrapped within the clot and desiccated tissue, where they become apoptotic and are phagocytosed by macrophages (57-61). A number of cytokines, including GM-CSF, IL-1β, TNF-α, and IFN-γ that are expressed by the mesothelial cells and other wound cells and are present in peritoneal fluid, promote the uptake of neutrophils by macrophages (57-61). The mechanism involved in the clearance of macrophages after the completion of their function in the inflammatory reaction has not been defined, although macrophages can undergo apoptosis under in vitro conditions (25,26,57-61). The continued and appropriate expression of cytokines, chemokines and their receptors is critical to the resolution of peritoneal inflammation and the transition from an inflammatory to a reparative environment. Because peritoneal mesothelial cells and fibroblasts interact with peritoneal inflammatory and immune cells, identification of specific chemotactic, pro-inflammatory, and anti-inflammatory cytokines and chemokines which regulate their interactions may allow to design better strategies to prevent the development of adhesions. Such studies would further increase our understanding of other biological processes that result in either uncomplicated repair or the development of adhesions, involving cellular migration, proliferation, and differentiation of several cell types that promote angiogenesis, tissue remodeling and synthesis and deposition of ECM.

4. ANGIOGENESIS AND PERITONEAL ADHESION

In the healthy adult, angiogenesis is restricted to the corpus luteum, placenta, endometrium during the menstrual cycle, and during wound healing. Angiogenesis is a self-limited and strictly regulated process that occurs in a sequential manner. It involves degradation of the vascular basement membrane and interstitial matrix by endothelial cells, migration and proliferation of endothelial cells, and finally tubulogenesis and formation of capillary loops (62-66). The production of proteolytic enzymes in response to angiogenic factors is fundamental to angiogenesis, not only for the degradation of perivascular matrix and tissue stroma, but also for the migration and proliferation of endothelial cells. During angiogenesis, the initial migration and proliferation of endothelial cells occurs in a fibronectin rich ECM, whereas vascular maturation, which takes place at later stages, is laminin rich (9). These processes also involve integrins, the essential components of ECM-cell and cell-cell interactions that promote cell migration, gene expression, cell differentiation and other cellular activities (20,21,62-66). At the initial stage of angiogenesis, the induction of proteases such as matrix metalloproteinases (MMPs) and serine proteases (fibrinolytic system; plasminogen activators) in endothelial cells is necessary to degrade components of the ECM, including fibronectin and laminin (14-21,62,67-69). However, these proteases are produced in inactive forms and must become activated to initiate their local actions. Proteolytic activities of these enzymes are regulated by naturally occurring physiological inhibitors, tissue inhibitor of MMPs (TIMPs) and plasminogen activator inhibitors (PAIs) (14-21,67-71). Cytokines and growth factors, such as IL-1, IL-8, TNF-α, GM-CSF, VEGF, FGFs, EGF, TGF-α, TGF-β, PDGF, and IGF-I, are considered to be angiogenic enhancing factors due to their ability to, 1) regulate the expression of MMPs, the fibrinolytic system and their inhibitors, and 2) to modulate endothelial cell proliferation and migration (14-21,62-74). MMPs, tPA, uPA, TIMPs and PAIs are expressed in parietal peritoneum, peritoneal mesothelial cells and adhesion fibroblasts, and similar to other systems, their expression is regulated by various cytokines and growth factors (13,16-22,62-78). Human peritoneal capillaries and arteriole endothelial cells express VEGF and other angiogenic factors (79) that may regulate the proteolytic enzymes and their inhibitors (Figures 2 & 3).

Since VEGF plays a key role in coagulation, fibrinolytic, and angiogenic activities, it is considered to be a critical cytokine in the development of peritoneal adhesions. Four species of mRNA encoding VEGFs arising from alternative splicing have been identified (VEGF 188, 165, 145 and 120) of which VEGF188, 165 and 120 are expressed in peritoneal wounds. Up-regulated expression of VEGF188 and 120 occurs during the early stages of peritoneal healing, and down-regulation of VEGF165 expression has been observed 24 to 48 hrs following injury (80). Hypoxia, a condition that promotes peritoneal adhesion, alters the expression of several cytokines, chemokines and eicosanoids in the wound, including the expression of VEGF and TGF-β, shown to cause tissue fibrosis (81). The peritoneal mesothelial and vascular endothelial cells of blood vessels, which supply peritoneal adhesions, express both VEGF and FGF-2, supporting a role for these cytokines in mediating peritoneal angiogenesis during adhesion formation (79). Although the expression of FGFs has been documented in a wide variety of cells and tissues, including monocytes/macrophages, T lymphocytes, vascular endothelial cells, fibroblasts, keritonocyte, and mesothelial cells (71-73,79,80), a profile of their expression and biological activities during peritoneal wound healing and adhesion formation awaits investigation. FGF-1 and FGF-2 lack the classical secretory signal peptides, and their detection in the ECM or peritoneal fluid is of unknown cellular origin. However, they are released as a result of mechanically-induced injury (73,74). Peritoneal injury could serve as a mechanism to release FGF from mesothelial cells and/or infiltrating inflammatory cells, which along with VEGF and EGF/TGF-α act as angiogenic factors as has been shown in omental microvascular endothelial cells. Delayed wound angiogenesis and healing in aged animals have been associated with alteration of FGF-2 and VEGF expression. Subcutaneous administration of rFGF induced less capillary growth into matrigel in aged mice than in young mice (82). These findings further suggest that a decline in angiogenic

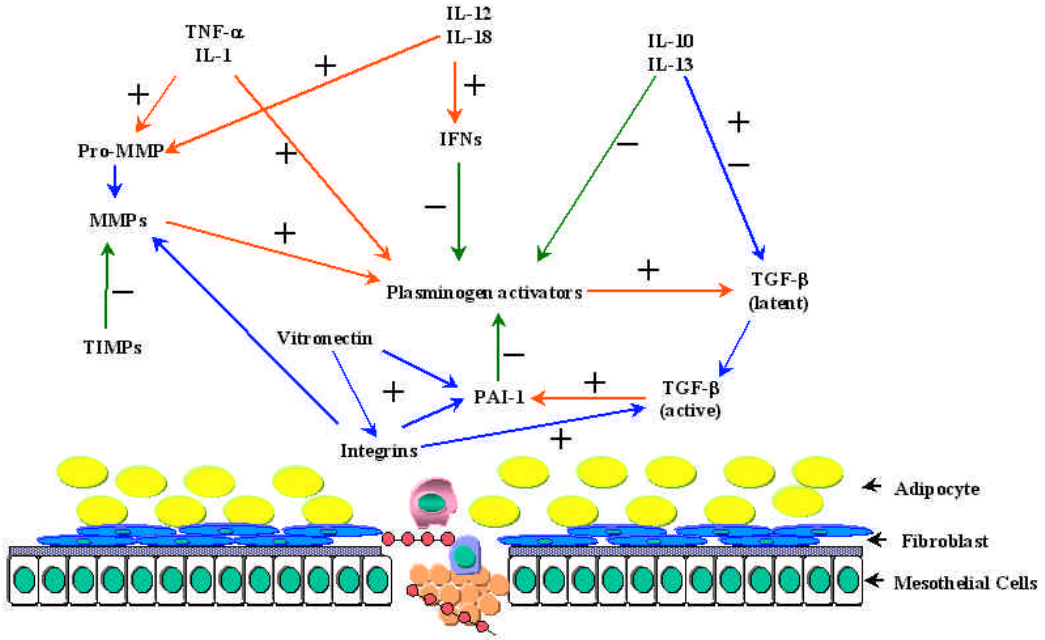

Figure 3. Schematic representation of and involvement of a number of cytokines, growth factors, proteases and adhesion molecules that regulate fibrinolysis resulting in either peritoneal wound healing and/or adhesion formation.

growth factor production and alteration in endothelial responsiveness may account for delayed wound angiogenesis and healing (82).

The angiogenic property of these growth factors is reflected in their ability to alter the expression of PA (uPA and tPA) and PAI-1 in microvascular endothelial cells and stimulate their invasion (16,17,66,71,73). For instance, VEGF stimulates von Willebrand and tissue factors in endothelial cells, and VEGF, FGF, EGF, and TGF-α either alone or through synergistic interactions, stimulate PA expression that converts TGF-β from its latent to active form (16,17,22,63,71,73). The active form of TGF-β inhibits PA expression, providing a feedback loop that modulates FGF, VEGF, TGF-α and PA expression. FGF-2 and TGF-β also have an opposing effect on PA activity; FGF acts as a potent inducer of uPA expression with a relatively modest effect on PAI-I synthesis, whereas TGF-β downregulates uPA and upregulates PAI-I synthesis (22,63). Kertitonocyte growth factor (KGF or FGF-7), a highly active mitogen for epithelial cells, also stimulates uPA expression (63,71,73,74). Several cytokines and chemokines also regulate the expression of fibrinolytic system, including several interleukins, M-CSF, GM-CSF and MCP-1 (11,12,65,66).

Angiogenesis is also dependent on the balance between angiogenic factors and their inhibitors. Among the angiogenic suppressors are cytokines, such as TGF-β, TNF-α, IFNs, and several other agents (71,83-85). These include collagen synthesis modifiers, protamine (an arginine rich protein that inhibits the mitogenic action of FGF), cyclosporin, PF-4, and HA (although its degradative products may be angiogenic), thrombospondin, which is released by platelets and is present around mature quiescent vessels, but absent from actively growing sprouts, and angiostatin, a tumor suppressor factor with angiogenic inhibiting property and considerable homology with plasminogen and hepatocyte growth factor (HGF), another angiogenic factor (83). In addition, an endogenous estrogen metabolite has been shown to inhibit angiogenesis in vivo and prevents endothelial cell proliferation and migration in vitro through a mechanism involving the induction of uPA (83,85). Vascular endothelial cells, including those in the peritoneum, contain receptors for ovarian steroids, suggesting that steroids can potentially regulate vascular activities that result in peritoneal healing and adhesion formation (79). Although many aspects of neovascularization in the peritoneum await investigation, the expression of these factors in peritoneal tissue and their presence in the peritoneal fluid imply that both direct and indirect action of these molecules can alter angiogenesis during peritoneal healing and adhesion formation. A number of anti-angiogenic factors are currently under intense investigation and results from these studies have revealed that factors which initiate, control and terminate the multi-stage process of angiogenesis may be useful in interfering with angiogenesis. Among these are compounds that inhibit angiogenic growth factors (IFN-α, suramin, and analogues) or their receptors (SU6668, SU5416), as well as endogenous inhibitors of angiogenesis (endostatin, IL-12), which are currently being tested for the treatment of a variety of disorders in clinical trials (86). Preoperative treatment with TNP-470, a potent endothelial cell inhibitor, has been reported to reduce vessel ingrowth, and associated sustained reduction in adhesion formation (87). In addition,

protease inhibitors, inhibitors of the endothelial cell proliferation, suppressors of angiogenic growth factors, and cytokines are also being investigated for their potential in controlling angiogenesis. These compounds may be useful as potential agents in the management of peritoneal wound healing by limiting tissue fibrosis such as adhesions.

5. PERITONEAL TISSUE REPAIR

The repair of denuded parietal peritoneum begins immediately after the injury. Various growth factors, cytokines, and chemokines, along with their specific receptors or binding proteins, appear to have the potential property of orchestrating such rapid and dramatic tissue reorganization. The expression of many of these cytokines and growth factors, and a limited number of chemokines, have been detected in parietal peritoneum and adhesions. Although direct evidence to link any of these molecules to normal physiological and/or pathophysiological processes which affects the outcome of wound repair and scar tissue formation is lacking, their local expression during the wound healing suggests their importance in various peritoneal biological activities. Although available information regarding the involvement of cytokines and chemokines in cellular activities of peritoneal mesothelial cells and adhesion fibroblasts is limited, the receptor signaling pathways that mediate their intracellular biological activities remain largely unknown.

5.1. Role of growth factors

EGF was the first growth factor to be identified during peritoneal wound healing and adhesion formation, followed by characterization of other members of the EGF family including TGF-α and HB-EGF and their common receptor, the EGF receptor (88-90). Studies of the expression and cellular distribution of EGF, TGF-α and HB-EGF, and their receptors, in parietal peritoneum and adhesions revealed a widespread association with various wound cells. These data suggest that the EGF family of growth factors, along with their receptors, might potentially affect a wide range of activities during peritoneal healing. Consistent with this possibility, EGF was found to act as a mitogenic factor for mesothelial and adhesion fibroblasts, which are synergistically enhanced by VEGF, IGF and PDGF (91). Although VEGF is expressed by mesothelial cells and during surgically-induced adhesion formation (79,80), its impact on the outcome of peritoneal adhesion formation is not known. However, anti-VEGF neutralizing antibody lowered VEGF expression in wound fluid and reduced vascularization without affecting granulation tissue formation (81,92), further implicating VEGF in angiogenesis. IGFs and their binding proteins (IGFBPs), which regulate IGF-I availability, are present in the circulation and in tissue fluids in association with IGFBPs through high affinity binding (93). IGFs, IGF-BPs, and the IGF receptor are expressed throughout the period of peritoneal wound healing, with significant increases in their expression during adhesion formation (94). Interference with IGF-I activity, both systemically and intraperitoneally through hypophysectomy (resulting in low IGF-1 levels) or IP administration of IGFBP-4, have been shown to reduce the severity of postoperative peritoneal adhesions (95). IGFBP-3 binds the native fibrin clot, fibrinogen, and fibrin through its interaction with the heparin binding domain and in the presence of plasminogen. Such interactions may concentrate IGF-1 at wound sites, and upon its release binds to IGF receptors that are present on stromal cells migrating into the fibrin clot (96). Fibrin clot associated IGF and other growth factors can become accessible for local biological activities through the proteolytic action of various proteases that are present in the early phase of tissue injury. Neutrophil proteases, cathepsin G, and elastase, in addition to their functions as ECM-degrading enzymes, can potentially regulate IGFs and IGFBPs during inflammation and wound healing (97). IGF-I has a growth promoting activity for many cell types; however, similar to EGF, IGF-1 is not a particularly strong mitogen for adhesion fibroblasts or mesothelial cells, and requires interaction with other growth factors such as EGF and PDGF (91). The requirement for growth factor interactions is related to the ability of an individual growth factor to act either as a competence (PDGF) or progression (EGF and IGF) factor. PDGF, TGF-α, HB-EGF and IGF-1, which are expressed and released by platelets and macrophages, can potentially act in this manner and influence cell migration and proliferation at the earliest stages of peritoneal wound healing (23,24).

Peritoneal mesothelial cell-derived HB-EGF, through its receptors HER-1 and HER-4, and associated proteins, integrin α3β1, regulate mesothelial cell migration by increasing β1 integrin expression and adhesion to collagen type I (90). Platelet-derived extract, which contains various wound-activating factors, has been reported to increase αvβ3 integrin expression, and to promote angiogenesis, revascularization and granulation tissue formation (98). Mesothelial cells and the serosal tissue of parietal peritoneum and several intraperitoneal organ express several integrins, including αvβ3. Following tissue or cellular injury, these integrins could participate in promoting angiogenesis, mesothelial cell migration, re-epithelialization and/or adhesion formation by altering fibroblast migration (99-101). The pattern of integrin subunit expression has been reported to be identical on mesothelial cells in the anterior peritoneum and uterine serosal, suggesting a bi-directional communication between peritoneal exudate cells and connective tissue fibroblasts (101). This pattern of integrin expression on mesothelial cells could be important since integrin α4-deficient T lymphocytes cannot migrate properly during thioglycolate–induced peritoneal inflammatory response (102). Furthermore, intergins could activate latent TGF-β, a key factor involved in all phases of the tissue repair processes including granulation tissue formation and tissue fibrosis (21,22,103,104). TGF-β is secreted as latent protein complex and stored at the cell surface in association with ECM proteins that regulate its availability and activity. TGF-β must become activated before binding to the TGF-β receptor, but the mechanism(s) underlying the conversion of latent to active TGF-β in vivo remain unknown. However, under in vitro conditions transient acidification and heating, as well as treatments with plasmin, thrombospondin-1, and mannose 6-phosphate have also

been reported to activate latent TGF-β (21,22,103-106). Activated TGF-β regulates integrin, ECM, and proteases such as the fibrinolytic system, MMPs and their inhibitors (9,21,22,103-107). TGF-βs have multiple biological activities depending on the cell type and the specific microenvironment. TGF-β has both stimulatory and inhibitory effects on cell growth and proliferation, and their mitogenic activity has been reported to be indirect and due to the induction of growth factors such as PDGF and PDGF α receptor (21,23). TGF-βs also enhance the expression of the EGF receptor, and are synergised with EGF-induced gene expression including the induction of TGF-β1, but not TGF-β2 expression, which in turn decreases the number of high affinity EGF receptors. Moreover, TGF-β upregulates its own expression in a variety of cell types, including adhesion fibroblasts and mesothelial cells (21,108,109).

Overexpression of TGF-β1 has been implicated in a number of disorders involving fibrotic abnormalities including pulmonary fibrosis, glomerulonephritis, cirrhosis of the liver, and dermal scarring (22,104,110-112). Deletion of the TGF-β1 gene through homologous recombination suggests that the release of TGF-β1 from degranulating platelets or its secretion by infiltrating macrophages and fibroblasts are not critical either to initiation or the progression of tissue repair, and further suggests that endogenous TGF-β1 may actually increase inflammation and retard wound healing (111). In contrast, TGF-β1-/- Scid-/- mice demonstrated a major delay in wound healing that involved wound inflammation, cell proliferation, and maturation. Immuno-deficient Scid-/- mice that express TGF-β1 do not experience a delay in wound healing, suggesting that the immune cells and TGF-β1 affect compensatory pathways in wound healing, and that the delayed expression of TGF-β2 and TGF-β3 that occurs in the absence of TGF-β1 is responsible for the delayed wound healing. In addition to TGF-β1, these observations further imply a key role for TGF-β2 and/or β3 in wound healing (112). In particular, TGF-β3 significantly increased wound cellular proliferation, breaking strength, and collagen deposition, indicating the importance of TGF-β3 in wound healing (113).

Evidence that implicates TGF-β in peritoneal adhesion formation results from experiments showing the expression of TGF-β in parietal peritoneum, and the serosal surface of several peritoneal organs. Adhesions with mesothelial cells and adhesion fibroblasts are major sites of TGF-β expression, and elevated levels of TGF-β have been observed in adhesion tissues and in the peritoneal fluid of patients with adhesions (114-116), as well as in surgically induced-adhesion formation in animal models (117-123). Mice heterozygous for TGF-β1 (+/-) experienced significantly lower adhesions and expressed at least two fold lower TGF-β1 protein in their peritoneal fluid compared with wild type (+/+) animals (122). Furthermore, postoperative peritoneal administration of TGF-β has been shown to increase adhesion formation, while neutralizing antibodies directed against TGF-βs reduced the incidence of adhesion formation (121,122). TGF-β1 and TGF-β3 are expressed in serosal tissue of parietal peritoneum, the uterus, oviducts, ovaries, omenta, the large and small bowels, as well as the adhesions, fascia and subcutaneous tissue in subjects with and without adhesions. Comparatively, there was more variation in TGF-β than TGF-β3 expression in the absence of a relationship to age or gender. Furthermore, adhesions express a significantly higher TGF-β1 and had the highest TGF-β1:TGF-β3 ratio, with lowest levels and ratio detected in omentum, small and large bowels, in contrast the uterus expresses higher TGF-β3, with lowest levels detected in subcutaneous tissue and large bowels. In subjects with adhesions, the adhesions express significantly more TGF-β1 compared to intact parietal peritoneum. Since TGF-β is expressed differently in these tissues and tissue injury often alters the expression of TGF-β, we proposed that tissues with a higher basal expression of TGF-β are more predisposed to develop more adhesions compared to others (114).

5.2. The role of Cytokines

The interleukins are another key group of regulators of peritoneal wound healing and adhesion formation. IL-1 is considered a key proinflammtory cytokine and both IL-1α and IL-1β are expressed during the early stage of wound healing. However, these cytokines are also expressed by mesothelial cells and fibroblasts, and are detectable in peritoneal fluid, suggesting their influence on other phases of wound healing including tissue fibrosis (9,10,12,26,108,115,120,124). The peritoneal expression of IL-1 increases following surgical-induced injury, peaking during the first week and its inhibition was shown to reduce the incidence of postsurgical adhesion formation. In contrast to IL-1α or IL-β, M-CSF, G-CSF and IL-6 failed to trigger the proliferation of mesothelial cells, although they are expressed by these cells (124). GM-CSF also induced its own expression and the expression of TGF-β1 in peritoneal fibroblasts. TGF-β is a potent chemoattractant factor for macrophages and fibroblasts, whereas GM-CSF promotes macrophage uptake of apoptotic neutrophils (118). The interactions between TGF-β and GM-CSF in the peritoneal environment may act as an important regulator to maintain a balance between the inflammatory and immunosuppressive activities of GM-CSF and TGF-β, respectively. Furthermore, GM-CSF markedly increased the expression of TGF-α by macrophages, while IL-1β, IFN-γ, IL-6 and TNF-α induced the expression of PDGF (125). Analysis of IL-1α, IL-1β, TNF-α, PDGF, TGF-β1, and bFGF expression in scar biopsies led to the hypothesis that impaired production of cytokines such as IL-1α decreases, whereas augmented PDGF production increases matrix formation in hypertrophic scars (126).

Another cytokine, IL-2, is synthesized by activated T helper cells and is a mitogen for T, NK, and B cells. In addition, IL-2 stimulates IFN-γ, IL-3, IL-4, IL-5, and GM-CSF production. Mesothelial cells have not been shown to express IL-2, IL-3, IL-4, or IL-5 (124); however, a human mesothelial cell line expresses these cytokines, and they are detected in human peritoneal fluid (11,12,127) that locally can act to modulate the expression of GM-CSF and other cytokines. In addition, IL-2 has been shown to

increase the survival of mice with peritonitis (128). IL-4, a key Th2 cytokine, produced by T cells, mast cells, and basophiles, suppresses the development of Th1 cells. IL-4 has been found to stimulate connective tissue fibroblasts, accumulation of ECM, production of IL-6 and inhibit the expression of IL-1, IL-8, and TNF-α, it is considered a key wound healing cytokine by influencing the pathogenesis of fibrotic disorders and inflammatory response (129-131). Topical application of IL-4 in experimental wounds appears to enhance, whereas, IL-4 antisense oligonucleotides inhibit the rate of wound healing (132). IL-5 is major product of NK cells that regulates eosinophil differentiation, and increases B cell proliferation and T cell cytotoxicity; and combined production of IL-4 and IL-5 results in mast cell and eosinophil stimulation (129). IL-6 is produced by a variety of cells including mesothelial cells and fibroblasts, and induces IL-2 and IL-2 receptor expression, while it inhibits TNF-α production, providing a negative feedback for limiting the acute inflammatory response. The upregulation of IL-6 production associated with a variety of chronic inflammatory and autoimmune disorders, including peritonitis (11,26,124-129), implicate another member of the interleukins family in mediating peritoneal mesothelial cells activities. IL-6 is positively regulated by IL-1, TNF-α VEGF and PDGF, whereas it is inhibited by IL-4 and IL-10 (133-136). Because IL-6 has a stimulatory action of endothelial cell proliferation and VEGF expression, it may alter the outcome of angiogenesis that results in adhesion formation (135). IL-6-deficiency has been reported to result in a significant delay in wound healing, characterized by minimal epithelial bridge formation, decreased inflammation, and granulation tissue formation, which was reversed by a single dose of recombinant IL-6 or IP injection of an expression plasmid containing the full-length IL-6 cDNA (136). Treatment with rIL-6 also reconstituted wound healing in dexamethasone-treated immunosuppressed mice (136). Elevated expression of IL-6 in peritonitis and following surgically-induced peritoneal injury further implicates IL-6 in peritoneal adhesion formation (11,12).

IL-8 which belongs to the chemokines family, is the main chemotactic factor for neutrophils. It upregulates the expression of cell-surface adhesion molecules, such as endothelial leukocyte adhesion molecule and intracellular adhesion molecule, that enhance neutrophil adherence to endothelial cells and facilitates their migration through vessel walls. IL-8 is expressed in parietal peritoneum and adhesions, and its expression in mesothelial cells is regulated by IL-1 and TNF-α (11,12,137). Increased production of IL-8 in nonhealing wounds has been shown to prevent keratinocyte replication without effecting fibroblasts. However, IL-8 inhibits the rate fibroblast migration into the collagen lattice, a process reversed by co-treatment with indomethacin through the inhibition of prostaglandin production (138). Although elevated levels of IL-8 may delay wound healing by retarding wound closure (138), topical application of IL-8 on human skin grafts in a chimeric mouse model has been reported to enhance re-epithelialization, though it induced a significant reduction in wound contraction (139). These results further indicate that IL-8 can sequentially influence all phases of wound healing, including tissue remodeling associated with adhesion formation.

IL-10 is produced by Th2 cells and inhibits Th1 function by preventing the production ofTh1 cytokine, such as IL-4 and IFN-γ Thus, it is considered a T cell cross-regulatory factor and a key anti-inflammatory cytokine (26,140,141). IL-10 is expressed by a variety of cell types, including CD4+ and CD8+ T cells, and activated B cells, mesothelial cells and fibroblasts, and it is detectable in wounds throughout the healing period (142). IL-10 expression peaks immediately after injury and returns to normal levels within 24h, but is increased again at a later time point. IL-10 has been shown to inhibit overexpression of MCP-1, MIP-1α, IL-1β, IL-6, and TNF-α following tissue injury, and neutralizing antibody to IL-10 inhibited the infiltration of neutrophils and macrophages at the site of injury (142). IL-10 is also expressed during peritoneal wound healing. Although postoperative application of IL-10 did not alter the outcome of peritoneal adhesion formation, it was found to be effective at reducing the incidence of adhesion formation (143). Interestingly, peritoneal fluid contains low levels of IL-10 during the postoperative period that do not correlate with adhesion scores, suggesting that the endogenous IL-10 may not play a role mediating adhesion-free peritoneal healing (143). In contrast, IL-10 has been reported to disseminate bacterial outgrowth during peritonitis, and it protected mice from lethality by attenuating a systemic inflammatory response through a mechanism involving inhibition of TNF-α release (144). Mice deficient in IL-10 that were subjected to ischemia and reperfusion injury experienced a higher rate of mortality and more severe tissue injury, characterized by epithelial hemorrhagic necrosis, upregulation of adhesion molecules, neutrophil infiltration, as well as the production of TNF-α and IL- 6 (152,145). It has been suggested that IL-10 exerts an anti-inflammatory action during reperfusion injury, possibly by regulating the early stress-related genetic response, adhesion molecule expression, neutrophil recruitment, and subsequent cytokine and oxidant generation (52,145,146).

IL-10, as well as IL-4, inhibits IL-12 production, an important cell-mediated inflammatory cytokine that plays a key role in wound healing. IL-12 is produced by activated macrophages, and among its key biological activities is the activation and proliferation of T cells, and NK cells, NK cell cytotoxicity, and IFN-γ production (147). Immunoneutralization of IL-12 in septic peritonitis not only resulted in increased mortality, but also promoted a shift away from IL-12 and IFN-γ in favor of IL-10 production (148). IL-13 like IL-10 is a key anti-inflammatory cytokine, and is closely related to IL-4; it is expressed primarily by T cells, mast cells, and activated basophils. The anti-inflammatory activity of IL-13 is due in part to its ability to inhibit inflammatory cytokines such as IL-1ß, TNF-α, IL-8, and IL-6 and induce IL-1 receptor antagonist and IL-1 type II receptor expression (149-150). IL-15 is another novel cytokine that shares many of its biological functions with IL-2 and is expressed in various cell types including mesothelial cells. IL-15 is expressed as two isoforms due to alternative splicing, with a different pattern of expression

and regulation than that reported for IL-2 (151-152). IL-15 is a key regulator of the local innate tissue inflammatory response and adaptive immunity, in particular that associated with NK cell proliferation, cytotoxic killing, and IFN-γ and TNF-α production (151-153). IL-13 and IL-15 are both expressed in peritoneal serosal tissue and adhesions, and are present in peritoneal fluid (154). A comparative analysis of peritoneal fluid content of IL-13 and IL-15 in women with endometriosis, which causes peritoneal inflammation and adhesion formation, in women who developed peritoneal adhesions unrelated to endometriosis, and in women with normal pelvic anatomy showed that these two cytokines may play a key role in mediating abnormalities associated with these disorders including adhesion formation (154). Interestingly, IL-13 and IL-15 differentially regulate TNF-α and TNF-α receptors expression, and TNF-α receptor type I content in the peritoneal fluid of women with peritoneal adhesions differs from those with endometriosis. The results of these studies, as well as reports demonstrating a diverse pattern of expression and distinct biological actions for IL-13 and IL-15 in other cells and tissues, suggest that these cytokines play a key role in both normal biologic functions and in pathologic conditions affecting the peritoneal cavity.

TNF-α also induced the expression of IL-18, another key inflammatory cytokine related to IL-1, while EGF and TGF-α inhibited its expression without influencing IL-18 protein release (155). There was also an increase in IL-18 expression during the cutaneous wound repair that was closely correlated to infiltration of neutrophils known to produce TNF-α (156). A genetically diabetic db/db mouse, with a prolonged wound inflammatory phase, had elevated expression of IL-18 during the late phase of repair and an absence of IFN-γ, despite the presence of subsets of leukocytic cells at the wound site that are known to produce IFN-γ in response to IL-18. Interestingly, TGF-β expression at the wound site may have a counterregulatory action on IL-18-induced IFN-γ expression, as TGF-β suppressed the production of IFN-γ by peripheral blood mononuclear cells following IL-18 induction (157). IL-10 and IFN-γ interaction also resulted in upregulation of TNF-α expression (158), a key proinflammatory cytokine with functions similar to IL-1 and IL-6. Patients with peritonitis had elevated levels of TNF-α, IL-1α, IL-6 and their soluble receptors, as well as IFN-α and IL-10 in the peritoneal fluids (11,12).

5.3. The role of Chemokines

Chemokines, such as MCP-1, IL-8, GRO*a*, IP-10, and Mig, are sequentially and differentially expressed during the phase-specific infiltration of leukocyte subsets in human wound healing (159,160). The timing of MCP-1 and MIP-1 expression in the wound suggests that MCP-1 is critical during the early phase, while MIP-1 may regulate the events occurring during the later phase of the repair process (161). In addition, the induction of MCP-1 in keratinocytes following wounding is attenuated by IL-1α- and TNF-α and augmented by IL-8, a functional human homolog to murine MIP-2 (162). Interestingly, wound re-epithelialization and collagen synthesis in MIP-1α deficient mice were nearly identical to that occurred in wild-type, whereas MCP-1 deficiency resulted in a significant delay in these parameters without affecting the number of wound macrophages (163), suggesting that monocyte recruitment into wounds is independent of MCP-1. IP-10 is expressed in human dermal wounds, with highly elevated levels in a number of chronic inflammatory conditions, including psoriasis. Transgenic mice that constitutively express IP-10 in keratinocytes developed normally and did not spontaneously recruit leukocytes into the skin or other organs that expressed the transgene. However, these mice had an abnormal wound healing response characterized by a more intense inflammatory phase and a prolonged and disorganized granulation phase associated with impaired blood vessel formation (164). IP-10 also inhibits EGF- and HB-EGF-induced dermal fibroblast motility without affecting either the basal or EGF receptor-mediated mitogenesis (165). These results suggest that IP-10, by inhibiting neovascularization, may have a novel biologic activity as an inhibitor of wound healing (164). The expression of MGSA/GRO and their receptor, the type B IL-8 receptor (IL-8RB), is restricted to sites populated by differentiated keratinocytes in normal skin. However, wounds that resulted in hypertrophic scaring expressed IL-8RB only in specific locations within epidermal and dermal compartments of normal and hypertrophic epidermal wounds and in granulation tissues (166). In addition, fibroblasts prepared from keloids or normal skin fibroblasts do not express MGSA/GRO; however, its expression induced following treatment with IL-1 and inhibited by glucocorticoid (167). Although these chemokines are synthesized and released by activated peritoneal fluid resident cells i.e. macrophages, neutrophils, mast cells and T cells, little is known about their expression during the peritoneal wound repair process. Intact peritoneum and adhesions express MCP-1, RANTES and IP-10 (168). During peritoneal wound healing, MCP-1 expression is detected within 48 hrs of postinjury that remained elevated until the fourth postoperative day and daily IP administration of anti-MCP-1 antibody for 6 days significantly reduced the incidence of adhesion formation (169). In addition, the peritoneal fluid content of MCP-1 was elevated in women with adhesions; however, there was no incremental effect of MCP-1 level on adhesion formation in patients with endometriosis (170). However, peritoneal serosal tissues and adhesions as well as mesothelial cells and adhesion fibroblasts, also express MCP-1, IP-10 and RANTES, and may turn serve as potential sources of these chemokines in peritoneal fluid. Elevated expression of MCP-1, IP-10 and RANTES in surgically-induced adhesions in rats and mice was directly proportional to the incidence of adhesions (168,169).

6. TISSUE REMODELING IN ADHESION DEVELOPMENT

Extracellular matrix (ECM) deposition and tissue remodeling are critical to all phases of normal wound healing most notably cell migration, growth and differentiation, angiogenesis and tissue fibrosis (9,67,69,108,171). During wound healing, wound cells are in dynamic contact with ECM, and through the interactions and communication mediated by integrins, promote

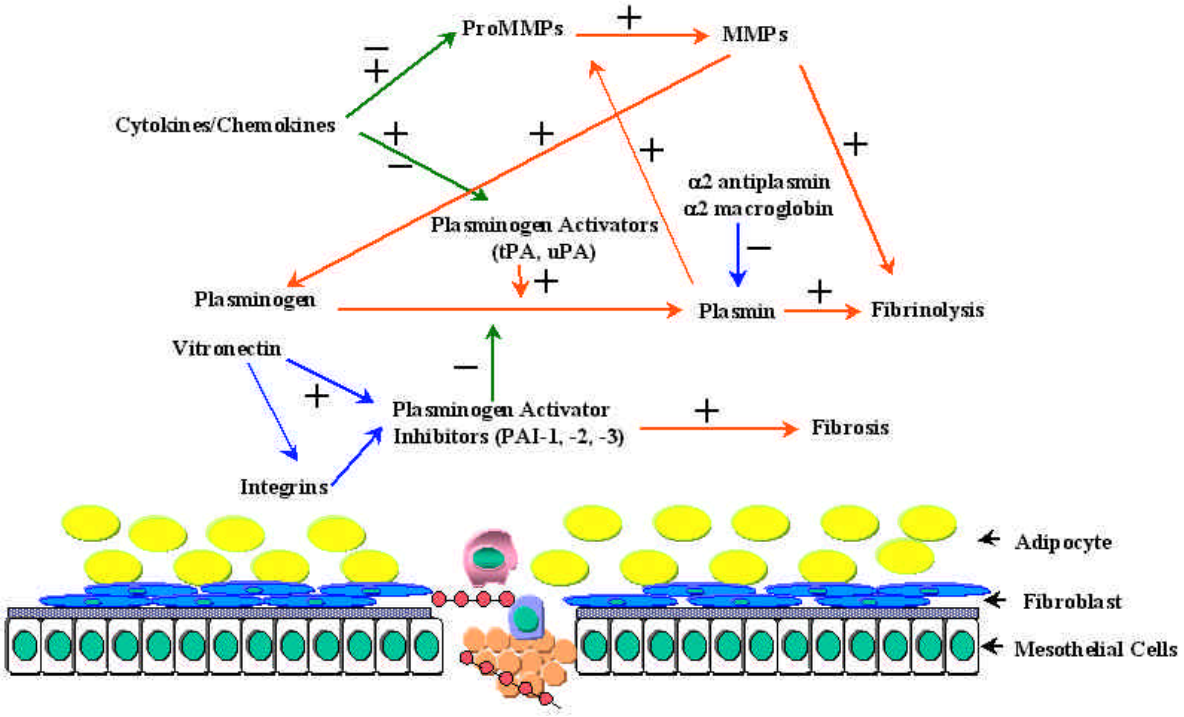

Figure 4. Schematic representation of fibrinolytic system and metrix metalloproteinases interactions, and their regulation by cytokines, chemokines and adhesion molecules that result in peritoneal wound healing and adhesion formation.

intracellular signals that regulate the above process (21,108,172). In addition, wound cells synthesize and release several proteolytic enzymes that hydrolyze various components of the ECM, such as collagens, fibronectin, vitronectin, laminin, elastin, and proteoglycans, allowing wound healing to proceed (108,171,172). Immediately after tissue injury, the exposure of fibrillar collagen to blood promotes aggregation and activation of platelets and the release of chemotactic factors, cytokines, chemokines, growth factors, proteases, etc., that regulate the ECM. Fibronectin, a major ECM component, is deposited in the wound as a part of preliminary matrix acting as a scaffold for cell migration and collagen deposition, and later regulation of re-epithlialization and wound contraction (9,108,171-176). Proteolytic degradation of collagens and fibronectin results in formation of smaller fragments that attract inflammatory cells, and later fibroblasts, into the injured area. Fibronectin fragments, but not intact fibronectin, also induce the expression of MMPs and PA, and increase proteoglycan content in a concentration-dependent manner (177,178). These fragments also enhance the release of several growth factors and cytokines that bind either to their specific cell surfaces or to the ECM following their release from producer cells (177,178). Many of these cytokines regulate proteases and ECM expression. Neutralization of the action of these cytokines reduced fibronectin fragment-mediated MMP-3 release and suppressed proteoglycan synthesis (178). As illustrated the interactions among components of the ECM, proteases and cytokines are regulated through a feedback mechanisms that are critical to the inflammatory response, angiogenesis and tissue repair occurring during peritonitis and peritoneal wound healing processes (Figure 4).

Although a very limited information is available regarding the expression and role of ECM in peritoneal environment during wound healing and adhesion development, collagen type I, type III and fibronectin are localized in the peritoneal wall and their expression is detected in peritoneal mesothelial cells and adhesion fibroblasts (101,109,179). TGF-β in a cell specific manner increases the expression of fibronectin and procollagen I in human adhesion fibroblasts, whereas it induces collagen type III, with a limited effect on collagen I in mesothelial cells (179,180). Because overproduction of TGF-β is associated with increased incidence of adhesion formation, modulation of the expression of ECM in adhesion fibroblasts may partly account for TGF-β induced adhesions (179,180). Peritoneal mesothelial cells and the serosal surface of several peritoneal organs and parietal peritoneum also express integrins, such as αv and β3 (99,101). These integrins specifically bind fibronectin and vitronectin expressed by peritoneal serosal tissue and mesothelial cells, and are regulated by TGF-β (79,107). Vitronectin deficiency has been reported to cause only a slight delay in dermal wound healing and was associated with a temporal increase in uPA and tPA activity in microvessels and decreased angiogenesis in response to tissue injury (180). The interaction of vitronectin with PAI-1, integrins and MMPs, factors having individual and interactive biological activities critical to the outcome of wound healing, further imply the

importance of vitronectin in peritoneal wound healing and adhesion formation.

ECM components are also important in regulating the expression of growth factors and cytokines in an interactive manner (175,176). In vitro experiments have shown that the expression of TNF-α and PDGF are dependent on the existence of ECM proteins (176,181). In contrast, the expression of TGF-β is constitutive, and IL-1, which is stimulated by bacterial endotoxin, is ECM-independent (182). The importance of ECM becomes further apparent when considering the association between numerous cytokine and growth factor soluble receptors with the ECM compartment (183-185). In some cases these specific ligand-receptor interactions involve either a single subunit or a complex of two or more subunits that are commonly shared among certain cytokines to initiate a cascade of intracellular signal transduction. However, many of these receptor molecules, often representing the ligand binding domain of the receptor complex, are detectable in naturally-occurring soluble forms in the serum, plasma, urine and in various cell culture-conditioned media. These include soluble receptors for interleukins, G-CSF, M-CSF, GM-CSF, TNF-α, EGF, TGF-α, TGF-β and PDGF, as well as IGFBPs. For instance, the protoglycan, decorin, as well as other ECM components such as biglycan and fibromodulin, associate and result in inactivation of TGF-β (22,103-105). TGF-β can downregulate the expression of decorin, while upregulating biglycan and inhibiting fibrilogenesis. Although the in vivo functions of these soluble receptors are unknown, potentially they can restrict the biological activities of their receptive ligands (antagonists), act as scavengers, control the physiological levels of these cytokines, or function as transport proteins to be presented to cell surface receptors.

Tissue remodeling and the induction of fibrosis occur not only by promoting chemotactic recruitment of fibroblasts and increasing in the deposition of ECM proteins, but also through the differential regulation of proteolytic enzymes that degrade the ECM (9,11,67,108). The earliest event in the wound repair process involves deposition of fibrin-rich exudates, referred to as provisional matrix (9,108). This matrix contains a variety of substances that are key elements in the initiation of wound healing; however, the resolution of this matrix is also necessary for the continuation of the healing process (9,108). The components of the fibrinolytic system and MMPs interact in a variety of ways to regulate many of the processes that are required for ECM proteolysis (16,17,108).

6.1. The role of Fibrinolytic System

Plasmin, a broad-range serine protease, cleaves a number of ECM components and degrades fibrin and its proenzymes, tPA and u-PA, are inhibited by PAIs, in particular, PAI-1 (16,17). Different cell types, including wound cells, express components of the fibrinolytic system (9,11,12,16,17). In the peritoneal cavity, peritoneal macrophages produce tPA, PAI-1, and their receptors, but the major contributor to fibrinolytic activity in the abdomen appears to be the mesothelial cell (11,12,186). Mesothelial cells mainly express the activators of fibrinolysis, whereas its inhibitors are widely distributed in a variety of tissues (11,12,187). This suggests that an intact mesothelium seems to be crucial in maintaining the balance of fibrin deposition and degradation. Gene targeting has provided important evidence supporting the role of fibrinolytic components in various physiological and pathological events including wound healing. Studies of individual and combined deficiencies of uPAR and tPA have shown that a deficiency in uPAR/tPA does not induce as profound an impairment in wound repair as that seen with uPA/tPA deficiency following full-thickness skin injury (188). These studies suggest that uPA alone is sufficient to clear fibrin deposits and support wound healing without the benefit of either uPAR or tPA. However, plasminogen deficiency causes severe thrombosis with delayed wound healing, in part due to impaired keratinocyte migration. Deficiency in both plasminogen and fibrinogen revealed that removal of fibrinogen from the extracellular environment alleviates the impairment associated with plasminogen deficiency and corrects healing, implying a fundamental physiological role for plasminogen in fibrinolysis (189,190). Furthermore, in vitro studies strongly support the importance of plasmin in angiogenesis; however, angiogenesis and fibrinolytic activity do not always appear to correlate with in vivo observations as suggested by the evidence of normal angiogenic activity during wound healing in the presence of plasminogen-deficiency (189,190). Deficiency in vitronectin was also associated with an increase in uPA and tPA activity (180). A further role of fibrinolysis in wound healing is suggested by the action of D dimer, a plasmin proteolytic fragment of fibrin, on the induction of PAI-1 expression, constituting a negative feedback mechanism in which specific fibrin fragments control the persistence fibrin at sites of inflammation and fibrosis (191). In addition, the annexin II heterotetramer (AIIt) that binds tPA, plasminogen, and plasmin stimulates the tPA-dependent conversion of plasminogen to plasmin and AIIt is the key physiological receptor for plasminogen on the extracellular surface of endothelial cells (192)(Figure 4).

Tissue trauma, ischemia and infection, events that increase the incidence of adhesion formation, are associated with reduced peritoneal fibrinolytic activity due to the rapid reduction in tPA activity and the increase in PAI-1 production that occurs shortly after post-operation (11,193). It seems possible that reduced tPA activity, in conjunction with over-expression of PAI-1 and quenching of t-PA, may explain the high frequency of adhesion reformation after adhesiolysis (1,11). A lower tPA to PAI-1 ratio was also observed immediately after surgery in patients who underwent hepatic versus colorectal resection. Extensive hypercoagulation and hypofibrinolytic activation during the late postoperative period also impair wound healing (9,11,194,195). Because impairment of peritoneal fibrinolysis following tissue injury leads to the development of adhesions, the effectiveness of intraperitoneal applications of recombinant tPA has been tested for the prevention of adhesions (194-198). Intraoperative IP administration of tPA has been shown to alter the strength, and extent of fibrous bands and wound strength with significantly lower plasma PAI-1 and PAI-2 production one to two weeks postsurgery. These findings

suggest that tPA can reduce the incidence of postoperative peritoneal adhesions without impairing wound healing. The effect of rtPA in overcoming the inhibition of fibrinolysis was dose related, as low levels showed no effect on adhesion formation, while higher concentrations reduced adhesions that paralleled wound hydroxyproline content (198). However, as indicated in many other studies, the levels of rtPA required to alter or prevent peritoneal adhesion formation not only impair the early phase of wound healing, but can also be detrimental (199). Intraperitoneal administration of anti-PAI-1 antibodies has also been reported to reduce the incidence of adhesion formation in surgically-induced peritoneal injury, possibly by limiting the availability of PAI-1 to inhibit tPA (200).

6.2. The role of Matrix Metalloproteinases

In addition to the fibrinolytic system, MMPs, a family of zinc-dependent endopeptidases, as a group can degrade essentially all components of the ECM that control tissue remodeling of newly formed granulation tissue. So far, 28 members of the MMP family have been identified. Based on their structure and substrate specificity, MMPs are divided into subgroups that include: collagenases (MMP-1, MMP-8, MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-7, MMP-10, MMP-11), matrilysin (MMP-9), and the membrane-type MMPs (MT-MMP) such as MT-MMP-1, etc (9,67,69,108,201). The catalytic activity of MMPs is controlled, at least in part, by their physiological inhibitors, TIMPs, which are comprised of TIMP-1 –2, -3 and -4 (67-70,202). MMPs typically are not constitutively expressed; however, they are induced in tissues that normally undergo extensive remodeling, such as wound tissue, and in response to various inflammatory conditions. In addition, MMPs are regulated by cytokines, growth factors, hormones, and cell-cell and cell-matrix interactions (67-70,201,202). In contrast, the expression of TIMPs is widespread in many tissues and is regulated in coordination with MMPs expression (67-70). TIMPs have growth factor-like activity in various cell types, alter angiogenesis and are associated with several other cellular activities (70,202). MMPs are produced as inactive proenzymes and require activation, achieved by various factors including serine proteases, such as plasmin, trypsin and neutrophil elastase (67-70,201,202). The temporal expression profiles for MMP-1, MMP-2, MMP-7, MMP-9, MT1-MMP, as well as TIMP-1, TIMP-2, and TIMP-3 observed during the inflammatory, granulation, and early tissue remodeling phases of excisional skin repair indicated that their induction and peak expression coincided with the well-characterized inflammatory and granulation phases (203). The expression of MMP-2, MMP-14, and TIMP-1 mRNAs was increased in inflamed areas in ulcerated colonic mucosa, with substantial increases in MMP-1 and MMP-3 expression that correlated with the histological degree of acute inflammation (204). Studies of the temporal activity of MMP-2, MMP-3, MMP-7, and MMP-9 during normal dermal repair also revealed that MMP-2 is a major gelatinolytic MMP (205) that participates in physiological turnover of ECM, whereas MMP-9 is important in the early phase of ulceration and in the healing process (206). Furthermore, the cellular distribution of MMPs suggests that these enzymes may contribute to anastomotic dehiscence, but only in the immediate postoperative period (207).

TIMP-1 is not expressed in the epidermis, but is co-localized with MMP-9, and MMP-3 during the hyperproliferative phase at the mesenchymal/epidermal border of granulation tissue. At the later phases of wound healing there is an increase in MMP-8 and MT1-MMP expression, and TIMPs are colocalized with MMPs in granulation tissue. At the completion of re-epithelialization, the expression of MMPs and TIMP-1 in epidermal and dermal compartments declined to near-basal levels, whereas the macrophage-specific metalloelastase (MMP-12) reached maximum expression. Systemic glucocorticoid treatment, which results in impaired wound healing, led to a nearly complete shut-off of MMP-12 expression (67). Infected and chronic wounds have been associated with persistent elevation of MMP-9, and its detection in postoperative wound fluids is considered to be an early indicator of impaired healing and relates to the amount of collagen deposition later in the wound healing process (208). Other studies have shown that MMP-2 is localized to the connective tissue fibroblasts and endothelial cells during all phases of wound healing. In contrast, mucosal epithelium is practically devoid of MMP-2, and only the basal cell layer expresses MMP-9 in the migrating epithelial cells, with strong expression in granulation tissue. Cytokines such as TGF-β1, IL-1β, bFGF, TNF-α, and IFN-γ differentially regulate the expression of MMPs and TIMPs in various cells types; IL1-β and TNF-α enhance MMP expression (67-70,201,202). Human dermal wound healing has also been shown to be associated with age-related increases in MMP-2 and MMP-9, MMP-2 was found in association with epidermal structures, while MMP-9 was observed in inflammatory cells (210). MMP-8 and MMP-13 were clearly reduced in the skin wound extracts of chemically modified tetracycline (CMT-8) treated rats compared to ovraictomized rats, suggesting that CMT-8 and estrogen have a beneficial effect on skin wound healing, possibly by increasing the collagen content and by reducing MMP-mediated collagenolysis (211).

We have provided evidence that peritoneum and adhesion tissues express MMPs and TIMPs with TIMP-1 levels significantly higher in fibrous adhesions than in the peritoneal serosal tissue (76,77). This increase in TIMP-1 expression in fibrous adhesions paralleled the expression of TGF-β1 and integrin (99,114). Under in vitro conditions, TGF-β1 inhibits the expression of MMPs and increases TIMPs, decreasing matrix degradation and increasing tissue fibrosis (22,67). Studies using isolated peritoneal mesothelial cells and adhesion fibroblasts indicated that TGF-β1 also increases the level of collagens, fibronectin and TIMP-1, while it reduces the expression of MMP-1 mRNA by these cells (75,107,109,179). Mesothelial cells also express MMP-1, MMP-3, TIMP-1 and TIMP-2 mRNA and protein at various levels, with the expression TIMPs being quantitatively the highest (75,107,109,179). Treatment with TGF-β1 resulted in a significant increase in the expression of TIMP-1, but not TIMP-2, mRNA in mesothelial cells, while down-regulating MMP-1 and MMP-3 (75). In contrast, TGF-β1

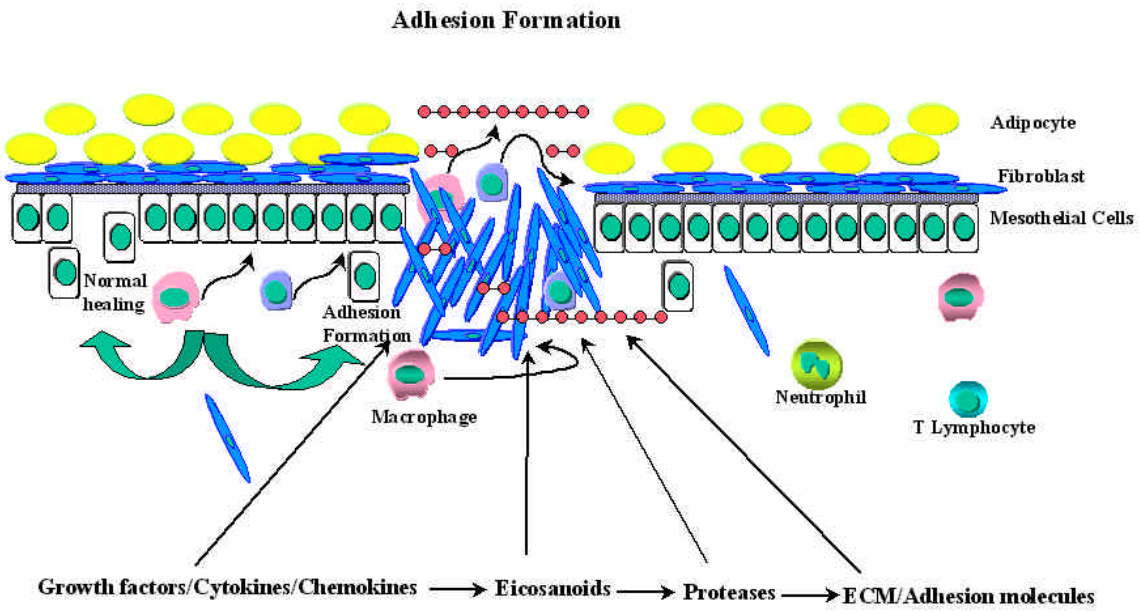

Figure 5. Schematic representation of events, and cytokines, growth factors, eicosanoids, proteases, adhesion molecules that regulate the excess migration and proliferation of fibroblasts that leads to peritoneal adhesion formation.

increased the release of MMP-1 and TIMP-1 by mesothelial cells, but majority of MMP-1 was complexed with TIMP-1 **(**75). These data provide further evidence that proteolytic enzymes, whose expression is partly regulated by TGF-β and other cytokines, may influence ECM turnover and the incidence of adhesion formation (Figures 4 & 5).

The blocking of MMP activity has been studied for its potential therapeutic efficacy in controlling pathologic processes. Synthetic MMP inhibitors, most notably the hydroxymates, have been engineered for this purpose and are presently undergoing clinical trials. These inhibitors may have broad or specific MMP inhibitory activity. However, non-matrix degrading capabilities of MMP have also been recognized, and include cytokine activation, processing of proteins to molecules of distinct biologic function. Thus it is less clear whether nonselective inhibition of MMP activity for all pathologic processes involving MMP is appropriate (67,212). Treatment of wounds with tetracycline analogues that inhibit MMP-2, but not MMP-9 production, is reported to inhibit migration and growth of mucosal and skin keratinocytes and keratinocyte growth. TGF-β treatment increased keratinocyte migration as well as the cell-associated and secreted MMP-2 production with partial conversion into an active form. Batimastat totally blocked TGFβ-induced keratinocyte migration (212). Another MMPs inhibitor, GM 6001 topically applied to the dermal wound did not influence the degree of dermal inflammatory cell infiltrate or epithelial proliferation, although it reduced re-epithelialization which was not due to interference with the inflammatory response or epithelial proliferation (212). In a comparative study of GM-6001 action in dermal and peritoneal wound healing and peritoneal adhesion formation, it has been found that MMP inhibitor has limited effect on the outcome of peritoneal healing and adhesion formation (213). In colonic anastomosis treatment with BB-1101, a synthetic broad-spectrum MMP inhibitor, increased anastomotic breaking strength without affecting collagen accumulation or infiltration of neutrophils in anastomotic area. The result indicates that alteration in MMPs expression during the critical early postoperative phase may increase the risk of anastomotic dehiscence (214).

As illustrated these proteases and their inhibitors are key regulators of many phases of wound healing. Since they are expressed differently in peritoneal wounds and adhesion tissues, and tissue injury is often associated with altered expression of proteases, we proposed that tissues with a higher basal expression of these molecules are more predisposed to develop more adhesions compared to others.

7. PROSPECTIVE

Whether induced by infection, inflammation, ischaemia, and/or surgical injury, peritoneal adhesions are the leading cause of pelvic pain, bowel obstruction and infertility, and cause a substantial burden during reoperative procedures as well as increasing medical cost. It is also clear that while postsurgical peritoneal wounds heal without adhesions in some patients, others develop severe scarring from seemingly equal procedures; in addition in the same patient, adhesions can develop at one surgical site and not in another. The mechanisms underlying the predisposition to form adhesions as well as their site specificity are completely unknown. However, a large number of intraperitoneal surgical procedures are performed each day in the USA, and thus many patients are at risk of developing postoperative adhesions. Therefore, the understanding of adhesion formation at the molecular level is essential and in the absence of such information,

attempts to prevent patients from developing adhesions will remain an empirical process.

The unprecedented advancement in molecular biology during the past decade has led to the identification of many biologically active molecules with the potential of regulating inflammatory and immune responses, angiogenesis and tissue remodeling, events that are central to normal wound healing as well as to tissue fibrosis associated with adhesion formation. The list of molecules that modify the wound healing process has also grown substantially, with increasing insight into their importance in the development of tissue fibrosis. However, their major roles in peritoneal biological functions and the adhesion formation remain speculative at best. Interestingly, there are common and overlapping biological functions among many growth factors, cytokines, chemokines and proteases that are evolved from the recruitment of multiple signaling molecules with similar downstream pathways. These molecules are also able to compensate for the function of a deleted gene product by using alternative pathways to trigger the full-scale activation of cellular response. Such functional pleiotropy and redundancy, a characteristic feature of many of these molecules, has been attributed to the molecular structure of their receptor and to the binding protein system. Elucidating how key regulatory molecules of the signaling pathways cooperate and interact, as well as factors involved in their downstream cascades, in peritoneal mesothelial cells and adhesion fibroblasts may allow the identification of molecules that are potentially vital in peritoneal repair, cellular invasion, and tissue fibrosis. This information could allow design and exploration of the use of specific modulators (inhibitor/stimulator) for their potential therapeutic applications in adhesion prevention and other disorders such as endometriosis and peritoneal cancers. For instance, several therapeutic interventions have been shown to be effective in modulating cellular behavior in disorders that could be potentially useful in modulating peritoneal wound healing and adhesion formation. These include synthetic inhibitors of cell invasion (marimastat, Neovastat, AG-3340), adhesion (Vitaxin), or proliferation (TNP-470, thalidomide, Combretastatin A-4). Compounds that interfere with angiogenic growth factors (IFN-α, suramin, and analogues) or their receptors (SU6668, SU5416), as well as endogenous inhibitors of angiogenesis (endostatin, IL-12), are currently being evaluated in clinical trials for the treatment of a variety of disorders (215). Modification of cellular function through gene targeting is also a promising therapeutic modality. The incorporation of genetically modified peritoneal mesothelial cells might be useful in preserving the normal physiological function of peritoneum during peritoneal dialysis, and through the production of proteins with therapeutic property could safeguard the peritoneal membrane against injury. Such local genetic modification could also be applied to alter the behavior of a variety of pathological conditions affecting the peritoneum, including carcinomas, such as ovarian cancers mesotheliomas, and endometriosis. For instance, IP administration of antisense oligonucleotides, plasmids, and viral vectors that are been evaluated for their efficacy in cancer biology have a potential application in adhesion prevention. IP administration of Semliki Forest virus (SFV) particles encoding recombinant murine GM-CSF to alter recruitment and activation of inflammatory cells and ovarian tumor growth, revealed a high level of incorporation in peritoneal mesothelial cells (216). Due to the exclusive uptake of viral particles by mesothelial cells, ex vivo gene transfer into mesothelial cells isolated from the peritoneal membrane, genetically modified in vitro and subsequently re-implanted back onto the peritoneal cavity of syngeneic recipients for in vivo gene therapy. This use of genetically modified peritoneal mesothelial cells may be of therapeutic value in maintaining the fibrinolytic balance in the peritoneal cavity, altering peritoneal inflammation, angiogenesis, cell migration, and the development of peritoneal adhesions or peritoneal physiology to prevent membrane damage and to maintain dialyzing performance (217,218). Another therapeutic approach has been the IP delivery of recombinant therapeutic proteins from a universal microencapsulated cell line as an alternate method for gene therapy. This approach has proved effective for the treatment of several murine models of human genetic diseases. However, in scaling up to large animal models, IP implantations of microcapsules have been associated with an excessive inflammatory response and rapid degradation, although implantation at a surgical site induced less inflammation, and permitted longer-term survival of microcapsules. However, adhesions consist mainly of migrating fibroblasts and it appears that cell type-specific gene delivery is essential for in vivo gene therapy. Interestingly, retroviral vector particles derived from the spleen necrosis virus, which display the antigen-binding site of an antibody on the viral surface have been used to infect cells expressing a receptor recognized by such as antibody, an approach that can be applied using mesothelial cells (219). Mesothelial cells are easy to obtain and propagate from individual patients, and following their genetic manipulation to express therapeutic proteins at useful levels such cells appear to provide a tool with clinical potential based on ex vivo gene therapy (220).

In recent years several biodegradable devices have been either marketed or are in clinical trials to prevent postoperative adhesion formation (Table 1). Although their addition to the field of adhesion preventative devices is welcomed, they appear to have either a limited or insignificant usefulness in the prevention of adhesion formation. In addition, the choice and utilization of these devices most often has been determined by their availability to the investigator, rather than on the basis of rigorous scientific investigation. Because these devices are of potential use for site-specific delivery of bioactive molecules, a clear understanding of their biocompatablity with the peritoneal environment as well the most effective forms (membrane, gel or liquid) to carry and appropriately release these molecules is required.

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Table 1. Current Products and Devices to Reduce Adhesion Formation

Product & Company	Composition	Indications
Interceed J&J/Eithicon)	Cellulose	Gynecological surgery
Preclude (W. L. Gore)	PTFE	Peritoneal reconstruction
Seprafilm (Genzyme)	Hyaluronic acid (Genzyme)	Gynecological & General surgery
Hydrogel (Biomatrix)	„ „ „ „ „ „ „ „	Preclinical
Lubricoat (Lifecore Biomedical)	„ „ „ „ „ „ „ „	IDE trials
Incert (Anika)	„ „ „ „ „ „ „ „	Preclinical (de nova adhesions)
Adcon P (Gliatech)	Polysaccharide	General surgery
Atrigel (Atrix)	Polylactic acid-based polymers	Preclinical (denova adhesion)
Repel (Life Medical)	„ „ „ „ „ „ „ „	IDE trail (Gyn/abd/pelvic surgeries)
Focal gel (Focal)	„ „ „ „ „ „ „ „	Gynecological surgery
Atrigel (Atrix)		Preclinical
Flogel (Alliance Pharmaceutical)	Pluronics	IDE trials (Gyn & General surgeries)
BioElastics (Bioelastics Res. Ltd)	Elastin peptides	Preclinical
Sepragel (Genzyme)	HA	IDE (Gyn & General surgery)
Sepracoat (Genzyme)	„ „ „ „ „ „ „	Preclinical
Intergel (Lifecore)	„ „ „ „ „ „ „	Preclinical

9. REFERENCES

1. M.P. Diamond. & L.B. Schwartz: "Prevention of Adhesion Development". In: Endoscopic Surgery for Gynecologists. Eds: Sutton C and Diamond M.P, 2nd Edition, W.B. Saunders, London, England, 398-406 (1994)
2. Ellis H., B.J. Moran, J.N. Thompson, M.C. Parker, M.S. Wilson , D. Menzies, A. McGuire, A.M. Lower, R. J. S. Hawthorn, F. O'Brien, S. Buchan, & A.M Crowe. Adhesion-related hospital readmissions after abdominal and pelvic surgery: a retrospective cohort study. *Lancet* 353, 1476-1480 (1999)
3. Lower A.M, R.J. Hawthorn, H. Ellis, F. O'Brien, S. Buchan, A.M. Crowe. The impact of adhesions on hospital readmissions over ten years after 8849 open gynecological operations: an assessment from the surgical and clinical adhesions research study. *Brit J Obstet Gynecol* 107, 855-862 (2000)
4. Diamond M.P., & M.L. Freeman. Clinical implications of postsurgical adhesions. *Hum Reprod Update*.7,567-576 (2001)
5. Franklin RR, M.P. Diamond, LR. Malinak, B. Larsson, R.P.S. Jansen, S. Rosenberg, & B.W. Webster. Reduction of ovarian adhesions by the use of interceed. *Obstet Gynecol* 86, 335-340 (1995)
6. Diamond M.P and the Seprafilm Adhesion Study Group: Reduction of adhesions after uterine myomectomy by Seprafilm (HAL-F) A blinded prospective, randomized, multicenter clinical study. *Fertil Steril* 66, 904-910 (1996)
7. Diamond M.P. and the Sepracoat Adhesion Study Group. Reduction of De Novo Postsurgical Adhesions by Intraoperative Precoating with Sepracoat (HAL-C) Solution, A Prospective, Randomized, Blinded, Placebo-Controlled Multicenter Study. *Fertil Steril* 69, 1067-1073 (1998)
8. Becker JM, M.T. Dayton, V.W. Fazio, D.E. Beck, S.J. Stryker, S.D. Wexner, B.G. Wolff, P.L. Roberts, L.E. Smith, S.A. Sweeney, & M. Moore. Prevention of postoperative abdominal adhesions by a sodium hyaluronate-based bioresorbable membrane: a prospective randomized, double-blind multicenter study. *J Am Coll Surg* 183, 297-306 (1996)
9. R.A.F Clark. Wound repair. Overview and general consideration. In: The molecular biology of wound repair. Ed: Clark R.A.F. 2nd ed. New York: Plenum Press, 3-50 (1996)
10. Raftery AT. Regeneration of peritoneum: a fibrinolytic study. *J Anat* 129,659-64 (1979)
11. Topley N, R.K. Mackenzie, & J.D. Williams. Macrophages and mesothelial cells in bacterial peritonitis. *Immunobiology* 95,63-573 (1996)
12. Holmdahl L, & L.M. Ivarsson. The role of cytokines, coagulation and fibrinolysis in peritoneal tissue repair. *Eur. J Surg*, 165, 1012-9 (1999)
13. Muller S.A. K.H. Treutner, L. Tietze, M Anurov, S. Titkova, M. Polivoda, A.P. Oettinger, & V. Schumpelick. Efficacy of adhesion prevention and impact on wound healing of intraperitoneal phospholipids. *J Surg Res* 96, 68-74 (2001)
14. Furie B, & B.C. Furie. The molecular basis of blood coagulation. *Cell* 53, 505-518 (1998)
15. Gaffney P.J.Fibrin degradation products. A review of structures found in vitro and in vivo. *Ann N Y Acad Sci*. 936, 594-610 (2001)
16. Lijnen H.R. Elements of the fibrinolytic system. *Ann N Y Acad Sci* 936, 226-236 (2001)
17. Herrick S, O. Blanc-Brude, A. Gray, & G Laurent. Fibrinogen. *Int J Biochem Cell Biol* 31, 741-746 (1999)
18. Ruggeri Z.M. von Willebrand factor and fibrinogen. *Curr Opin Cell Biol* 5,898-906 (1993)
19. Bennett J.S. Platelet-fibrinogen interactions. *Ann N Y Acad Sci.* 936, 340-354 (2001)
20. Clemetson K.J, & J.M Clemetson. Platelet collagen receptors. *Thromb Haemost* 86, 189-197 (2001)
21. Holly S.P, M.K. Larson, & L.V. Parise. Multiple roles of integrins in cell motility. *Exp Cell Res* 261, 69-74 (2000)
22. Blobe G.C, W.P. Schiemann, & H.F. Lodish. Role of transforming growth factor beta in human disease. *N Engl J Med* 342, 1350-1358 (2000)
23. Heldin C.H, & B. Westermark. Mechanism of action and in vivo role of platelet-derived growth factor. *Physiol Rev* 79, 1283-1316 (1999)
24. Iida N, M. Haisa, A. Igarashi, D. Pencev, & GR Grotendorst. Leukocyte-derived growth factor links the

PDGF and CXC chemokine families of peptides. *FASEB J.* 1996; 10: 1336-1345 (1996)
25. Jones G.E. Cellular signaling in macrophage migration and chemotaxis. *J Leukoc Biol* 68, 593-602 (2000)
26. Lowry SF. Cytokine mediators of immunity and inflammation. *Arch Surg* 128, 1235-1241 (1993)
27. Iwamoto R, & E. Mekada. Heparin-binding EGF-like growth factor: a juxtacrine growth factor. *Cytokine Growth Factor Rev* 11, 335-44 (2000)
28. Fitzpatrick F.A, & R. Soberman. Regulated formation of eicosanoids. *J Clin Invest* 107, 1347-1351 (2001)
29. Peplow P.V. Actions of cytokines in relation to arachidonic acid metabolism and eicosanoid production. *Prostaglandins Leukot Essent Fatty Acids* 54,303-317 (1996)
30. Sherman M, B. Weber, R. R. Datta & D.W. Kufe. Transcriptional and posttranscriptional regulation of macrophage-specific colony stimulating factor gene expression by tumor necrosis factor. Involvement of arachidonic acid metabolites. *J Clin Invest* 85, 442-447 (1990)
31. Gagnon L, L. Filion, C. Dubois, & M. Rola-Pleszczynski. Leukotrienes and macrophage activation: augmented cytotoxic activity and enhanced interleukin 1, tumor necrosis factor, and hydrogen peroxide production. *Agent Action* 26, 142-147 (1989)
32. Hori T, Y. Yamanaka, M. Hayakawa, S. Shibamoto, M. Tsujimoto, N. Oku, & F Ito. Prostaglandin antagonize fibroblast proliferation stimulated by tumor necrosis factor. *Biochem Biophys Res Commun* 174,758-766 (1991)
33. Chegini N, M.J. Rossi & L.E. Holmdahl. Cellular distribution of 5-lipoxygenase and leukotriene receptors in postsurgical peritoneal wound repair. *Wound Repair Reg* 5, 235-242 (1997)
34. Chegini N, & H. Rong. Postoperative exposure to glove powders modulates production of peritoneal eicosanoids during peritoneal wound healing. *Eur J Surg* 165, 698-704 (1999)
35. Muzii L, Marana R, Brunetti L, Margutti F, Vacca M, Mancuso S. Postoperative adhesion prevention with low-dose aspirin: effect through the selective inhibition of thromboxane production. *Hum Reprod* 13, 1486-1489 (1998)
36. Douma C.E, D.R. de Waart, D. Zemel, D.G. Struijk & R.T Krediet. Prostaglandin inhibition by intraperitoneal indomethacin has no effect on peritoneal permeability during stable CAPD. *Nephrol Dial Transplant* 16, 803-808 (2001)
37. Peng H, A.K. Cheung, L.G. Reimer, C.D. Kamerath & J.K. Leypoldt. Effect of indomethacin on peritoneal protein loss in a rabbit model of peritonitis. *Kidney Int* 59, 44-51 (2001)
38. Tager A.M, J.H. Dufour, K. Goodarzi, S.D. Bercury, U.H. von Andrian, & A.D. Luster. BLTR mediates leukotriene B(4)-induced chemotaxis and adhesion and plays a dominant role in eosinophil accumulation in a murine model of peritonitis. *J Exp Med* 192, 439-446 (2000)
39. Haribabu B, M.W. Verghese, D.A. Steeber, D.D. Sellars, C.B. Bock & R. Snyderman. Targeted disruption of the leukotriene B(4) receptor in mice reveals its role in inflammation and platelet-activating factor-induced anaphylaxis. *J Exp Med* 192, 433-438 (2000)
40. Gerard C, & B.J. Rollins. Chemokines and disease. Nat Immunol 2, 108-115 (2001)
41. Horuk R. Chemokine receptors. *Cytokine Growth Factor Rev* 12, 313-335 (2001)
42. Smiley S.T., J.A. King & W.W. Hancock. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. *J Immunol* 167, 2887-2894 (2001)
43. Matsukawa A, C.M. Hogaboam, N.W. Lukacs, P.M. Lincoln, R.M. Strieter, & S.L. Kunkel. Endogenous monocyte chemoattractant protein-1 (MCP-1) protects mice in a model of acute septic peritonitis: cross-talk between MCP-1 and leukotriene B4. *J Immunol* 163,6148-6154 (1999)
44. Fine JS, Jackson JV, Rojas-Triana A, Bober LA. Evaluation of chemokine- and phlogistin-mediated leukocyte chemotaxis using an in vivo sponge model. *Inflammation* 24, 331-346 (2000)
45. Call D.R, J.A. Nemzek, S.J. Ebong, G.B. Bolgos, D.E. Newcomb, G.K. Wollenberg, & D.G. Remick. Differential local and systemic regulation of the murine chemokines KC and MIP2. *Shock*.15, 278-284 (2001)
46. Kopydlowski K.M, C.A. Salkowski, M.J. Cody, N. van-Rooijen, J. Major, T.A. Hamilton & S.N. Vogel. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. *J Immunol*.163, 1537-1544 (1999)
47. Foussat A, K. Balabanian, A. Amara, L. Bouchet-Delbos, I. Durand-Gasselin, F. Baleux, J. Couderc, P. Galanaud, & D Emilie. Production of stromal cell-derived factor 1 by mesothelial cells and effects of this chemokine on peritoneal B lymphocytes. *Eur J Immunol* 31, 350-359 (2001)
48. Poznansky M.C, I.T. Olszak, R. Foxall, R.H. Evans, A.D. Luster, & D.T. Scadden. Active movement of T cells away from a chemokines. *Nat Med* 6, 543-548 (2000)
49. Steinhauser M.L, C.M. Hogaboam, A. Matsukawa, N.W. Lukacs, R.M. Strieter, & S.L. Kunkel. Chemokine C10 promotes disease resolution and survival in an experimental model of bacterial sepsis. *Infect Immun* 68, 6108-6114 (2000)
50. Orlofsky A, Y. Wu &M.B. Prystowsky. Divergent regulation of the murine CC chemokine C10 by Th(1) and Th(2) cytokines. *Cytokine* 12, 220-228 (2000)
51. Matsukawa A., C.M. Hogaboam, N.W. Lukacs, P.M. Lincoln, H.L. Evanoff, R.M. Strieter, & S.L. Kunkel. Expression and contribution of endogenous IL-13 in an experimental model of sepsis. *J Immunol* 164, 2738-2744 (2000)
52. Witowski J., A. Thiel, R. Dechend, K. Dunkel, N. Fouquet, T.O. Bender, J.M. Langrehr, G.M. Gahl, U. Frei, & A. Jorres. Synthesis of C-X-C and C-C chemokines by human peritoneal fibroblasts: induction by macrophage- derived cytokines. *Am J Pathol* 158, 1441-1450 (2001)
53. Visser C.E., J. Tekstra, J.J. Brouwer-Steenbergen, C.W. Tuk, D.M. Boorsma, S.C Sampat-Sardjoepersad, S. Meijer, R.T. Krediet, & R.H. Beelen. Chemokines produced by mesothelial cells: huGRO-α, IP-10, MCP-1 and RANTES. *Clin Exp Immunol* 112, 270-275 (1998)
54. McColl SR, Clark-Lewis I. Inhibition of murine neutrophil recruitment in vivo by CXC chemokine receptor antagonists. *J Immunol*.163,2829-2835 (1999)
55. Haslinger B, S. Mandl-Weber, A. Sellmayer, & T. Sitter. Hyaluronan fragments induce the synthesis of MCP-1 and IL-8 in cultured human peritoneal mesothelial cells. *Cell Tissue Res* 305, 79-76 (2001)
56. Ishihara K, Y. Yamaguchi, K. Okabe, & M. Ogawa. Neutrophil elastase enhances macrophage production of

chemokines in receptor-mediated reaction. *Res Commun Mol Pathol Pharmacol* 103,139-147 (1999)
57. Lee A, M.B.K. Whyte, & C. Haslett. In hibition of opoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. *J Leuk Biol* 54,283-288 (1993)
58. Savill J, V. Fadok, P. Hersar & C. Haslett. Phagocyte recognition of cell undergoing apoptosis. *Immunol Today* 14.131-136 (1993)
59. Savill J, I. Dransfield, N. Hogg, & C. Haslett. Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. *Nature* 343, 170-173 (1990)
60. Collins M.K, G.R. Perkins, G. Rodriguez-Tarduchy, M.A. Nieto, & A. Lopez-Rivas. Growth factors as survival factors: Regulation of apoptosis. *Bioassays* 16,133-138 (1994)
61. Hegyi L, S.J. Hardwick, R.C. Siow, & J.N. Skepper. Macrophage death and the role of apoptosis in human atherosclerosis. *J Hematother Stem Cell Res* 10,27-42 (2001)
62. Cockerill GW, J.R. Gamble, & M.A. Vadas. Angiogenesis: Models and modulators. *Internatl Rev Cytol* 159,113-160 (1995)
63. Ferrara N. Role of vascular endothelial growth factor in regulation of physiological angiogenesis. *Am J Physiol Cell Physiol* 280,C1358-1366 (2001)
64. Shibuya M. Structure and function of VEGF/VEGF-receptor system involved in angiogenesis. *Cell Struct Funct* 26,25-35 (2001)
65. Szekanecz Z., & A.E. Koch. Chemokines and angiogenesis. *Curr Opin Rheumatol* 13:202-208 (2001)
66. Kurz H. Physiology of angiogenesis. *J Neurooncol* 50,17-35 (2000)
67. Parks W.C. Matrix metalloproteinases in repair. *Wound Repair Regen* 7,423-432 (1999)
68. Vincenti M.P. The matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) genes. Transcriptional and posttranscriptional regulation, signal transduction and cell-type-specific expression. *Methods Mol Biol* 151,121-148 (2001)
69. Lund L.R., J. Romer, T.H. Bugge, B.S. Nielsen, T.L. Frandsen, J.L. Degen, R.W. Stephens, & K Dano. Functional overlap between two classes of matrix-degrading proteases in wound healing. *EMBO J* 18,4645-4656 (1999)
70. Gomez D.E, A.F. Alonso, H. Yoshiji, & U.P. Torgeirsson. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur J Cell Biol* 74,111-122 (1997)
71. Tonnesen M.G, X. Feng, & R.A. Clark. Angiogenesis in wound healing. *J Investig Dermatol Symp Proc* 5, 40-46 (2000)
72. Takehara K. Growth regulation of skin fibroblasts. *J Dermatol Sci* 24(Supp1): S70-77 (2000)
73. Cross M.J, & L Claesson-Welsh. FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition. *Trends Pharmacol Sci* 22, 201-207 (2001)
74. Ornitz D.M., & N. Itoh. Fibroblast growth factors. *Genome Biol* 2, 3005 (2001)
75. Ma C., R.W. Tarnuzzer & N. Chegini. Expression of matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases in mesothelial cells and their regulation by transforming growth factor-beta1. *Wound Repair Regen* 7, 477-485 (1999)
76. Chegini N. K. Kotseos, Y. Zhao, C. Ma, F. McLean, M.P. Diamond, L. Holmdahl, & J. Burns. The expression of matrix metalloproteinase (MMP-1) and tissue inhibitor of MMP (TIMP-1) in serosal tissue of intraperitoneal organs and adhesions. *Fertil Steril* 76, 1212-1219 (2001)
77. Chegini N, K. Kotsesos, B. Bennett, F.W. McLean, M.P. Diamond, L. Holmdahl & J. Burns. Matrix Metalloproteinase (MMP-1) and tissue inhibitor of MMP (TIMP-1) in peritoneal fluids and sera, and correlation with peritoneal adhesions. *Fertil Steril* 76, 1207-1211(2001)
78. Sharpe-Timms K.L., R.L. Zimmer, W.J. Jolliff, J.A. Wright, W.B. Nothnick, & T.E. Curry. 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 (1998)
79. Wiczyk H.P, D.R. Grow, L.A. Adams, D.L. O'Shea, & M.T. Reece. Pelvic adhesions contain sex steroid receptors and produce angiogenesis growth factors. *Fertil-Steril* 69, 511-516 (1998)
80. Rout U.K, K. Oommen & M.P. Diamond. Altered expressions of VEGF mRNA splice variants during progression of uterine-peritoneal adhesions in the rat. *Am J Reprod Immunol* 43, 299-304 (2000)
81. Howdieshell T.R, C. Riegner, V. Gupta, D. Callaway, K. Grembowicz, & P.L. Sathyanarayana-McNeil. Normoxic wound fluid contains high levels of vascular endothelial growth factor. *Ann Surg* 228, 707-715 (1998)
82. Swift M.E, H.K. Kleinman, & L.A. DiPietro. Impaired wound repair and delayed angiogenesis in aged mice. *Lab Invest* 79, 1479-1487 (1999)
83. O'Reilly M.S, L. Holmgren, Y. Shing. C. Chen, R.A. Rosenthal, M. Moses, W.S. Lane, Y Cao, E.H. Sage, & J Folkman. Angiostatin: A novel angiogenesis inhibitor that modulates the suppression of metastases by a Lewis Carcinoma. *Cell* 79, 315-328 (1994)
84. Fotsis T., M.S. Pepper, H. Aldercrutz, G. Fleischmann T. Hase , R. Montesano & L. Schweigerer. Genistein, a deitry-derived inhibitor of in vivo angiogenesis. *Proc Natl Acad Sci USA* 90, 2690-2694 (1993)
85. Fotsis T, Y. Zhang, M.S. Pepper, H. Adlercreutz, R. Montesano, P.P. Nawroth, & L. Schweigerer. The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppress tumor growth. *Nature* 368, 237-239 (1994)
86. Liekens S, E. De Clercq, & J. Neyts. Angiogenesis: regulators and clinical applications. *Biochem Pharmacol* 61,253-270 (2001)
87. Chiang S.C, C.H. Cheng, K.S. Moulton, J.M. Kasznica, & S.L. Moulton. TNP-470 inhibits intraabdominal adhesion formation. *J Pediatr Surg* 35, 189-196 (2000)
88. Chegini N., M.J. Rossi, G.S. Schultz, W.A. Dunn Jr. & B. J. Masterson. Cellular distribution of EGF, TGF-α and EGF receptor in fascia and peritoneum during healing: An autoradiographic and immunocytochemical study. *Wound Repair Reg* 1, 27-40 (1993)
89. Chegini N., J. Simms, R.S. Williams, & B.J. Masterson. Identification of epidermal growth factor, transforming

growth factor-a, and epidermal growth factor receptor in surgically induced pelvic adhesions in the rat and intraperitoneal adhesions in the human. *Am J Obstet Gynecol* 171, 321-328 (1994)
90. Faull R.J, J.M. Stanley, S. Fraser, D.A. Power, & D.I. Leavesley. HB-EGF is produced in the peritoneal cavity and enhances mesothelial cell adhesion and migration. *Kidney Int.* 59, 614-624 (2001)
91. Chegini N. The effect of growth factors on peritoneal cell growth. (perssonal observations)
92. Howdieshell T.R, D. Callaway, W.L. Webb, M.D. Gaines, C.D. Procter Jr, Sathyanarayana, J.S. Pollock, T.L. Brock, & P.L. McNeil. Antibody neutralization of vascular endothelial growth factor inhibits wound granulation tissue formation. *J Surg Res* 96, 173-182 (2001)
93. Jones J., & D. Clemmons. Insulin-like growth factors and their binding proteins, biological actions. *Endocrine Rev* 16, 3-34 (1994)
94. Chegini N., X-M. Tang, M.J. Rossi, S. Barnes, C.J.M. Kane, & B.J. Masterson. Detection of relaxin in rat and human Skin wound and its effect in skin fibroblast in vitro. *Wound Repair Reg*. Abst #33 (1993)
95. Gimbel M.L, D. Chelius, T.K. Hunt, & E.M. Spencer. A novel approach to reducing postoperative intraperitoneal adhesions through the inhibition of insulin like growth factor I activity. *Arch Surg* 136, 311-317 (2001)
96. Campbell P.G., S.K. Durham, J.D. Hayes, A. Suwanichkul, & D.R. Powell. Insulin-like growth factor-binding protein-3 binds fibrinogen and fibrin. *J Biol Chem* 274, 30215-30221 (1999)
97. Gibson T.L, & P Cohen. Inflammation-related neutrophil proteases, cathepsin G and elastase, function as insulin-like growth factor binding protein proteases. *Growth Horm IGF Res* 9, 241-253 (1999)
98. Herouy Y., P. Mellios, E. Bandemir, C Stetter, S. Dichmann, M. Idzko, C. Hofmann, W. Vanscheidt, E. Schopf, & J. Norgauer. Autologous platelet-derived wound healing factor promotes angiogenesis via *a*v**b**3-integrin expression in chronic wounds. *Int J Mol Med* 6, 515-519 (2000)
99. Chegini N., K. Kotseos, C. Ma, R.S. Williams, M.P. Diamond, L. Holmdahl & K Skinner. Differential expression of integrin αv and β3 in serosal tissue of human intraperitoneal organs and adhesion. *Fertil Steril* 75, 791-796 (2001)
100. Tietze L., J. Borntraeger, B. Klosterhalfen, B. Amo-Takyi, S. Handt, K. Gunther, & S Merkelbach-Bruse. Expression and function of β1 and β3 integrins of human mesothelial cells in vitro. *Exp Mol Pathol* 66, 131-139 (1999)
101. Witz C.A, I.A. Montoya-Rodriguez,, D.M. Miller, B.G. Schneider, & R.S. Schenken. Mesothelium expression of integrins in vivo and in vitro. *J Soc Gynecol Investig* 5, 87-93 (1998)
102. Arroyo A.G., D. Taverna, C.A. Whittaker, U.G. Strauch, B.L. Bader, H. Rayburn, D. Crowley, C.M. Parker, & R.O Hynes. In vivo roles of integrins during leukocyte development and traffic: insights from the analysis of mice chimeric for α5, αv, and α4 integrins. *J Immunol* 165, 4667-4675 (2000)
103. Sheppard D. Integrin-mediated activation of transforming growth factor-beta(1) in pulmonary fibrosis. *Chest* 120(Suppl): 49S-53S (2001)
104. Sime P.J, & K.M. O'Reilly. Fibrosis of the lung and other tissues: new concepts in pathogenesis and treatment. *Clin Immunol* 99, 308-319 (2001)
105. Murphy-Ullrich J.E, & M. Poczatek. Activation of latent TGF-β by thrombospondin-1: mechanisms and physiology. *Cytokine Growth Factor Rev*. 11,59-69 (2000)
106. Koli K, J. Saharinen, M. Hyytiainen, C. Penttinen, & J. Keski-Oja. Latency, activation, and binding proteins of TGF-β. *Microsc Res Tech* 52,354-362 (2001)
107. Dou, Q., R.S. Williams & N. Chegini. The expression of integrin messenger ribonucleic acid in human endometrium; a quantitative reverse transcription polymerase chain reaction study. *Fertil. Streil* 71, 347-353 (1999)
108. Singer A.J & R.A. Clark. Cutaneous wound healing. N *Engl J Med* 341,738-746 (1999)
109. Saed G.M, W. Zhang, N. Chegini, L. Holmdahl, & M.P. Diamond. Alteration of type I and III collagen expression in human peritoneal mesothelial cells in response to hypoxia and transforming growth factor-beta1. *Wound Repair Regen* 7, 504-510 (1999)
110. Branton M.H., & J.B. Kopp. TGF-β and fibrosis. *Microbes Infect* 1, 1349-1365 (1999)
111. Koch R.M., N.S. Roche, W.T. Parks, G.S. Ashcroft J.J. Letterio, & A.B. Roberts. Incisional wound healing in transforming growth factor-β1 null mice. *Wound Repair Regen* 8, 179-191 (2000)
112. Crowe M.J., T Doetschman, & D.G. Greenhalgh. Delayed wound healing in immunodeficient TGF-β1 knockout mice. *J Invest Dermatol* 115, 3-11 (2000)
113. Tyrone J.W, J.R. Marcus, S.R. Bonomo, J.E. Mogford, Y. Xia, & T.A. Mustoe. Transforming growth factor β3 promotes fascial wound healing in a new animal model. *Arch Surg* 135,1154-1159 (2000)
114. Chegini N, K. Kotseos, Y. Zhao, B. Bennett, F.W. McLean, M.P. Diamond, L. Holmdahl, & J. Burns. Differential expression of TGF-β1 and TGF-β3 in serosal tissues of human intraperitoneal organs and peritoneal adhesions. *Hum Reprod* 6, 1291-300 (2001)
115. Chegini N, H. Rong, B. Bennett, & I.K. Stone. Peritoneal fluid cytokine and eicosanoid levels and their relation to the incidence of peritoneal adhesion. *J Soc Gynecol Investig* 6,153-157 (1999)
116. Holmdahl L, K. Kotseos, M. Bergstrom, P. Falk, M.L. Ivarsson, & N Chegini. Overproduction of transforming growth factor-beta1 (TGF-β1) is associated with adhesion formation and peritoneal fibrinolytic impairment. *Surgery* 129,26-32 (2001)
117. Chegini N., L.I. Gold, R.S. Williams, & B.J. Masterson. Localization of transforming growth factor beta isoforms TGF-β1, TGF-β2, and TGF-β3 in surgically induced pelvic adhesions in the rat. *Obstet Gynecol* 83, 449-454 (1994)
118. Chegini N. The role of growth factors in peritoneal healing: transforming growth factor beta (TGF-β) Eur J Surg (Suppl) 577,17-22 (1997)
119. Ghellai A.M., A.F. Stucchi, N. Chegini, C. Ma, C.D. Andry, J.M. Kaseta, J.W. Burns, K.C. Skinner, & J.M. Becker. Role of transforming growth factor beta-1 in peritonitis-induced adhesions. *J Gastrointest Surg* 4,316-23 (2000)
120. Rong H, X-M.Tang, Y. Zhao, S.C. Juneja, M.F. Fay, R.S. Williams & N. Chegini. Peritoneal exposure to surgical glove powders differentially modulate the inflammatory and immune related cytokines production during surgically-induced adhesion formation. *Wound Repair Reg* 5, 89-96 (1997)

121. Lucas P.A., D.J. Warejcka, H.E. Young, & B.Y. Lee. Formation of abdominal adhesions is inhibited by antibodies to transforming growth factor-beta1. *J Surg Res* 65,135-138 (1996)
122. Williams R.S, A.M. Rossi, N. Chegini, & G. Schultz. Effect of transforming growth factor beta on postoperative adhesion formation and intact peritoneum. *J Surg Res* 52, 65-70 (1992)
123. Krause T.J, D. Katz, C.J. Wheeler, S. Ebner, & R.D. McKinnon. Increased levels of surgical adhesions in TGFb1 heterozygous mice. *J Invest Surg* 12,31-38 (1999)
124. Lanfrancone L., D. Boraschi, P. Ghiara, B. Falini, F. Grignani, G. Peri, A. Mantovani, & P.G. Pelicci. Human peritoneal mesothelial cells produce many cytokines (granulocyte colony-stimulating factor (CSF), granulocyte-monocyte-CSF, macrophage-CSF, interleukin-1 (IL-1), and IL-6) and are activated and stimulated to grow by IL-1. *Blood* 80, 2835-2842 (1992)
125. D.W. H. Riches. Macrophages involvement in wound repair, remodeling and fibrosis. In: The molecular and cellular biology of wound repair. Ed. R.A.F. Clark 2nd ed New York: Plenum Press, 95-141 (1996)
126. Niessen F.B, M.P. Andriessen, J. Schalkwijk, L. Visser, & W Timens. Keratinocyte-derived growth factors play a role in the formation of hypertrophic scars. *J Pathol* 194, 207-216 (2001)
127. Ma C, Q. Duo & N Chegini. Modulation of interleukin (IL), IL-2, IL-5, IL-6, IL-12, IL-13 and IL-16 expression in human mesothelial cells, macrophage and lymphocyte exposed to glove powders, latex proteins and LPS. *Fertil Steril.* 74, Abst. # P892 (1998)
128. Shadrin O.V, O.Y. Kaufman, O.M. Dronova, & S.N. Bykovskaya. Recombinant interleukin-2 significantly increases the survival of mice with peritonitis, but not acute Staphylococcus aureus peritoneal infection. *Mol Biother* 4, 151-154 (1992)
129. Cohen M.C, & S. Cohen. Cytokine Function. A study in biologic diversity. *Am J Clin Pathol* 105, 589-598 (1996)
130. Miossec P. Interleukin 4. A potential anti-inflammatory agent. *Rev Rheum* 60, 119-124 (1993)
131. Beckmann M.P, D. Cosman, W. Fanslow, C.R. Maliszewski, & S.D. Lyman. The interleukin-4 receptor: structure, function, and signal transduction. *Chem Immunol* 51, 107-134 (1992)
132. Salmon-Ehr V., L. Ramont, G. Godeau, P. Birembaut, M. Guenounou, P. Bernard, & F.X Maquart. Implication of interleukin-4 in wound healing. *Lab Invest* 80, 1337-1343 (2000)
133. Hirano T. The biology of interleukin-6. Chem *Immunol* 51, 153-180 (1992)
134. Donnelly RP, L.J. Crofford, S.L. Freeman, J. Buras, E. Remmers, R.L. Wilder, & M.J. Fenton.Tissue-specific regulation of IL-6 production by IL-4. Differential effects of IL-4 on nuclear factor-kappa B activity in monocytes and fibroblasts. *J Immunol* 151, 5603-5612 (1993)
135. Saba A.A., A.A.Kaidi, V. Godziachvili, G.W. Dombi, E.J. Dawe, J.H. Libcke, & Y.J. Silva. Effects of interleukin-6 and its neutralizing antibodies on peritoneal adhesion formation and wound healing. *Am Surg* 62,569-572 (1996)
136. Gallucci R.M, P.P. Simeonova, J.M. Matheson, C. Kommineni, J.L. Guriel, T. Sugawara, & M.I. Luster. Impaired cutaneous wound healing in interleukin-6-deficient and immunosuppressed mice. *FASEB J* 14,2525-2531 (2000)
137. Arici A., S.I. Tazuke, E. Attar, H.J. Kliman, & D.L. Olive. 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 (1996)
138. Iocono J.A, K.R. Colleran, D.G. Remick, B.W. Gillespie, H.P. Ehrlich, & W.L. Garner. Interleukin-8 levels and activity in delayed-healing human thermal wounds. *Wound Repair Regen* 8, 216-225 (2000)
139. Rennekampff H.O., J.F. Hansbrough, V. Kiessig, C. Dore, M. Sticherling, & J.M. Schroder. Bioactive interleukin-8 is expressed in wounds and enhances wound healing. *J Surg Res* 93,41-54 (2000)
140. Holzheimer R.G, & W. Steinmetz. Local and systemic concentrations of pro- and anti-inflammatory cytokines in human wounds. *Eur J Med Res* 5,347-355 (2000)
141. Cavaillon J.M. Pro- versus anti-inflammatory cytokines: myth or reality. *Cell Mol Biol* 47, 695-702 (2001)
142. Sato Y, T. Ohshima, & T. Kondo. Regulatory role of endogenous interleukin-10 in cutaneous inflammatory response of murine wound healing. *Biochem Biophys Res Commun* 265,194-199 (1999)
143. Holschneider C.H., P.M. Cristoforoni, K. Ghosh, M. Punyasavatsut, E. Abed, & F.J. Montz. Endogenous versus exogenous IL-10 in postoperative intraperitoneal adhesion formation in a murine model. *J Surg Res* 70, 138-143 (1997)
144. Sewnath M.E, D.P. Olszyna, R. Birjmohun, F.J. ten Kate, D.J. Gouma, & T. vanDer Poll. IL-10-deficient mice demonstrate multiple organ failure and increased mortality during Escherichia coli peritonitis despite an accelerated bacterial clearance. *J Immunol* 166, 6323-6331 (2001)
145. Zingarelli B., Z. Yang, P.W. Hake, A. Denenberg, & H.R. Wong. Absence of endogenous interleukin 10 enhances early stress response during post-ischaemic injury in mice intestine. *Gut* 48: 610-622 (2001)
146. Opal S.M, & V.A. DePalo. Anti-inflammatory cytokines. *Chest* 117, 1162-1172 (2000)
147. Scott P. IL-12: initiation cytokine for cell-mediated immunity. *Science* 260, 496-507 (1993)
148. Steinhauser ML, C.M. Hogaboam, N.W. Lukacs, R.M. Strieter, & S.L. Kunkel. Multiple roles for IL-12 in a model of acute septic peritonitis. *J Immunol* 162, 5437-5443 (1999)
149. Brombacher F. The role of interleukin-13 in infectious diseases and allergy. *Bioessays* 22,646-56 (2000)
150. Chomarat, P. & J. Banncherеau. Interleukin-4 and interleukin-13: their similarities and discrepancies. *Int Rev Immunol* 17, 1-52 (1998)
151. Waldmann T.A, & Y. Tagaya. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens1. *Annu Rev Immunol* 17, 19-49 (1999)
152. Fehniger T.A, & M.A. Caligiuri. Interleukin 15: biology and relevance to human disease. *Blood* 97,14-32 (2001)
153. Marks-Konczalik J., S. Dubois, J.M. Losi, H. Sabzevari, N. Yamada, L. Feigenbaum, T.A. Waldmann, &

Y Tagaya. IL-2-induced activation-induced cell death is inhibited in IL-15 transgenic mice. *Proc. Natl. Acad. Sci. USA* 97, 11445-11450 (2000)
154. Chegini N, M. Roberts, B.A. Ripps. Differential expression of interlukines (IL) IL-13 and IL-15 in endometrial implants and peritoneal fluids of women with endometriosis. *J Reprod Immunol* 2002.
155. Swain S.L. Interleukin 18: tipping the balance towards a T helper cell 1 response. *J Exp Med* 194, F11-4 (2001)
156. Kampfer H., H. Muhl, M. Manderscheid, U. Kalina, D. Kauschat, J. Pfeilschifter, & S Frank. Regulation of interleukin-18 (IL-18) expression in keratinocytes (HaCaT): implications for early wound healing. *Eur Cytokine Netw* 11, 626-633 (2000)
157. Kampfer H., J. Paulukat, H. Muhl, C Wetzler, J. Pfeilschifter, & S Frank. Lack of interferon-gamma production despite the presence of interleukin-18 during cutaneous wound healing. *Mol Med* 6,1016-1027 (2000)
158. Ma X. TNF-α and IL-12: a balancing act in macrophage functioning. *Microbes Infect* 3, 121-129 (2001)
159. Gillitzer R, & M. Goebeler. Chemokines in cutaneous wound healing. *J Leukoc Biol* 69, 513-521 (2001)
160. Engelhardt E, A. Toksoy, M. Goebeler, S. Debus, E.B. Brocker, & R Gillitzer. Chemokines IL-8, GROα, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. *Am J Pathol* 153, 1849-1860 (1998)
161. Jackman S.H., M.B. Yoak, S. Keerthy, & B.L. Beaver. Differential expression of chemokines in a mouse model of wound healing. *Ann Clin Lab Sci*. 30, 201-207 (2000)
162. Wetzler C, H. Kampfer, J. Pfeilschifter, & S. Frank. Keratinocyte-derived chemotactic cytokines: expressional modulation by nitric oxide in vitro and during cutaneous wound repair in vivo. *Biochem Biophys Res Commun* 274: 689-696 (2000)
163. Low Q.E., I.A. Drugea, L.A. Duffner, D.G. Quinn, D.N. Cook, B.J. Rollins, E.J. Kovacs, & L.A. DiPietro. Wound healing in MIP-1a (-/-) and MCP-1(-/-) mice. *Am J Pathol* 159, 457-463 (2001)
164. Luster A.D., R.D. Cardiff, J.A. MacLean, K. Crowe, & R.D. Granstein. Delayed wound healing and disorganized neovascularization in transgenic mice expressing the IP-10 chemokine. *Proc Assoc Am Physicians*. 110: 183-196 (19998)
165. Shiraha H., A. Glading, K. Gupta, & A. Wells. IP-10 inhibits epidermal growth factor-induced motility by decreasing epidermal growth factor receptor-mediated calpain activity. *J Cell Biol* 146, 243-254 (1999)
166. Nanney L.B, S.G. Mueller, R. Bueno, S.C. Peiper, & A. Richmond. Distributions of melanoma growth stimulatory activity of growth-regulated gene and the interleukin-8 receptor B in human wound repair. *Am J Pathol* 147, 1248-1260 (1995)
167. Nirodi C.S, R. Devalaraja, L.B. Nanney, S Arrindell, S. Russell, J. Trupin, & A. Richmond. Chemokine and chemokine receptor expression in keloid and normal fibroblasts. *Wound Repair Regen* 8,371-382 (2000)
168. Zeyneloglu H.B., E Seli, L.M. Senturk, L.S. Gutierrez, D.L. Olive, A. Arici. The effect of monocyte chemotactic protein 1 in intraperitoneal adhesion formation in a mouse model. *Am J Obstet Gynecol* 179, 438-443 (1998)
169. Mrstik M, K. Kotseos C. Ma & N. Chegini. Increased expression of interferon-gamma inducible protein-10 (IP-10) mRNA during surgically-induced peritoneal injury. *J Soc Gynecol Invest* ,7;Abst # 828 (2000)
170. Zeyneloglu H.B, L.M Senturk, E. Seli, E. Oral, D.L. Olive & A. Arici. The role of monocyte chemotactic protein-1 in intraperitoneal adhesion formation. *Hum Reprod* 13, 1194-1199 (1998)
171. Gallo R.L. Proteoglycans and cutaneous vascular defense and repair. *J Investig Dermatol Symp Proc* 5,55-60 (2000)
172. Yamada K.M, J. Gailit, & R.A. F. Clark. Integrin in wound repair. In: The molecular biology of wound repair. Ed. R.A.F. Clark. 2nd ed. New York: Plenum Press, 311-330 (1996)
173. Idell S., C. Zwieb, J. Boggaram, D. Holiday, A.R. Johnson, & G. Raghu. Mechanisms of fibrin formation and lysis by human lung fibroblasts: influence of TGF-b and TNF-*a. Am J Physiol*. 263: L487-494 (1992)
174. Keene D.R., M.P. Marinkovich, & L.Y. Sakai. Immunodissection of the connective tissue matrix in human skin. *Microsc Res Tech* 38, 394-406 (1997)
175. Iozzo R.V., & A.D. Murdoch. Proteoglycans of the extracellular environment: clues from the gene and protein side offer novel perspectives in molecular diversity and function. *FASEB* 10,598-614 (1996)
176. Raghow R. The role of extracellular matrix in post-inflammatory wound healing and fibrosis. *FASEB* 8,823-831 (1994)
177. Hu B., Y.L. Kapila, M. Buddhikot, M. Shiga, & S. Kapila. Coordinate induction of collagenase-1, stromelysin-1 and urokinase plasminogen activator (uPA) by the 120-kDa cell-binding fibronectin fragment in fibrocartilaginous cells: uPA contributes to activation of procollagenase-1. *Matrix Biol*.19,657-669 (2000)
178. Olman M.A, J.S. Hagood, W.L. Simmons, G.M. Fuller, C. Vinson, & K.E. White. Fibrin fragment induction of plasminogen activator inhibitor transcription is mediated by activator protein-1 through a highly conserved element. *Blood* 94, 2029-2038 (1999)
179. Saed G., W. Zhang, K.A. Wie, F.D. Yelian, & M.P. Diamond. The effect of TGF-β and hypoxia on fibronectin expression. *J Soc Gynecol Invest* 7, 348-354 (2000)
180. Jang Y.C., R. Tsou, N.S. Gibran, & F.F. Isik. Vitronectin deficiency is associated with increased wound fibrinolysis and decreased microvascular angiogenesis in mice. *Surgery* 127, 696-704 (2000)
181. Shaw R.J, D.E. Doherty, A.G. Ritterl, S.H. Benedict & R.A. Clark. Adherance-dependent increase in human monocyte PDGF(B) mRNA is associated with increases in c-fos, c-jun and EGR-2 mRNA. *J Cell Biol* 111, 2139-2148 (1990)
182. Gharaee-Kermani M, & S.H. Phan. Role of cytokines and cytokine therapy in wound healing and fibrotic diseases. *Curr Pharm Des*.7, 1083-1103 (2001)
183. Ramanathan M. A pharmacokinetic approach for evaluating cytokine binding macromolecules as antagonists. *Pharm Res* 13, 84-90 (1996)
184. Arend W.P. Inhibiting the effects of cytokines in human diseases. Adv Intern Med 40, 365-394 (1995)
185. Gehr G, T. Braun, & W. Lesslauer. Cytokines, receptors, and inhibitors. *Clin Invest* 70, 64-69 (1992)

186. Holmdahl L. The role of fibrinolysis in the formation of adhesions. *Eur J Surg* (Suppl) 577, 24-31 (1997)
187. Falk P., C. Ma, N. Chegini, & L. Holmdahl. Differential regulation of mesothelial cell fibrinolysis by transforming growth factor beta 1. *Scand J Clin Lab Invest* 60, 439-47 (2000)
188. Bugge T.H., M.J. Flick, M.J. Danton, C.C. Daugherty, J. Romer, K. Dano, P. Carmeliet, D. Collen, & J.L. Degen. Urokinase-type plasminogen activator is effective in fibrin clearance in the absence of its receptor or tissue-type plasminogen activator. *Proc Natl Acad Sci USA*. 93,5899-904 (1996)
189. Bugge T.H, K.W. Kombrinck, M.J. Flick, C.C. Daugherty, M.J. Danton, & J.L. Degen. Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. *Cell* 87, 709-719 (1996)
190. Carmeliet P, & D. Collen. Molecular genetics of the fibrinolytic and coagulation systems in haemostasis, thrombogenesis, restenosis and atherosclerosis. *Curr Opin Lipidol* 8,118-125 (1997)
191. Horton M.R, M.A. Olman, & P.W Noble. Hyaluronan fragments induce plasminogen activator inhibitor-1 and inhibit urokinase activity in mouse alveolar macrophages: a potential mechanism for impaired fibrinolytic activity in acute lung injury. *Chest* 116 (Suppl),17S (1999)
192. Fitzpatrick S.L, G. Kassam, K.S. Choi, H.M. Kang, D.K. Fogg, & D.M. Waisman. Regulation of plasmin activity by annexin II tetramer. *Biochemistry* 39,1021-1028 (2000)
193. Holmdahl L, E. Eriksson, B.I. Eriksson, & B. Risberg. Depression of peritoneal fibrinolysis during surgery is a local response to trauma. *Surgery* 123,539-544 (1998)
194. Tsuji K, Y. Eguchi, & M. Kodama. Postoperative hypercoagulable state followed by hyperfibrinolysis related to wound healing after hepatic resection. *J Am Coll Surg* 183,230-238 (1996)
195. Lai H.S, Y. Chen, K.J. Chang, & W.J. Chen. Tissue plasminogen activator reduces intraperitoneal adhesion after intestinal resection in rats. *J Formos Med Assoc* 97, 323-327 (1998)
196. Montz F.J, J.M. Fowler, A.J. Wolff, S.M. Lacey, & M. Mohler. The ability of recombinant tissue plasminogen activator to inhibit post-radical pelvic surgery adhesions in the dog model. *Am J Obstet Gynecol* 165, 1539-1542 (1991)
197. Dunn R.C, & M. Mohler. Effect of varying days of plasminogen activator therapy on the prevention of postsurgical adhesions in a rabbit model. *J Surg Res* 54, 242-245 (1993)
198. Evans D.M, K. McAree, D.P. Guyton, N. Hawkins, & K. Stakleff. Dose dependency and wound healing aspects of the use of tissue plasminogen activator in the prevention of intra-abdominal adhesions. *Am J Surg* 165, 229-232 (1993)
199. Arumugam S., Y.C. Jang, C. Chen-Jensen, N.S. Gibran, & F.F. Isik. Temporal activity of plasminogen activators and matrix metalloproteinases during cutaneous wound repair. *Surgery* 125,587-593 (1999)
200. Falk K., P. Bjorquist, M. Stromqvist, & L. Holmdahl. Reduction of experimental adhesion formation by inhibition of plasminogen activator inhibitor type 1. *Br J Surg* 88, 286-289 (2001)
201. Ravanti L., & V.M. Kahari. Matrix metalloproteinases in wound repair. *Int J Mol Med*. 6,391-407 (2000)
202. Raza S.L, & L.A. Cornelius. Matrix metalloproteinases and their native or pharmacologic inhibitors. *Adv Dermatol* 16, 185-208 (2000)
203. Soo C., W.W. Shaw, X. Zhang, M.T. Longaker, E.W. Howard, & K. Ting. Differential expression of matrix metalloproteinases and their tissue-derived inhibitors in cutaneous wound repair. *Plast Reconstr Surg* 105, 638-647 (2000)
204. von Lampe B., B. Barthel, S.E. Coupland, E.O. Riecken, S. Rosewicz. Differential expression of matrix metalloproteinases and their tissue inhibitors in colon mucosa of patients with inflammatory bowel disease. *Gut* 47, 63-73 (2000)
205. Makela M, H. Larjava, E. Pirila, P. Maisi, T. Salo, T. Sorsa, & V.J. Uitto. Matrix metalloproteinase 2 (gelatinase A) is related to migration of keratinocytes. *Exp Cell Res* 251, 67-78 (1999)
206. Lempinen M., K. Inkinen, H. Wolff, & J. Ahonen. Matrix metalloproteinases 2 and 9 in indomethacin-induced rat gastric ulcer. *Eur Surg Res* 32, 169-176 (2000)
207. Savage F.J, D.L. Lacombe, R.M. Hembry, & P.B. Boulos. Effect of colonic obstruction on the distribution of matrix metalloproteinases during anastomotic healing. *Br J Surg* 85,72-75 (1998)
208. Tarlton J.F, C.J. Vickery, D.J. Leaper, & A.J. Bailey. Postsurgical wound progression monitored by temporal changes in the expression of matrix metalloproteinase-9. *Br J Dermatol* 137, 506-516 (1997)
209. Agren M.S. Matrix metalloproteinases (MMPs) are required for re-epithelialization of cutaneous wounds. *Arch Dermatol Res* 291: 583-90 (1999)
210. Ashcroft G.S, M.A. Horan, S.E. Herrick, R.W. Tarnuzzer, G.S. Schultz, & M.W. Ferguson. Age-related differences in the temporal and spatial regulation of matrix metalloproteinases (MMPs) in normal skin and acute cutaneous wounds of healthy humans. *Cell Tissue Res* 290, 581-591 (1997)
211. Pirila E., N. Ramamurthy, P. Maisi, S. McClain, A. Kucine, J. Wahlgren, L. Golub, T. Salo, & T Sorsa. Wound healing in ovariectomized rats: effects of chemically modified tetracycline (CMT-8) and estrogen on matrix metalloproteinases -8, -13 and type I collagen expression. *Curr Med Chem* 8, 281-294 (2001)
212. Agren M.S, L.N. Jorgensen, M. Andersen, J. Viljanto, & F. Gottrup. Matrix metalloproteinase 9 level predicts optimal collagen deposition during early wound repair in humans. *Br J Surg* 85, 68-71 (1998)
213. Mirastschijski U, M.A. Karsdal, B. Jeppsson, & M.S. Agren. Matrix metalloproteinses (MMPs) in epidermal and peritoneal wound healing. *Wound Repair Reg*, 6 Abst #A321 (1998)
214. Syk I., M.S. Agren, D. Adawi, & B. Jeppsson. Inhibition of matrix metalloproteinases enhances breaking strength of colonic anastomoses in an experimental model. *Br J Surg* 88,228-234 (2001)
215. Liekens S., E. De Clercq, & J. Neyts. Angiogenesis: regulators and clinical applications. *Biochem Pharmacol* 61,253-270 (2001)
216. Klimp AH, E. van der Vaart, P.O. Lansink, S. Withoff, E.G. de Vries, G.L. Scherphof, J. Wilschut, & T.

Daemen. Activation of peritoneal cells upon in vivo transfection with a recombinant alphavirus expressing GM-CSF. *Gene Ther* 8, 300-307 (2001)
217. Hoff C.M. Ex vivo and in vivo gene transfer to the peritoneal membrane in a rat model. *Nephrol Dial Transplant* 16,666-668 (2001)
218. Jackman R.W., T.D. Stapleton, E.M. Masse, V.S. Harvey, M.S. Meyers, T.R. Shockley, & J.A. Nagy. Enhancement of the functional repertoire of the rat parietal peritoneal mesothelium in vivo: directed expression of the anticoagulant and anti-inflammatory molecule thrombomodulin. *Hum Gene Ther* 9,1069-1081 (1998)
219. Jiang A, & R. Dornburg. In vivo cell type-specific gene delivery with retroviral vectors that display single chain antibodies. *Gene Ther* 6,1982-1987 (1999)
220. Murphy J.E., & J.G. Rheinwald. Intraperitoneal injection of genetically modified human mesothelial cells for systemic gene therapy. *Hum Gene Ther* 8,1867-1879 (1997)

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